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Combined Particle Analysis as a New Tool to Predict Gushing Shown with Alcohol-Free Beverage Products

Combined particle analysis was applied as a tool to predict gushing in alcohol-free beverage products. Gushing is known as spontaneous overfoaming of carbonated beverages that is an intermittent problem for brewers and the beverage industry. Therefore, preventive tests to examine raw materials for their gushing risk are of interest for the industry. Previously, the focus of gushing tests was on the overfoaming of carbonated samples in specifically shaken bottles, after which the amount of overfoaming was recorded as the measure of gushing. As these tests require a number of days to be performed, it often happens that the raw materials have already been processed before the results are available. Here the combined particle analysis is presented as a “real time” alternative to such tests. This testing methodology was applied in beverage products (for producing spritzers) where it was shown that this analysis was successful in predicting gushing. In the test samples for producing apple spritzers putative gushing causing particles with sizes of only 1–2 nm were identified. Significantly higher stray light intensities for these particles with sizes of 1–2 nm were detected for the gushing-positive compared to gushing-negative samples. The particle charge titration method revealed higher titrated volumes for the gushing-positive samples (to neutralize the entire particle surface charge) than for the gushing-negative ones. Therefore, the higher titrated volumes were associated with significantly higher amounts of 1–2 nm-particles. Accordingly the titrated volume for charge-neutralization was proposed as measurement parameter to “quantify” gushing-relevant 1–2 nm-particles in order to estimate the gushing propensity. In further beverage products (for producing apple-cherry-spritzers) small particles were not detected, which explains why considerably lower titrated volumes were used and no gushing was observed. The results demonstrate that the particle size and the particle surface charge are promising analytical parameters to predict the gushing propensity of beverage products.

Descriptors: beverage, combined particle analysis, fruit spritzer, gushing, particle charge analyzer, particle size measurement

1 Introduction

The brewing and beverage industry is faced with the problem of gushing, a phenomenon of carbonated beverages that is characterized by the eruptive overfoaming after opening the bottle. A variety of influencing factors originating from the raw materials and during the production process contribute to the complexity of this phenomenon [1–6]. Gushing of beer can be induced by substances from raw materials, especially malt, which is named primary gushing. During some years and in specific regions the cereal raw materials can be qualitatively changed due to fungal infection [7–9]. Biological factors such as microorganisms including lactic acid bacteria and yeast have been suggested by previous studies to influence gushing during the malting and brewing process [10–12]. In general, the influencing factors in the production process of beer are described as secondary gushing. For

example, particles such as metal ions or calcium oxalate [13–17] can be responsible for this type of gushing. Another example is the fermentation process where the flocculation of yeast is associated with gushing [18].

In regard to primary gushing two groups of proteins, fungal specific hydrophobins and plant typical non-specific lipid transfer proteins (ns-LTPs) were identified as gushing-positive [19–22]. It is assumed that ns-LTP1 induces gushing when they are glycosylated and degraded by proteolysis during the brewing process [19]. These surface active peptides can be responsible for gushing. The property of surface activity can also be found for hydrophobins [23] which makes them important as gushing inducing substances. Both classes are suggested as indicators for the infection of cereal and malt grains by mould, which can be useful to assess the gushing risk prior to the beer production [19, 21, 22, 24, 25].

There is an agreement of a common gushing mechanism in carbonated beverages [26–29]:

Surface active substances stabilize CO₂-microbubbles by agglomeration at the interface gas-liquid of the CO₂-bubble. A recent study [30] suggested a new mechanism for the formation of these stabilized microbubbles where micelles grow to stabilized microbubbles by diffusion of CO₂ molecules into the hydrophobic core of the micelles.

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Tables and figures see Appendix

Despite of intensive investigations of the gushing phenomenon [7, 8, 19, 26, 31] a timely quality control and preventative solution has yet to be developed. Various substances are known to have a gushing reducing effect [32–38]. A recent investigation by *Hanke et al.* [34] about gushing suppressing hop substances indicated that appropriate hop technology can be an effective technological measure to minimize gushing. Previous studies confirmed the gushing reducing capability of hop [35–38]. Additionally, experiences in the brewing industry have shown that beer with higher hop addition can lead to beer with reduced gushing propensity (such as pilsner beer) [34].

The best way to reduce the risk of gushing is the early detection of raw materials with gushing potential. Brewers currently use two approved gushing tests according to MEBAK [39], the Weihenstephaner Test [39–41] and the Modified Carlsberg Test [39, 42] to determine the gushing potential of malt. With the Weihenstephaner Test the wort is first carbonated and then bottled, whereas in the Modified Carlsberg Test the wort is added to carbonated water. In both tests, the produced bottles are subjected to specific protocols for bottle shaking, followed by opening to provide the extent of overfoaming or not. The overfoaming amount serves as measure for the detected gushing potential of the examined malt.

Unfortunately, experiments have shown that the overfoaming amount can fluctuate, which makes it difficult to determine the gushing potential precisely. That is why two methods have been developed by a modification of the Modified Carlsberg Test [43]. The concept of the first method is based on the hypothesis that gushing can only occur when gushing inducing substances reach a minimum concentration. The existence of a minimum concentration for observing gushing has already been shown by hydrophobins [22] and by an aliphatic cationic surfactant [30]. In the first method the volume of wort (added to carbonated table water) is determined when gushing appears the first time. For example, if the volume of wort is very low when gushing is observed, a very high concentration of gushing inducing substances is expected. This modification makes the volume of wort a useful measurement parameter to quantify the gushing potential. In the second method gushing (induced by a constant volume of wort) is gradually reduced by the increasing amount of a gushing suppressing hop product (CO₂ hop extract). For example, if low amounts of this hop product are sufficient to neutralize gushing entirely, then a low concentration of gushing inducing substances is expected. In this method the amount of hop product to neutralize gushing for the first time is proposed as measurement parameter to quantify the gushing potential. In both methods, not the overfoaming amount, but the “zero point” is determined when gushing starts (from a minimum volume of wort, first method) or is neutralized (by a certain amount of a hop product, second method). Both methods have the advantage that the problem of fluctuating overfoaming amounts does not exist any more.

For the beverage industries, the most desirable solution would be a rapid analysis to predict the gushing potential of raw materials before they are processed. As the current gushing tests are time consuming, this study focused on developing a rapid analysis for gushing prediction. To achieve this objective, gushing-relevant particles were assessed by size and surface charge by the combined particle analysis. It has been experimentally demonstrated

by the use of an aliphatic cationic surfactant with known chemical structure that particles with sizes of only 1–2 nm are important in initiating gushing [30].

By combining the particle size measurement with the particle charge titration, the detected particles can be “quantified” by the titrated volume that was necessary to neutralize the particle surface charge. The particle charge titration method and the concept were first introduced as a long term stability measurement of filtered beer by *Titze et al.* [44]. Another application in the brewing process was also presented for characterizing the content of high-molecular protein fractions (coagulable nitrogen) in wort by using the charge analyzing system [45]. Recent investigations have shown the advantages of the combined particle analysis for estimating the physico-chemical behavior of particles in beer samples like the agglomeration process regarding haze-forming [46].

Previously, the particle charge analyzer was successfully applied to sparkling wine to estimate its gushing propensity [47]. Gushing-positive were distinguished from gushing-negative sparkling wines by higher amounts of a cationic titration solution (Poly-DADMAC) for charge-neutralization. Higher consumptions of Poly-DADMAC for gushing-positive sparkling wines were associated with higher amounts of negatively charged colloids playing an important role in the occurrence of gushing. Further examinations of the sparkling wines confirmed that more surface active anionic substances were present in the gushing-positive samples [47]. *Bach et al.* [47] presented the particle charge titration as a reliable method to detect the gushing propensity of sparkling wine.

In this study primary products for producing fruit spritzers were examined by the combined particle analysis. These materials can also be of interest for brewers as they are commonly used for creating beer “shandies”, mixtures of beer and spritzers. As reference method to the particle analysis, a conventional gushing test was applied which detected gushing by overfoaming of alcohol-free beverages.

2 Materials and methods

2.1 Raw materials and chemicals

Primary products were examined that are used in the industry for producing spritzers. Two different types of these products were applied, on the one hand for producing apple spritzers and on the other hand for producing apple-cherry spritzers. For the apple spritzers five primary products (named A 1–5) and for the apple-cherry spritzers two primary products (B 1–2) were examined.

2.2 Gushing test for alcohol-free beverages

At R&D of the Research Center Weihenstephan for Brewing and Food Quality a gushing test for alcohol-free beverages was optimized as “rapid test”. Beverage materials can be examined for their gushing propensity within one day. The development based on gushing tests that have been already applied by manufacturing companies for beverage products and by brewers [39–42]. In this test the overfoaming amount serves as positive signal for present gushing.

In the following, the test execution is described: The primary products were added to carbonated water (500 ml) that was bottled in disposable PET-bottles and have a content of CO₂ from 6.5 to 7.0 g/l. The primary product and the carbonated water were stored at $T \approx 4$ °C before mixing. Using pipettes the carbonated water (500 ml) was substituted by the same amount (50 ml) as the primary product was added to. These prepared bottles were rotated by 360 degrees for 10 minutes using a bottle turning device (Dr. Thiedig & Co., Berlin, Germany) with a frequency of 0.66 Hz. After this rotation the bottles were subjected to room temperature (approx. 3 h). Prior to opening, the bottles were rotated again (three times) by the bottle turning device and let stand for 30 seconds. For each experiment six identically prepared bottles ($n = 6$) were examined within one day.

2.3 Particle Size Analyzer

A Nanotrak Particle Size Analyzer from Microtrac of Particle Metrix GmbH, Meerbusch (Germany) with the measurement range from 0.8 to 6500 nm was applied to determine the particle size distribution. The analyzer allowed the detection of all particles in the medium that differ in the refraction index of the liquid.

The intensity of the dynamic straylight was graphically illustrated as a sum distribution being a function of the particle size. Existing particle sizes were identified by the slope of the graph, while a horizontal plot indicated that the corresponding particle sizes were not present.

2.4 Particle Charge Analyzer [44]

The surface potential of the particles in the applied samples were measured with a particle charge analyzer, model Charge Analyzing System (CAS) from AFG Analytic GmbH, Leipzig (Germany). The streaming potential was given in mV-units. The titrations were performed using 0.001 n polydiallyl dimethyl ammonium chloride (Poly-DADMAC) as the cationic solution. By titrating the polyelectrolyte solution, the net charge on the entire surface of the particles could be determined; the surface charge was expressed as the equivalent to the amount of 0.001 n Poly-DADMAC-solution required to achieve neutrality. The mechanism of the charge titration method was described in detail in previous publications [48, 49].

2.5 Combined particle analysis

The concept of a combined particle analysis was previously introduced by Titze et al. [46] using beer samples where particle size measurement was combined with a particle charge analysis. Particle size measurement enables the identification of particles with particular sizes, but the quantity of the detected particles is not indicated. Titze et al. [46] also showed that the particle size distribution of a beer sample did not change despite of different degrees of dilution. The distribution of all particles remains equal even if lower concentrations of particles can be found in all size classes. Further examples were presented that in aging beer (accelerated by a forcing test according to MEBAK [50]) particle sizes increase due to agglomeration of present particles [44]. A decrease of the entire particle surface (charge) was expected and

was confirmed by a lower titrated volume for the charge-neutralization [44].

2.6 Statistical evaluation

To work out statistical significance the Student's *t*-test was applied, so that each mean value (arithmetic mean) is provided with a confidence interval with a probability of $P = 0.95\%$ [51]. The confidence interval serves as statistical tool to estimate the repeatability of each measurement according to DIN EN ISO 5725 [52]; the smaller the confidence intervals the better the repeatability.

2.6.1 Particle size measurement

For the depiction of the particle size distribution, 20 single measurements ($n = 20$) were performed consecutively to provide an arithmetic mean for each class of particles. The sum distributions in the diagrams were illustrated with the confidence intervals.

2.6.2 Particle charge titration

For each primary product three titration curves were determined. From these values the arithmetic means were calculated to produce the mean value curve. Each mean value curve was approximated by a characteristic function (approximation curves) according to Titze et al. [44]. The agreement of the approximation curve with the respective mean value curve was expressed by the coefficient of determination (R^2). In addition to the approximation curve, the respective confidence intervals were illustrated in the diagrams (Fig. 2 and 4) in order to show the range of variation. The coefficients of determination (R^2) of all chosen approximation curves are above 99%.

2.7 Sample preparation for particle size measurement and charge titration

For an optimal signal-to-noise ratio of the particle size measurement a dilution ratio of between 1:2 and 1:3 was chosen. Before measuring, the diluted samples were homogenized by a magnetic stirrer.

For the charge titration the primary products were diluted with distilled water with the same mixing ratio (approx. 1:10) as in the gushing test for producing fruit spritzers.

3 Results and Discussion

3.1 Gushing propensity of the primary products for fruit spritzers

Five primary products (A 1–5) for producing apple spritzers and two primary products (B 1 and 2) for apple-cherry spritzers were examined for their gushing characteristics by application of the gushing test. The result of each “bottled” product is presented in table 1.

The primary products A 1, 2 and 5 showed gushing, so they were identified as gushing-positive samples, whereas the remaining

samples (A 3 and 4, B 1 and 2) were gushing-negative without overfoaming.

3.2 Application of the combined particle analysis for primary products for apple spritzers

3.2.1 Particles in primary products for apple spritzers with sizes of 1–2 nm are suspicious for gushing

Five different primary products for producing apple spritzers were examined for their particle size distribution (Fig. 1).

It is obvious that the particle size distribution of the gushing-positive samples (A 1, 2 and 5) differ from the distribution of the gushing-negative ones (A 3 and 4) by sizes of only 1 to 2 nm: For the gushing-positive samples significantly higher stray light intensities for these particle sizes (1–2 nm) were detected than for the gushing negative ones. If the amounts of larger particles in these examined samples were nearly identical, it would be expected that the detected higher intensities signify higher amounts of these 1–2 nm-particles. In the following the particle charge titration was applied for examining its feasibility to “quantify” the detected 1–2 nm-particles and to predict gushing.

3.2.2 Charge titration as complementary analysis to predict gushing

For the primary products A 1–5 the charge titration was performed in order to examine if the detected 1–2 nm-particles could be “quantified”: For a higher amount of 1–2 nm-particles a higher entire particle surface (charge) was expected that may be equivalent to a higher titrated volume consumed for charge-neutralization. The results of the charge titration for the primary products A 1–5 are illustrated in figure 2 and presented in table 1.

For the gushing-positive samples (A 1, 2 and 5) higher volume of the titration solution (2.5 to 3.9 ml) was necessary for charge-neutralization than for the gushing-negative ones (A3 and 4) which required only 2.0 ml. The higher consumption of the titration solution for the gushing-positive samples was in agreement with the expected higher amount of the detected 1–2 nm-particles, which contribute to a higher entire particle surface (charge). This correlation that particles with decreasing diameters exhibit higher surfaces in relation to their volume is illustrated by Titze et. al. [46].

The results indicate once more that particles with sizes of 1–2 nm are relevant for gushing [30].

In the following the question should be answered why particles with detected sizes of 1–2 nm in the gushing-negative samples did not lead to overfoaming in the corresponding prepared spritzers. Two reasons are proposed:

1. Not all particles of these sizes are able to induce gushing.
2. The concentration of gushing inducing substances was too low to cause gushing in the spritzers. The necessity for a minimum concentration of respective substances to observe gushing was shown in previous studies [22, 30] and supports this assumption.

Also both aspects can be the reasons why the samples A 3 and 4 show no gushing despite of the presence of 1–2 nm-particles.

Below further beverage materials are examined to estimate the combined particle analysis as a predicting method for gushing.

3.3 Application of the combined particle analysis for primary products for apple-cherry spritzers

Two primary products (B 1 and 2) for producing apple-cherry spritzers were examined by the combined particle analysis. The particle size distribution is illustrated in figure 3.

The particle size distributions of both primary products B 1 and 2 were statistically identical. Particles with sizes of 1–2 nm as in the primary products A 1–5 were not detected. While in the samples B 1 and 2 larger particles (around 1000 nm and above 1000 nm) are mostly detected in comparison to A 1–5 the entire surface (charge) of all particles may be lower. Thus, considerably lower titrated volumes are expected for charge-neutralization. The results are shown in figure 4 and the experimental data in table 3.

The results of the charge titration for the primary products B 1 and 2 confirm the expectations. Considerably lower titrated volumes were necessary: for B 1 approx. 0.24 ml and for B 2 approx. 0.17 ml titration solution.

For the apple-cherry spritzers the gushing test indicated no overfoaming (Table 1), which was to be expected as no particles with sizes of 1–2 nm were detected in the respective primary products B 1 and 2.

The results demonstrate that beverage samples with detected particle sizes of 1–2 nm can lead to gushing. It has been proven that the titrated volume is a useful measurement parameter to “quantify” these gushing-relevant 1–2 nm-particles and thus to give further information about the gushing propensity of the examined samples. Therefore, the particle size and particle surface charge as analytical parameters play decisive roles in the prediction of gushing.

4 Conclusion

In the experiments the combined particle analysis was successfully applied with alcohol-free beverage products to predict gushing. The overfoaming amount (according to the gushing test) served as positive signal for the presence of gushing. In the samples A (for apple spritzers) particles with sizes of 1–2 nm were detected. The detected stray light intensities of these particle sizes (1–2 nm) were significantly higher for the gushing-positive samples than for the gushing-negative ones. The results of the charge titration showed further that higher titrated volumes for charge-neutralization were necessary for the gushing-positive samples than for the gushing-negative ones. In this case the particle charge titration method was useful in distinguishing the gushing-positive samples from the gushing-negative ones by a higher titrated volume which were associated with higher amounts of 1–2 nm-particles. Therefore, the titrated volume for charge-neutralization was pro-

posed as measurement parameter to “quantify” gushing-relevant 1–2 nm-substances. It must be assumed that not all particles with sizes of 1–2 nm are gushing inducing substances. Further experiments with other beverage samples are necessary to evaluate the combined particle analysis as rapid methods to predict gushing. In further beverage samples (primary products B for apple-cherry spritzers) larger particles and medium-sized particles, but no 1–2 nm-particles were detected than in the samples A (for apple spritzers). The expected low titrated volumes for charge-neutralization for the samples B were confirmed and no gushing (according to the gushing test) was observed.

The results demonstrate that the particle size and the particle surface charge are promising analytical parameters to predict the gushing propensity of beverage products. For further evaluation of the combined particle analysis the overfoaming amount of conventional gushing tests is the obvious reference value to prove present gushing.

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Appendix

Table 1 Overfoaming amounts ($n = 6$) of the primary products (A 1–5 and B 1–2) according to the gushing test for alcohol-free beverages

Primary product	Bottle					
	1	2	3	4	5	6
A1	40	26	32	19	35	64
A2	72	61	58	64	51	76
A3	0	0	0	0	0	0
A4	0	0	0	0	0	0
A5	50	52	41	65	49	73
B1	0	0	0	0	0	0
B2	0	0	0	0	0	0

Table 2 Experimental data of the charge titration for the samples A 1–5. The potential, the titrated volume, the conductivity and coefficient of determination R^2 of the approximation curve are presented. The conductivities for all samples A 1–5 were nearly identical

Sample	Potential [mV]	Titrated volume [ml]	Conductivity [$\mu\text{S}/\text{cm}$]	Coefficient of determination R^2
A1	-1088	2.502	1660	0.998
A2	-950	3.874		0.998
A3	-845	1.949		0.996
A4	-882	1.992		0.996
A5	-940	3.260		0.994

Table 3 Experimental data of the charge titration for the samples B 1 and 2: The potential, the titrated volume, the conductivity and the coefficient of determination R^2 of the approximation curve are presented. The conductivities of each sample (B 1 and 2) were nearly identical

Sample	Potential [mV]	Titrated volume [ml]	Conductivity [$\mu\text{S}/\text{cm}$]	Coefficient of determination R^2
B1	-761	0.235	515	0.992
B2	-474	0.169	504	0.995

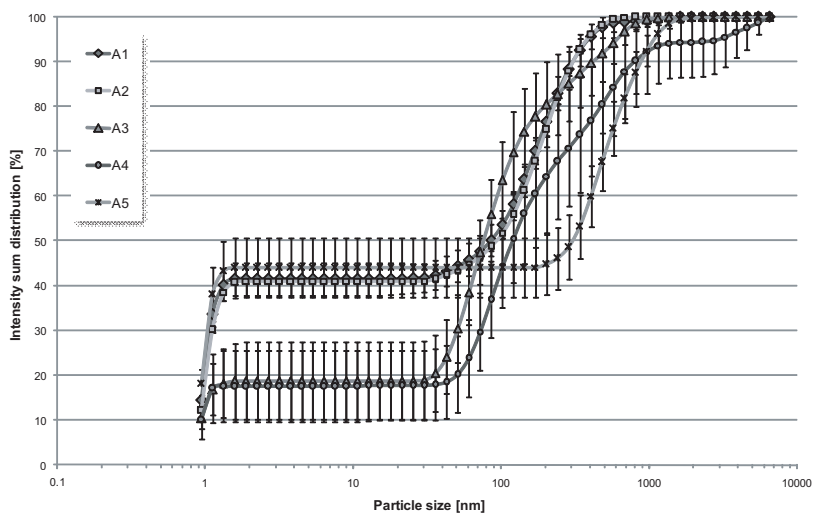


Fig. 1 Intensity sum distribution (arithmetic mean with confidence interval) of the primary products (A 1–5) for producing apple spritzers in relation to the particle size. The primary products A 1, 2 and 5 showed gushing according to the gushing test, whereas A 3 and 4 did not lead to overfoaming

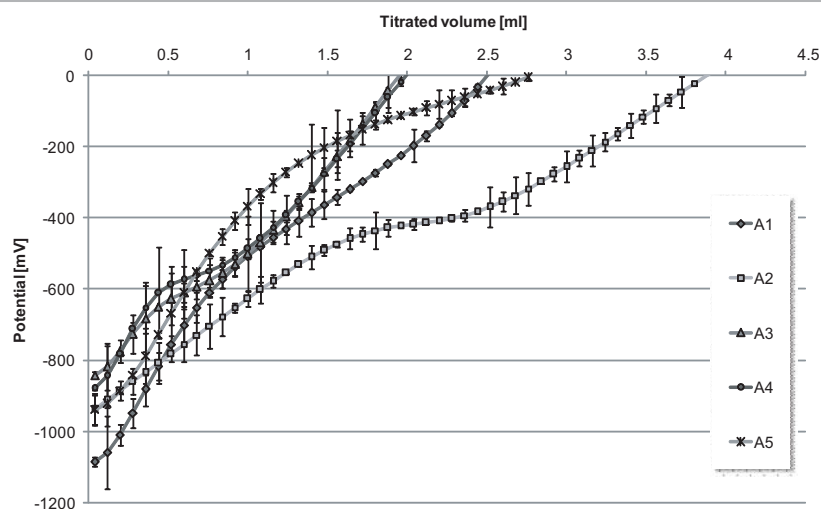


Fig. 2 Titration curves of the primary products (A 1–5) for apple spritzers. For the gushing-positive samples A 1, 2 and 5 significant higher stray light intensities of particle sizes of 1–2 nm were detected than for the gushing-negative samples (A 3 and 4)

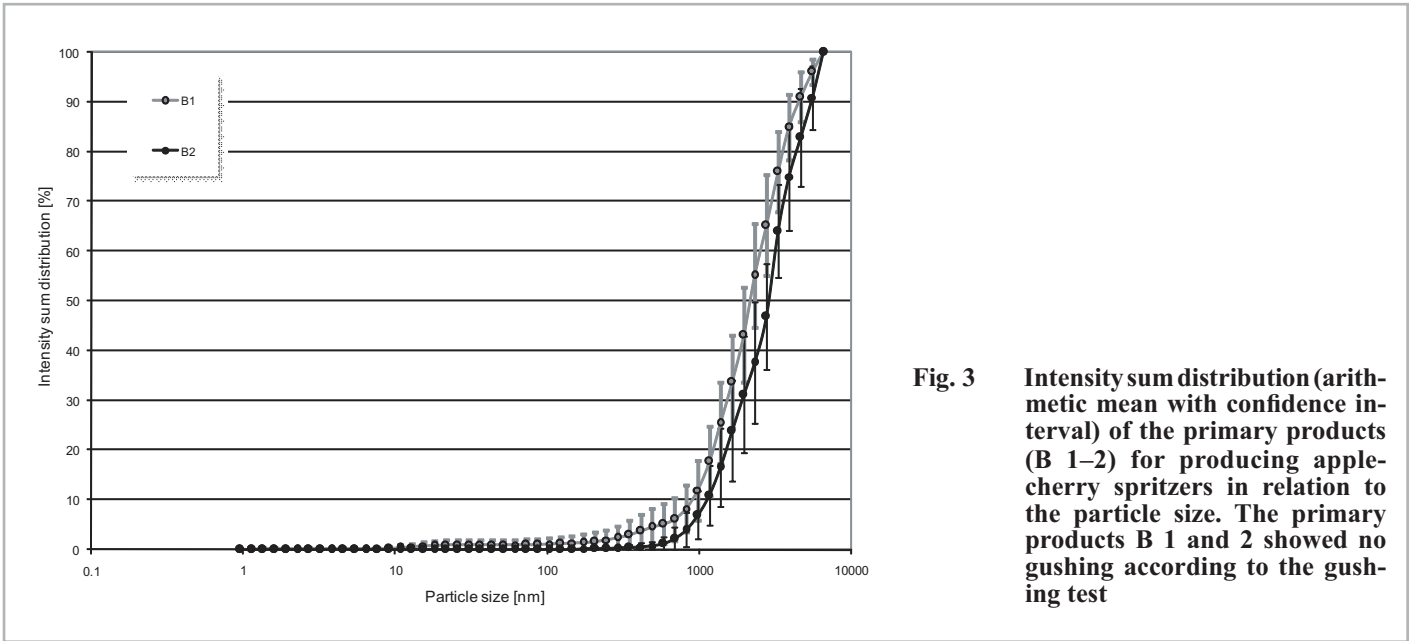


Fig. 3 Intensity sum distribution (arithmetic mean with confidence interval) of the primary products (B 1–2) for producing apple-cherry spritzers in relation to the particle size. The primary products B 1 and 2 showed no gushing according to the gushing test

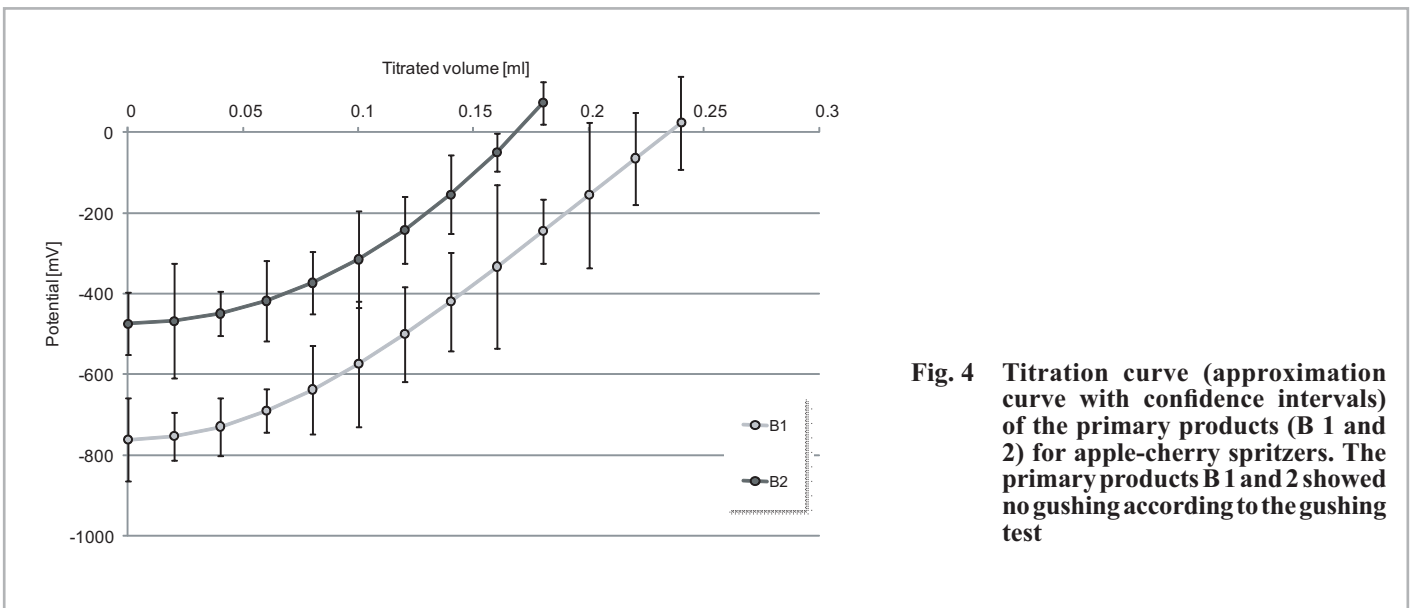


Fig. 4 Titration curve (approximation curve with confidence intervals) of the primary products (B 1 and 2) for apple-cherry spritzers. The primary products B 1 and 2 showed no gushing according to the gushing test