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Fractionated boiling technology: wort boiling of different lauter fractions

In the past few years, many innovations in wort boiling have been introduced in order to minimize thermal stress associated with the staling of beer. Many of these innovations were based on gentle wort boiling. These concepts were very successful for retaining beer flavor stability and saving energy. However, the possibilities are limited, because the quality of wort after lautering (sweet wort) itself cannot be altered. If sweet wort were separated into several groups according to each one's staling sensitivity, it would be possible to achieve much more in the area of wort boiling. In the framework of this research, the first wort and the spargings were boiled separately. The first wort was treated as a staling-sensitive wort, and the spargings as a less staling-sensitive wort. No hops were added when boiling either the first wort or the spargings. The following was measured during boiling: extract, color, pH, polyphenols, nitrogen content and fractions, amino acids, carbohydrates, free dimethylsulfide (DMS) and carbonyl compounds. As a result of these investigations, distinct differences were observed between both of the boiled fractions, e.g. high amounts of substances resulting from lipid degradation in the boiled spargings. Additionally, the creation of different aroma compositions was possible through wort aroma simulations using Fractionated Boiling Technology (FBT). Some of these aroma components in wort are later important for the flavors typically associated with beer staling.

Descriptors: wort boiling, first wort, sparging, flavor stability, staling flavor, aging, brewing

1 Introduction

For over 50 years, research has been done on the origins of the staling flavors in beer, and today it is still one of the biggest challenges facing the brewing industry. Almost 30 years ago, Dalgliesh [1] proposed the model of sensory changes in beer flavor during aging, which includes a decrease in sensory bitterness, an increase in sweet notes, an increase and subsequent decrease in ribes aroma and increase in cardboard flavor. The best-characterized substance is (E)-2-nonenal [2-5] derived from malt lipids, which was thought to be the main source of cardboard flavor. The generally accepted opinion was that this substance develops during the storage of beer. However, Schieberle [13, 20] recently pointed out that this substance is not generated during storage and the role of (E)-2-nonenal as an important staling flavor remains an open question. In any case, researchers have strived to minimize cardboard flavor by focusing their research on the lipid oxygenation substance (E)-2-nonenal itself or by analyzing the potential for (E)-2-nonenal formation. Important information has also been gained from research which focused on the technological influences during malt and beer production, e.g. high temperature kilning during malting, pH control during mashing, high temperature mashing (> 62 °C) to suppress lipoxygenase activity [2, 51], lowering of the pH of spargings [3], influence of pH on beer [6], influence of sulfite on (E)-2-nonenal adducts [4], effect of wort boiling on free (E)-2-nonenal [5] and (E)-2-nonenal potential [7]. Recently, the withering of green malt through reduced air humidity and high air flow rates was shown to have a positive effect on reducing potential (E)-2-nonenal formation [8]. Presently, it still remains unclear whether or not (E)-2-nonenal is important for cardboard flavor.

On the other hand, in recent years sweet aroma components have been frequently discussed. These flavor components are primarily carbonyl compounds, such as Strecker aldehydes, products of lipid oxidation or the Maillard reactions [9]. These key flavors and their flavor intensity were measured by the gas chromatography-olfactometry (GCO) method with aroma extract dilution analysis (AEDA) [10, 11] or by means of multivariate modeling like Projection to Latent Structures algorithms (PLC) [12]. Through these studies 3-methylbutanal (malty, unripe banana-like), 2-methylbutanal (green grass-like), methylpropanal (green grass-like) [13], phenylacetaldehyde (honey-like) [14, 15], (E,E)2,4-nonadienal (fatty, waxy) [14], methional (cooked potato-like) [15], γ-nonalactone and dihydro-5-pentyl-2(3H)-furanone, respectively (coconut-like) [12, 16, 17], 2-furfural (almond-like, fruity), 5-hydroxymethyl furfural: 5 HMF (grainy) [12, 18] and heptanal (wine-like) [12] have been identified as key staling flavors. These carbonyl compounds are originally derived from malt [19] and most are released during the wort boiling process.

From the viewpoint of the concentration and the odor activity of these flavors, wort boiling plays a significant role in the evaporation of these volatile carbonyl compounds and in the regeneration of these substances in Maillard and Strecker reactions [26]. Therefore, the connection between wort boiling and staling flavors in beer has been the focus of many research projects.

According to Back [21], the thiobarbituric acid number (TBN) correlated with 5 HMF [22, 23] has been used as a means for measuring thermal stress with regard to flavor stability; the increase of this amount (Δ-TBN) during wort boiling should be less than 15. Fritsch [24] has studied the sensory difference between unboiled wort and boiled wort without a hop addition. He found that boiled wort exhibited less malty and grainy notes than unboiled wort. One of the reasons was the decrease in concentrations of 3-methylbutanal, hexanal and (Z)-4-heptanal. Liégeois [5]

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Tables and Figures see Appendix

suggested that wort boiling contributed approximately 70% of the free (E)-2-nonenal in aged beer. Drost [2] and Narziß [26] mentioned the volatile carbonyl compounds are removed during the wort boiling process. Morikawa [25] reported that the nonenal potential in cold wort and beer decreases slightly in accordance with the length of wort boiling time and the total evaporation rate, and additionally, a lower pH of the sweet wort results in a lower TBN after boiling. According to Ogane, Strecker aldehydes in aged beer tended to increase with longer the boiling times and greater thermal stress [7].

There are also some opinions to the contrary, but reduction of thermal stress during wort boiling is generally considered to improve beer flavor stability.

The most recent ideas regarding the principle of homogeneous wort heating and boiling have been well researched. As a result, new types of boiling systems have been developed in order to reduce thermal stress [21, 26-39]. Recent innovations incorporate improved wort convection during wort boiling, especially during the heating phase. Improved convection helps eliminate partial overheating (pulsation) in an internal calandria and dead zones in the wort kettle [36-38]. In this way, unnecessary thermal stress is avoided and the wort boiling process is gentle and homogeneous.

These advances in wort boiling technology are limited in their capacity to maintain flavor stability in beer because manipulation of the wort boiling process still cannot change the initial quality of the sweet wort. However, if it were possible to separate the sweet wort into staling-sensitive wort fractions and staling-insensitive wort fractions prior to boiling and subsequently boil each of them using modern wort boiling technology, increased flavor stability would be expected. Narziß and Schneider [40] described their findings on high temperature wort boiling (HTW) 25 years ago. They collected and boiled the first wort and 3 spargings separately, then mixed them before pitching. The beer proved to be better in quality than the control sample due to an overall reduction in the "boiled" flavors, which are caused by the excessive production of certain compounds, e.g. 2-furfural and heterocyclic nitrogen compounds. The authors concluded that low gravity worts are better suited for HTW from the viewpoint of beer quality. This could mean that the high gravity first wort is not stable at high temperatures, such as those present during wort boiling. In contrast it appears that low gravity wort spargings are not as susceptible to these effects during boiling.

In our research, in order to reduce the staling of beer, we treated the first wort (high gravity) as a staling-sensitive wort during boiling and the spargings (low gravity) as less staling-sensitive. Each was boiled separately. No hops were added when boiling either the first wort or the spargings. A 60 L pilot system was used. The following was measured during boiling: extract, color, pH, polyphenols, nitrogen compounds, amino acids, carbohydrates, free dimethylsulfide (DMS) and aroma carbonyl compounds. The concentration of each of these substances was measured in the first wort and the spargings at designated sampling times. These data were used as the basis for calculating different boiling time combinations for the first wort fraction and sparging fractions in order to find the final concentration of wort aroma compounds in the cast-out wort. These measurements have provided new insight into the behavior of individual aroma components during wort boiling. We simulated undesirable aroma compounds, e.g. Strecker degradation substances, with different boiling times of the first wort and spargings, then we were able to determine the optimal length of time for boiling the first wort as well as for boiling the spargings in order to minimize these undesirable flavor components.

2 Material and methods

2.1 Wort sample production (60 L pilot system)

The wort boiling experiments were carried out in an identical fashion, with the exception that the boiling time was varied, (100 min and 120 min), and sampling times were varied with the goal of determining the effects on the the components described above.

Milling of the malt

14.5 kg of pale malt was milled using a two- roller mill (Künzel, Germany) with the roller gap adjusted to 0.8 mm. The malt specifications are as follows:

Water content:	6.2%
Extract content (dry weight):	82.1%
Protein content:	11.2%
Soluble N in malt (dry weight):	751 mg/100 g dry weight
Kolbach index:	41.9%
Friabilimeter value:	84.5%
Totally glassy fraction:	1.0%

Mashing

The 60 L pilot system consists of a mash kettle, a mash tun, a lauter tun, a wort kettle and a whirlpool made of stainless steel (Kamm, Germany) located at the Lehrstuhl für Technologie der Brauerei I Weihenstephan. The temperature of mash vessels and wort kettle is regulated by a PT100 sensor and are heated using saturated steam from a steam generator PS200 (Stritzel Dampftechnik, Germany).

The mash vessels are each equipped with one agitator blade.

The lauter tun is 335 mm in diameter and can accept a mash volume of 68 L. With 14.5 kg of grist, the specific grist load is 162.4 kg/m^2. To avoid a decrease in temperature in the settling mash, the lauter tun is equipped with a heating jacket regulated with hot water at a temperature of 78 °C. The lauter tun has a rake unit consisting of two arms and 3 knives, which corresponds to 33 knives per m^2 of lauter floor surface. The water used for sparging is supplied by the hot liquor tank and enters through a spray head at a temperature of 78 °C.

The wort kettle is 45 cm in diameter and 65 cm in high and is equipped with an agitator blade to guarantee homogeneous wort boiling.

The grist was mashed in with 58 L of mash water (grist : water = 1 : 4) at 52 °C. An infusion mashing process was used and corresponding temperatures and times are as follows:

52 °C:	30 min
62 °C:	30 min
72 °C:	30 min
78 °C:	mash out

The temperature was increased at a rate of 1 °C/min between stands.

A total of 40 L of first wort was collected and immediately boiled with no hop additions at atmospheric pressure. After the first wort was collected, sparge water was added twice to the lauter tun, 30 L and 20 L, respectively. A total of 50 L was collected using 16 L vessels filled with carbon dioxide to avoid oxidation. After the first wort boiling and the subsequent rinsing of the wort kettle, the total spargings were added to the wort kettle and boiled in exactly the same way as the first wort.

For the first boiling experiment (Brew 1), the wort boiling time of the first wort and spargings were 120 min each and for the second experiment (Brew 2), they were boiled 100 min each. The steam supply for the wort kettle remained constant during boiling. We took samples at defined times during wort boiling. In order to slow all chemical reactions, the samples were cooled down quickly in cold water and placed in a climate-controlled room at 0 °C.

2.2 Wort analysis

Prior to wort analysis, samples were centrifuged at 9000 rpm for 20 min at 4 °C.

Most of these analyses were performed according to procedures outlined in MEBAK [55]. Methods were also used which were developed by the institute at the Lehrstuhl für Technologie der Brauerei I Weihenstephan. Each analysis method is as follows:

Extract	MEBAK 2.10.6.1
pH	MEBAK 2.14
Color	MEBAK 2.13.2
Polyphenols	MEBAK 2.17.1
Anthocyanogens	MEBAK 2.17.2
Tannoids	MEBAK 2.17.3
TBN	MEBAK 2.4
Total-Nitrogen	MEBAK 2.8.1.1
FAN	MEBAK 2.8.4.1.1
Computer simulation	STATISTICA™ 06J, StatSoft, Japan

Amino acids

The amino acids were analyzed according to the institute method used by the HPLC-laboratory at the Lehrstuhl für Technologie der Brauerei I Weihenstephan based on high performance liquid chromatography with a diode array detector (HPLC-DAD). The following amino acids were analyzed: aspartic acid (Asp), glutamic acid (Glu), asparagine (Asn), serine (Ser), glutamine (Gln), histidine (His), glycine (Gly), threonine (Thr), arginine (Arg), alanine (Ala), γ-aminobutyric acid (GABA), tyrosine (Tyr), valine (Val), methionine (Met), tryptophan (Trp), phenylalanine (Phe), isoleucine (Ile), leucine (Leu), lysine (Lys)

Total Dimethylsulfide (DMS), Free DMS, S-methyl methionine (DMS-Precursor)

The above substances were analyzed according to the institute method developed by the GC-laboratory at the Lehrstuhl für Technologie der Brauerei I Weihenstephan based on gas chromatography with a flame photometric detector (GC-FPD).

Wort aroma components

The volatile aroma compounds in wort were concentrated through steam distillation and extracted with dichloromethane. Afterwards, the solvent phase was analyzed using the institute method developed by the GC-laboratory at the Lehrstuhl für Technologie der Brauerei I Weihenstephan based on gas chromatography with a flame ionization detector (GC-FID).

The following substances were analyzed: 3-methylbutanal, 2-methylbutanal, methional, benzaldehyde, 2-phenylacetaldehyde, pentanal, 2-pentanone, hexanal, heptanal, (E)-2-(Z)-6-nonadienal, 2-furfural, 2-acetylfuran, γ-nonalactone, 3-methylbutanol, 2-methylbutanol, 1-pentanol, 1-hexanol, 1-octanol, 1-octen-3-ol, phenylethanol.

To obtain the total amount of Strecker aldehydes, we added the amounts of the following five substances together, namely 3-methylbutanal, 2-methylbutanal, methional, benzaldehyde and 2-phenylacetaldehyde.

Fermentable sugars (low molecular weight sugars)

Fermentable sugars were analyzed using the institute method developed by HPLC-laboratorty at the Lehrstuhl für Technologie der Brauerei I based on an ion chromatography system. The following substances were analyzed: fructose, glucose, sucrose, maltose and maltotriose.

3 Results and discussion

3.1 Characterization of both lauter fractions (the first wort and spargings) during wort boiling

All data except pH were adjusted to correspond to a 12% original gravity to determine the wort aroma components present in the boiled first wort fraction and boiled spargings fraction.

3.1.1 Extract, pH, color

Extract

The gravities of the first wort and spargings measured before wort boiling were approximately 16% and 7%, respectively. The concentration of the extract increased in a constant and linear manner, confirming a constant evaporation rate during wort boiling

pH

Figure 1 shows that the pH of the spargings is 0.1–0.2 higher than that of the first wort due to the pH of the sparge water. The reductions in pH could be attributed to the presence of Maillard products, acidicity due to the presence of calcium ions or magnesium ions, or by the precipitation of tertiary phosphate [26].

Color adjusted to correspond to an original gravity of 12%

Figure 2 indicates that the color of the spargings was higher than that of the first wort. The reason might be the high amount of polyphenols in the spargings (Fig. 3).

3.1.2 Polyphenols, anthocyanogens and tannoids, adjusted to correspond to an original gravity of 12%

The amount of total polyphenols, anthocyanogens and tannoids was higher in the spargings than the first wort. It has already been confirmed [26, 41] that spargings contain higher amount of polyphenols. This fact might be one of the reasons why the spargings possess a darker color as mentioned above.

The concentration of anthocyanogens and tannoids decreased during wort boiling; however total polyphenols remained constant. This could be contributed to the polymerization of low molecular weight polyphenols such as anthocyanogens and tannoids during wort boiling.

It has been suggested that polyphenols from malt also contribute to the precipitation of protein [26], but we found no significant difference between the amount of polyphenols present before and after wort boiling (Fig. 3).

3.1.3 Total nitrogen, FAN and amino acids

Total nitrogen, adjusted to correspond to an original gravity of 12%

The total amount of nitrogen decreased slightly during wort boiling, and no significant difference was found between the levels in the first wort and in the spargings.

FAN, adjusted to correspond to an original gravity of 12%

During wort boiling, the amount of FAN present in the first wort decreased by approximately 10 mg/l, but the amount of FAN in the spargings was constant.

Amino acids, adjusted to correspond to an original gravity of 12%

The amount of total amino acids in the first wort increased during the first 10 minutes of wort boiling and then decreased gradually. In the spargings, it decreased during the first 20 minutes and then remained constant (Fig. 4)

Upon examination of specific amino acid concentrations during wort boiling, the following observations could be made: The individual amounts of Lys, Leu, Phe, Met, Ser, Gly, and Ile in the lauter fractions displayed the same behavior in both wort fractions during boiling as the total amount of amino acids. This means that the concentration decreased during the first 20 minutes and then remained constant. The amounts of Asp, Trp, GABA, Ala, Asn, Glu, His, Thr, Arg, Tyr, Val in the first wort also displayed the same pattern as the total amount of amino acids; however, these amounts did not changed during the boiling of the spargings. The decrease in Gln during wort boiling [21], during beer aging [42] and during flash pasteurization [52] is well-documented. Figure 4 shows the decrease of Gln during wort boiling, and there was a noticeable difference between the first wort and spargings.

It has long been established that Strecker aldehydes are created during wort boiling and that the reaction partners are amino acids and carbohydrates [43]. Schieberle [13] found that both oxygen and light have a direct influence on the formation of Strecker aldehydes in beer, and he proposed that the degradation of carbohydrates is not a crucial factor in initiating the formation of Strecker aldehydes during beer aging. In recent years, lipid oxidation products have also been found to be involved in the Strecker degradation of amino acids [44, 45].

With the exception of Gln, we found during the boiling of the spargings that the amount of amino acids decreased at the beginning of boiling but after 20 minutes remained constant. Although there were enough carbohydrates to serve as reaction partners during the boiling of the spargings fraction, no amino acid degradation occurred after 20 minutes of boiling. Generally, it should be noted that the amounts of amino acids and carbohydrates are over 1000 times higher than the concentration of carbonyl compounds. It is not possible to calculate the concentration of Maillard products by the decrease in educts.

3.1.4 TBN, adjusted to correspond to an original gravity of 12%

Increases in the amounts of Δ-TBN of the first wort and spargings were almost linear and displayed comparable results (Fig.5). This result underscores the fact that there is a high correlation between the rate of Gln reduction and increased levels of TBN during wort boiling [21] (Fig. 5).

3.1.5 Fermentable sugars (low molecular weight sugars), adjusted to correspond to an original gravity of 12%

The first wort and spargings possess an almost identical sugar composition. During wort boiling, the total amount of low molecular weight fermentable sugars was almost constant. Compared with Maillard aroma compounds, the amount of sugar was too high, therefore it was very difficult to ascertain whether or not the sugar reacted with other substances.

3.1.6 Free DMS

Free DMS was reduced to levels less than 50 µg/l within the first 10 minutes of wort boiling (Fig. 6). The decrease in the concentrations of free DMS and DMS precursor was almost identical during the boiling of both lauter fractions. With modern boiling technology [28–38], we can reduce and eliminate such compounds efficiently and also prevent their production through pre-cooling technology after wort boiling [39], so that DMS is no longer a problem.

3.1.7 Wort aroma components, adjusted to correspond to an original gravity of 12%

At the beginning of boiling, the spargings contained distinctly more wort aroma components than the first wort, in particular, more lipid oxidation products (Table I).

During boiling, 3-methylbutanal (Fig. 7) and 2-methylbutanal (Fig. 8) evaporated from the wort in the first 10 minutes. After about 50 minutes of boiling the first wort, the amount of these substances slightly increased, but during the boiling of the spargings the levels were constant. Methional and 2-phenylacetaldehyde (Fig. 9) gradually evaporated during wort boiling and showed an increase after about 50 minutes of boiling the first wort. The total amount of Strecker aldehydes also exhibited the same tendency.

We have also documented the same patterns in the normal boiling process on an industrial scale. Changes in certain wort aroma components were examined during wort boiling using eight different types of brewing systems (three with an internal calandria, five with an external calandria, with the following characteristics: cast-out wort volumes of 100–700 hl, steam temperatures of 101–107 °C, boiling times of 50–73 minutes, total evaporation of 2.6–8.5%). The amounts of 2-methylbutanal (Fig.10) decreased in the first 15–20 minutes and then remained constant at a level of 10–50 µg/l. Concentrations of 2-phenylacetaldehyde (Fig. 11) decreased gradually in the first 45 minutes and later increased again in some cases. The differences in phenylacetaldehyde concentration between our experiments and those performed on an industrial scale might be attributable to different malt combinations. In our tests, we used only pale malt, however, in addition to pale malt, the industrial scale breweries used darker malts such as caramel malt and dark malt, which contain more wort aroma components than pale malt.

The lipid oxidation products hexanal, heptanal, pentanal, 1-pentanol and 1-hexanol experienced a relatively rapid elimination at the start of the boiling process. The compounds 2-pentanone and γ-nonalactone (Fig. 12) gradually decreased at a very slow rate, and we found no significant differences in the levels of these two substances when comparing the boiling of first wort to the boiling of the spargings. Because of its low threshold (11.2 µg/l [17]) in beer, γ-nonalactone is considered to be a very important flavor in beer staling. The spargings contain more γ-nonalactone (Table 1) than the first wort. According to Hertel [46], aroma compounds from fatty acids are not created during boiling, so that if we want to minimize the concentration of this substance, it would be preferable to boil the spargings much longer. In this case, the concentration of long-chain fatty acids (C14 – C18:3) during lautering should be considered. The total concentration of fatty acids was adjusted to correspond to an original gravity of 12%. This concentration is at a moderate level of 8–10 mg/l during first wort collection (Fig. 13) and then increases rapidly as the extract level decreases at the point that spargings are added and raking begins. For different lautering regimes such as clear and turbid lautering, the relative concentrations of fatty acids experience large variations towards the end of lautering and peaks of up to 42 mg/l were observed during second spargings for turbid lautering (Fig. 13) [54].

In order to evaluate the influence of volatile compounds from the wort on the staling of beer, the individual thresholds should be taken into account. The threshold of each substance in beer is given in Table 2 [15, 17, 47-50]. Almost every threshold is high, but the substances, which have relatively low thresholds compared to the amount of wort, are 3-methylbutanal, methional, 2-phenylacetaldehyde, heptanal, (E)-2-(Z)-6-nonadienal and γ-nonalactone.

3.2 Simulation with a mixture of first wort and spargings and different boiling times

From the viewpoint of the amount of Strecker aldehydes, there was significant difference in the patterns between the first wort and spargings during wort boiling. This means that there is a possibility to produce much lower net amount of Strecker aldehydes by mixing of first wort and spargings which were boiled for different periods of time.

We simulated a mixture of first wort and spargings, boiled for different periods of time and represented the results with the help of three-dimensional graphs.

Figures 14 and 15 show the simulation of 3-methylbutanal. The mixture of first wort boiled for 45 minutes and the spargings boiled for 50 minutes yielded the lowest amount.

Figures 16 and 17 show the simulation of 2-phenylacetaldehyde and the minimum amount was reached using a combination of first wort boiled for 50 minutes and the spargings boiled for 80–100 minutes.

The simulation of total amount of Strecker aldehyde can be seen in Fig. 18–19. The minimum amount produced was with a mixture of first wort boiled for 50 minutes and the spargings boiled for 60 minutes. For the production of certain Strecker aldehydes, boiling the first wort for no more than 50 minutes is optimal. If increased evaporation of other volatile compounds is desired, e.g. γ-nonalactone, it is recommended that the spargings should be boiled as long as possible.

Of course, some of these carbonyl substances such as γ-nonalactone [13, 17, 53], phenylacetaldehyde [13] are produced later during fermentation or during beer aging. For many years brewers have attempted to find the precursors of these substances; however, their origin remains unclear at this time. For these reasons, the question remains unanswered as to whether or not wort boiling plays a significant role in increasing the concentration of these substances, and what their role is in beer flavor stability. In this research, we established that the levels of certain carbonyl compounds decrease and subsequently increase when the first wort is boiled, and that the level of amino acids remain constant when the spargings are boiled. These results show us that the Fractionated Boiling Technology (FBT) gives us a new tool with which wort composition can be influenced during boiling.

3.3 Conclusions

We analyzed the effects of boiling two different lauter fractions, namely the first wort fraction and the spargings fraction. We found a continuous decrease in the amount of amino acids during first wort boiling and a decrease and subsequent increase in the levels of certain carbonyl compounds. Contrary to the results from the first wort boiling, the spargings exhibited high levels of polyphenols and wort aroma components, mainly Strecker aldehydes and lipid oxidation products. The amino acid concentration in the spargings remained constant during boiling.

From the simulations done for of each of the carbonyl compounds, our findings suggest that boiling the first wort and spargings separately (FBT) could minimize volatile aroma compounds in wort.

In order to minimize Strecker aldehydes, the first wort should be boiled approximately 50 minutes, and to minimize lipid oxidation products, the spargings should be boiled as long as possible.

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Received 06. 07. 2006, accepted 24. 07. 2006

Appendix

Table 1 **Changes in wort aroma components during wort boiling – Brew 1; FW = first wort, SP = spargings, results from wort samples were adjusted to correspond to an original gravity of 12%**

Wort aroma components	Boiling time (min)	0	10	20	30	50	80	120
3-methylbutanal (µg/l)	Brew 1 FW	279.9	39.8	13.8	12.4	13.6	40.9	34.6
	Brew 1 SP	385.9	20.7	12.2	18.3	23.2	29.3	35.1
2-methylbutanal (µg/l)	Brew 1 FW	95.6	13.3	5	4.5	4.7	12.1	11.1
	Brew 1 SP	144.9	8.8	5.6	7	7.8	9.2	10.9
Methional (µg/l)	Brew 1 FW	62.2	58.2	48	35	29.9	33.4	35.6
	Brew 1 SP	65.1	62.2	60.9	44.2	49.3	45.3	43.5
Benzaldehyde (µg/l)	Brew 1 FW	6.6	4.9	2.5	1.4	1	1.2	0.9
	Brew 1 SP	12.9	6.2	4.1	4	2.6	2.3	2.1
2-phenylacetaldehyde (µg/l)	Brew 1 FW	110	95.8	72	54.9	40.2	57.6	55.8
	Brew 1 SP	125.4	106.8	96.9	94.4	79.7	74.6	73.8
Σ-Strecker aldehydes (µg/l)	Brew 1 FW	554.3	212.1	141.4	108.3	89.4	145	138
	Brew 1 SP	734.2	204.6	179.7	167.8	162.6	160.6	165.4
Pentanal (µg/l)	Brew 1 FW	28.1	5.6	1.9	2.4	1.2	1.7	1.4
	Brew 1 SP	45.6	4.8	3.3	3.4	2.8	3.2	2.7
2-pentanone (µg/l)	Brew 1 FW	8.3	7.1	6.3	6.3	5.6	5.5	4.4
	Brew 1 SP	16.3	15.1	15	13.7	12.6	12.7	11.1
Hexanal (µg/l)	Brew 1 FW	163.8	25.8	4.1	8.9	1.4	1.8	1
	Brew 1 SP	273.5	10.5	3.9	4.1	3.8	3.6	3.1
Heptanal (µg/l)	Brew 1 FW	7.4	2.4	1.4	1.6	1.4	1.7	1.4
	Brew 1 SP	16.3	3.2	2.8	3	2.8	3.2	3.4
(E)-2-(Z) 6-nonadienal (µg/l)	Brew 1 FW	1.1	0.8	0.8	0.8	0.6	0.4	0.4
	Brew 1 SP	2	1.9	1.3	1.1	1	1.1	0.8
2-furfural (µg/l)	Brew 1 FW	130.5	132.7	124.4	110.5	106.4	116.8	111.6
	Brew 1 SP	154.7	143.4	143.8	132.5	139.1	131.9	125.8
2-acetylfuran (µg/l)	Brew 1 FW	15.9	14	11.6	11.7	9.7	8.6	7.2
	Brew 1 SP	27.7	27.1	26.6	25.9	21.7	20	18.1
γ-nonalactone (µg/l)	Brew 1 FW	8.3	8.1	7.3	5.9	4.7	3	1.4
	Brew 1 SP	16.3	14.8	15.6	13.6	10.7	7.9	6
3-methylbutanol (µg/l)	Brew 1 FW	114.5	61.9	11.6	5.4	1.4	1.4	1.2
	Brew 1 SP	159.6	49.4	20.3	13.4	6.8	4.3	3.6
2-methylbutanol (µg/l)	Brew 1 FW	27.3	3.1	2.2	1.4	0	0	0
	Brew 1 SP	40.7	12.1	3.9	3.2	4.6	3.5	1.7
1-pentanol (µg/l)	Brew 1 FW	62.2	36.9	8	3.9	0.7	0.6	0.4
	Brew 1 SP	89.6	30.3	12.3	8.1	4.5	2.3	1.1
1-hexanol (µg/l)	Brew 1 FW	72.8	31.7	4.1	1	0.3	0.2	0.1
	Brew 1 SP	131.9	25.5	7.3	4.6	2.3	1.1	0.5
1-octanol (µg/l)	Brew 1 FW	1.9	1.5	0.7	0.3	0.3	0.3	0.2
	Brew 1 SP	3.7	1.6	2.7	1.2	2.3	1.1	0.5
1-octen-3-ol (µg/l)	Brew 1 FW	2.5	1.3	0.7	0.5	0.4	0.3	0.3
	Brew 1 SP	4.7	1.8	1.1	0.8	0.7	0.7	0.6
Phenylethanol (µg/l)	Brew 1 FW	110	112	105.5	92	85	67.9	52.4
	Brew 1 SP	153.1	148.2	154.7	152.3	129	106.5	98

Table 2 **Thresholds of various wort aroma components [15, 17, 47-50]**

	Threshold in beer (µg/l: ppb)	Boiling point (°C)	Aroma note	Origin
3-Methylbutanal	600	91-93	unripe banana-like, malty	Leucine
2-Methylbutanal	1250	70	Green grass-like	Isoleucine
Methional	<3*, 50-250	160-165	Cooked potato-like	Methionine
Benzaldehyde	2000	179	almond-like, fruity	Phenylglycine
2-Phenylacetaldehyde	<4*, 1600	195	honey-like	Phenylalanine
Pentanal	500	102	grass, banana-like	Linoleic acid C18:2
2-Pentanone	30000	101-103	geranium-like	Linolenic acid C18:3
Hexanal	350	129-131	bitter, vinous	Linolenic acid C18:2
Heptanal	75	153	bitter, vinous	Oleic acid C18:1
(E)-2-(Z) 6-Nonadienal	0.05	94-95	green leaf, cucumber-like	Linolenic acid C18:3
2-Furfural	150000	162	almond, fruity	Sugars, Amino acids
2-Acetylfuran	80000	67	burned	1-Deoxyoson pathway
γ-Nonalactone	11.2, 80	121-122	fruity, coconut-like	fatty acids
3-Methylbutanol	70000	131-132	banana-like	Leucine
2-Methylbutanol	65000	160-165	banana-like	Isoleucine
1-Pentanol	80000	138	medically	Linoleic acid C18:2
1-Hexanol	4000	157	Green	Linoleic acid C18:2
1-Octanol	900	188-198	coconut-like	Oleic acid C18:1
1-Octen-3-ol	200	173-175	green leaf-like	Linoleic acid C18:2
Phenylethanol	125000	218-220	rose, honey-like	Phenylalanine

*The threshold was estimated by triangular test with spiked samples. Assessors recognized differences between spiked samples and the control [15]

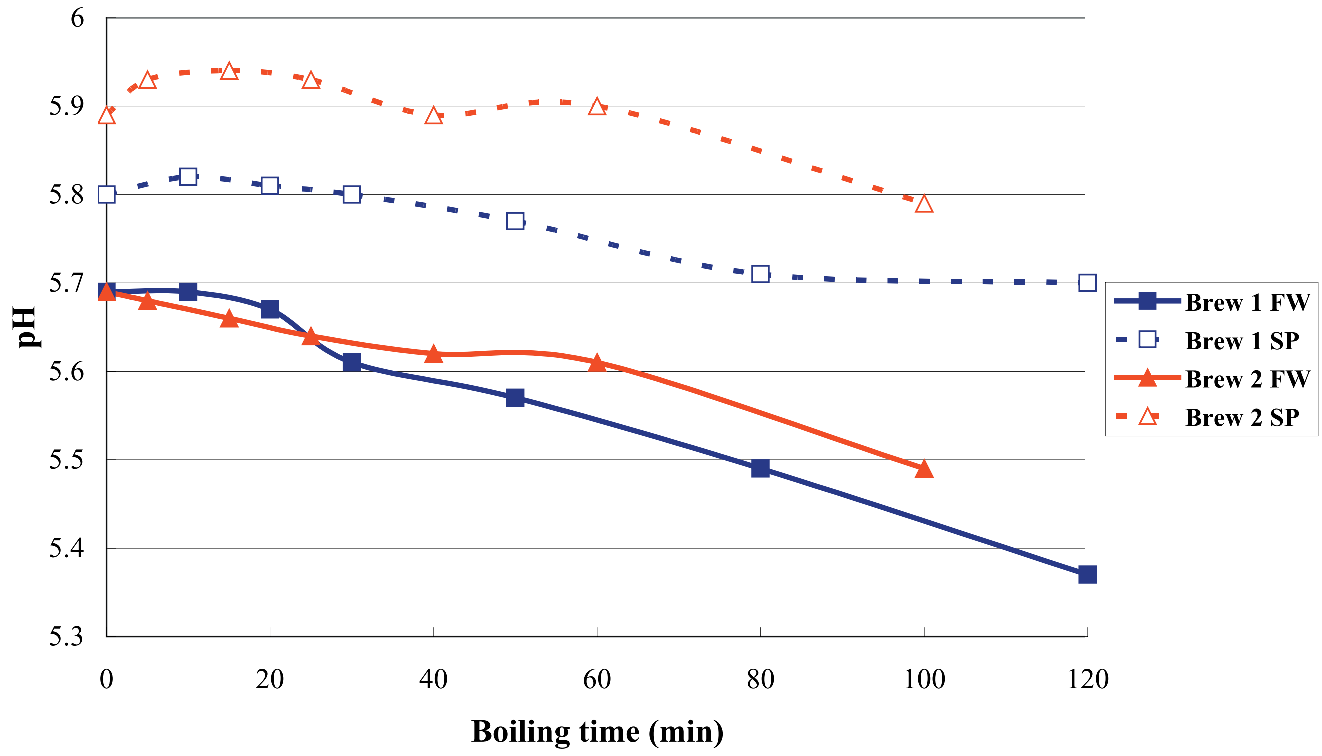

Fig. 1 **Changes in pH during wort boiling; FW = first wort, SP = spargings**

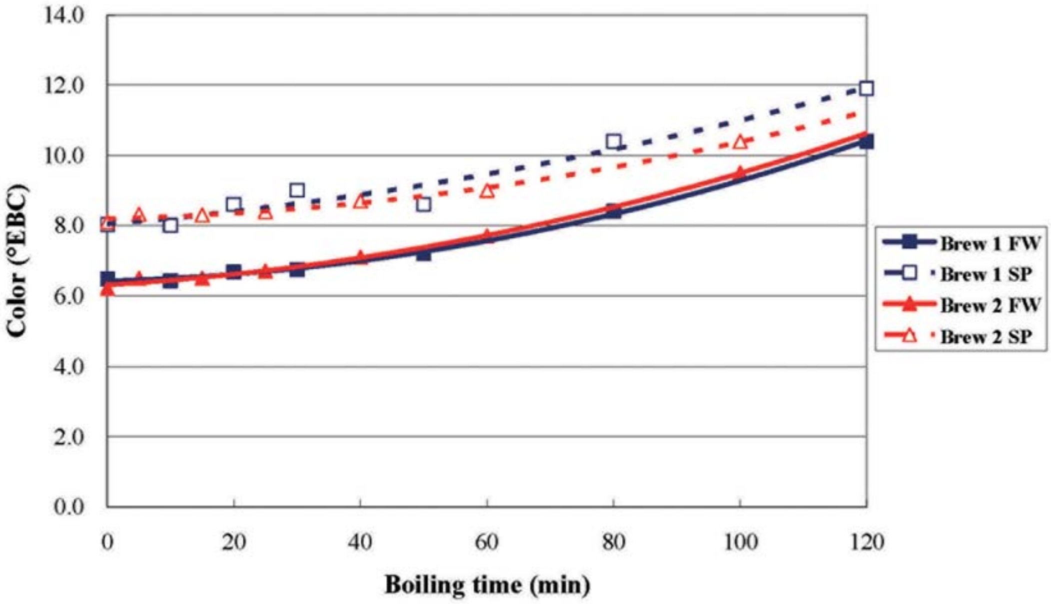

Fig. 2 Color development during wort boiling; FW = first wort, SP = spargings, results from wort samples were adjusted to correspond to an original gravity of 12%

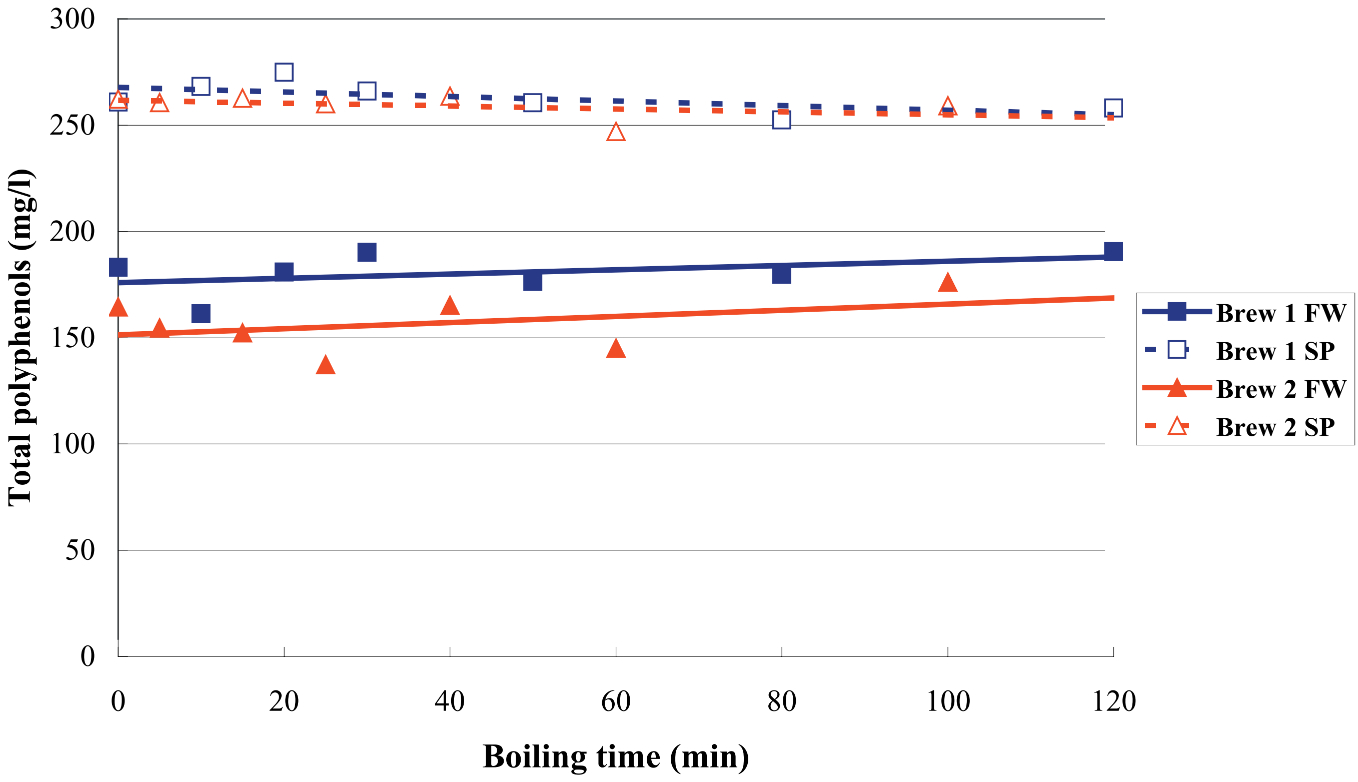

Fig. 3 Changes in the concentration of total polyphenols during wort boiling; FW = first wort, SP = spargings, results from wort samples were adjusted to correspond to an original gravity of 12%

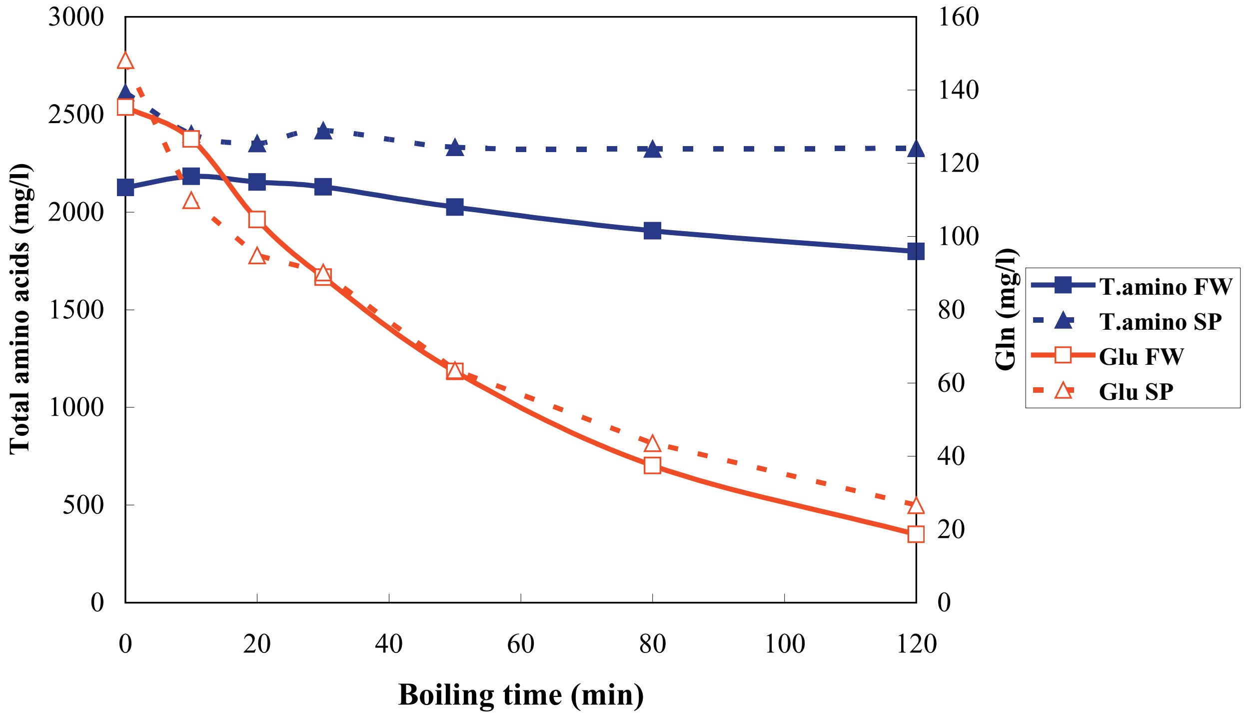

Fig. 4 Changes in the concentration of total amino acids (T.amino) and Glutamine (Gln) during wort boiling – Brew 1; FW = first wort, SP = spargings, results from wort samples were adjusted to correspond to an original gravity of 12%

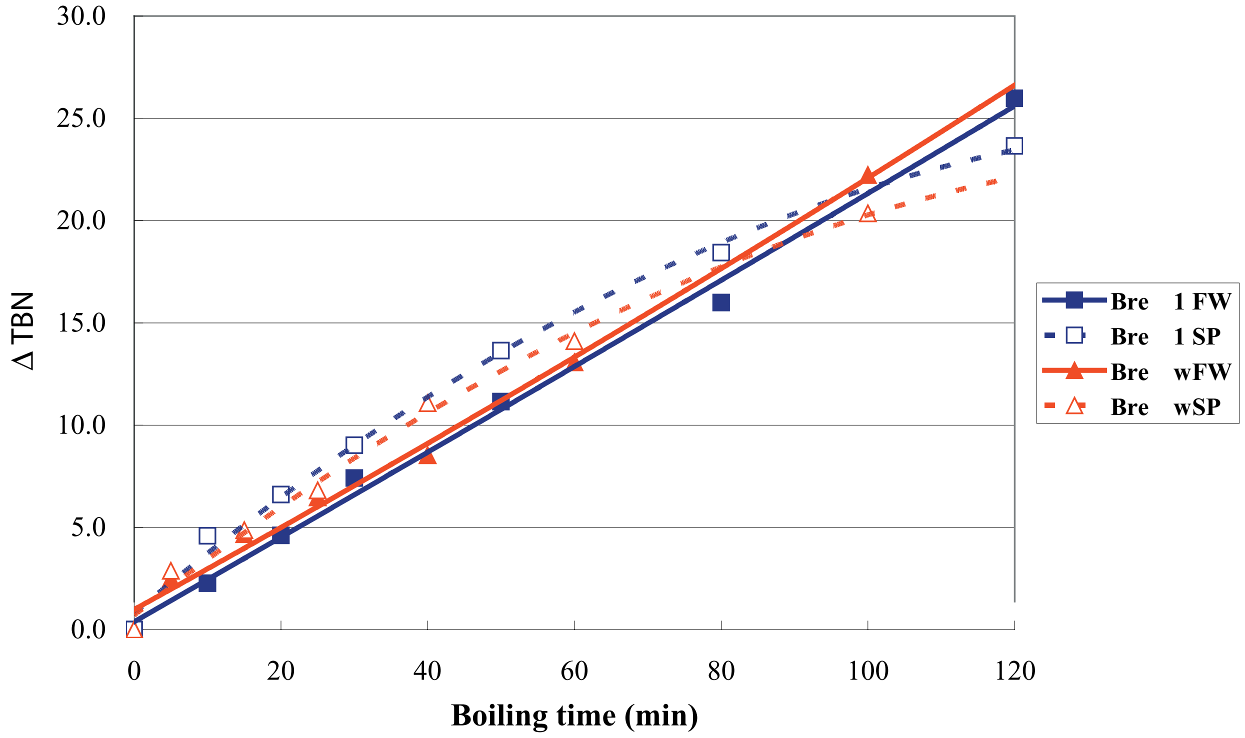

Fig. 5 TBN development during wort boiling; FW = first wort, SP = spargings, results from wort samples were adjusted to correspond to an original gravity of 12%

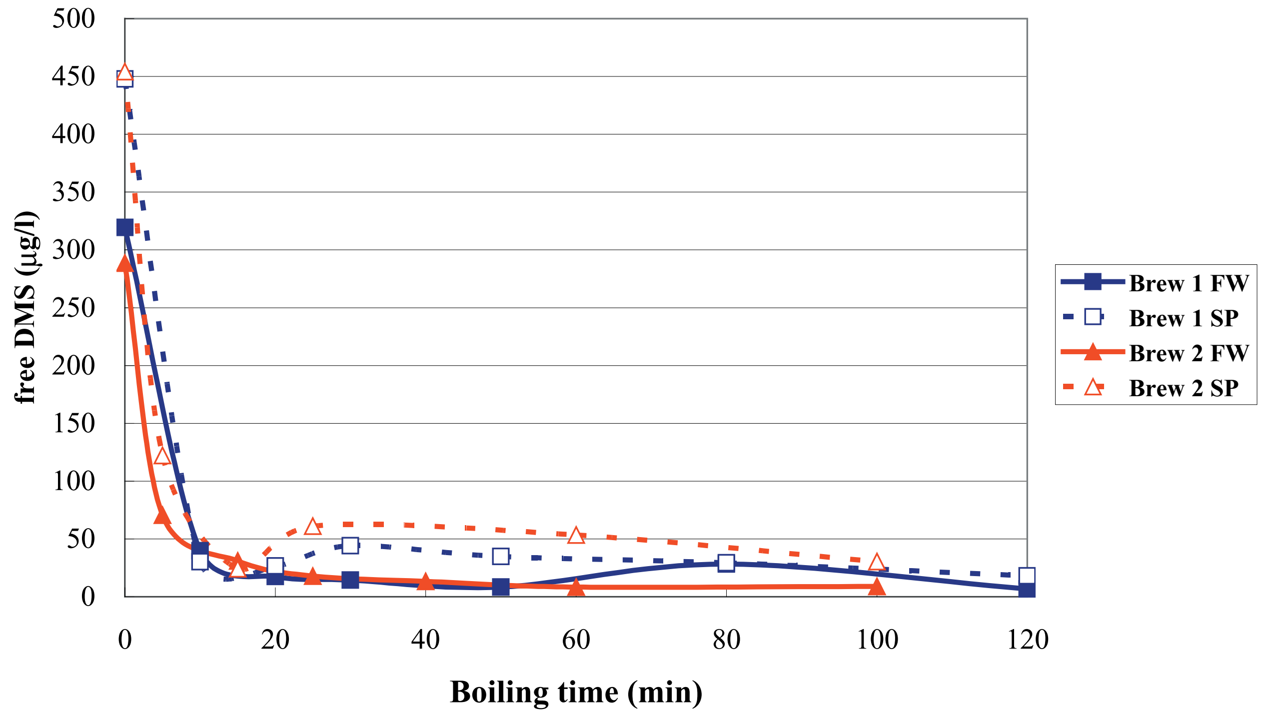

Fig. 6 Changes in the concentration of free DMS during wort boiling - Brew 1; FW = first wort, SP = spargings, results from wort samples were adjusted to correspond to an original gravity of 12%

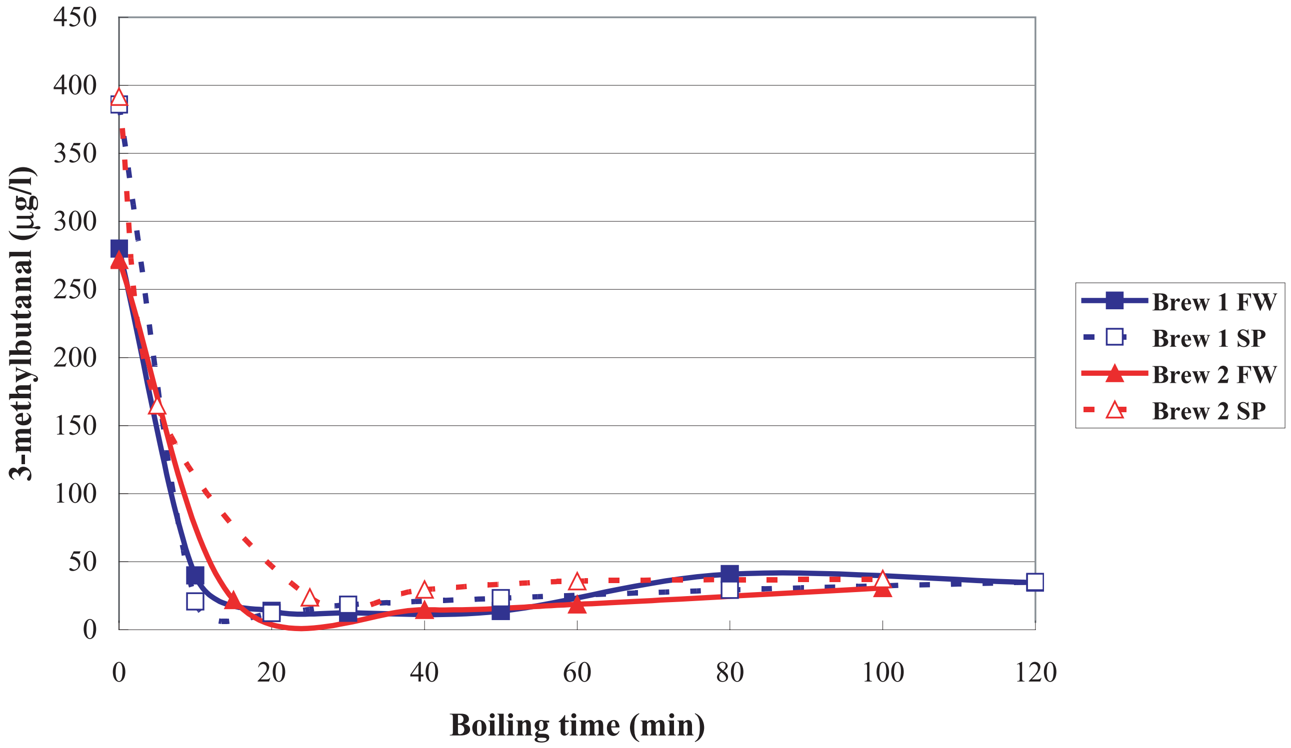

Fig. 7 Changes in the concentration of 3-methylbutanal during wort boiling; FW = first wort, SP = spargings, results from wort samples were adjusted to correspond to an original gravity of 12%

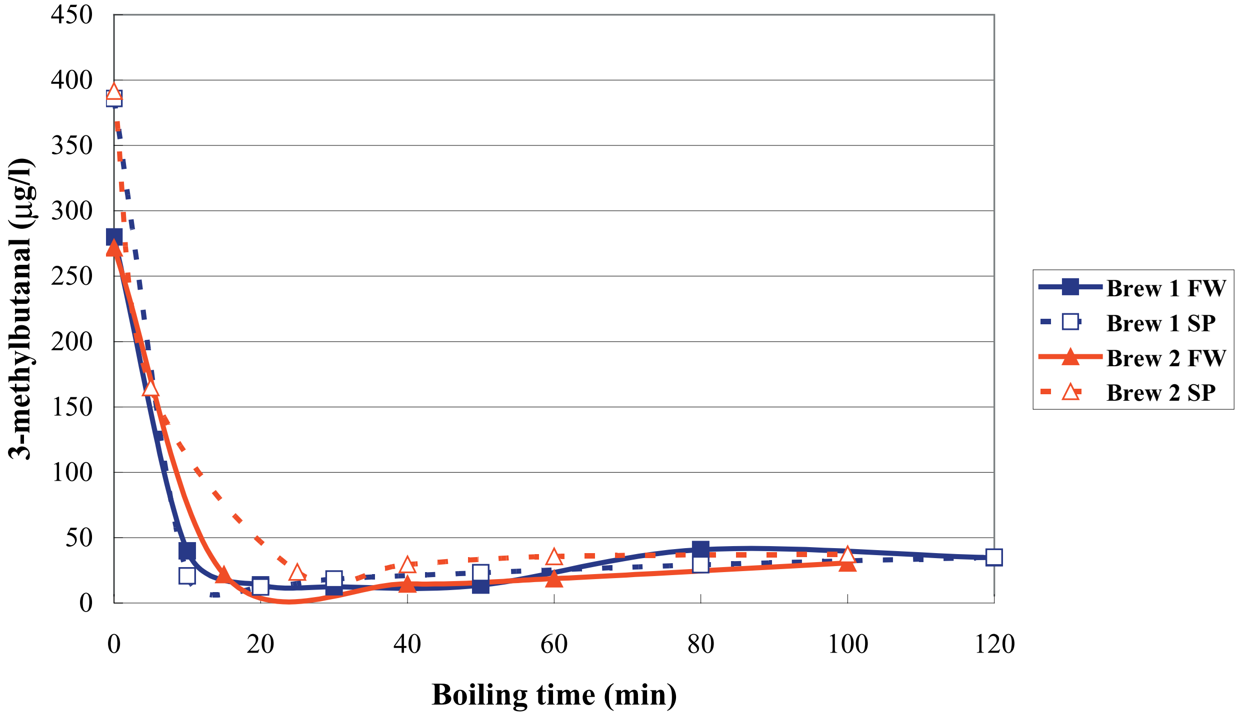

Fig. 8 Changes in the concentration of 2-methylbutanal during wort boiling; FW = first wort, SP = spargings, results from wort samples were adjusted to correspond to an original gravity of 12%

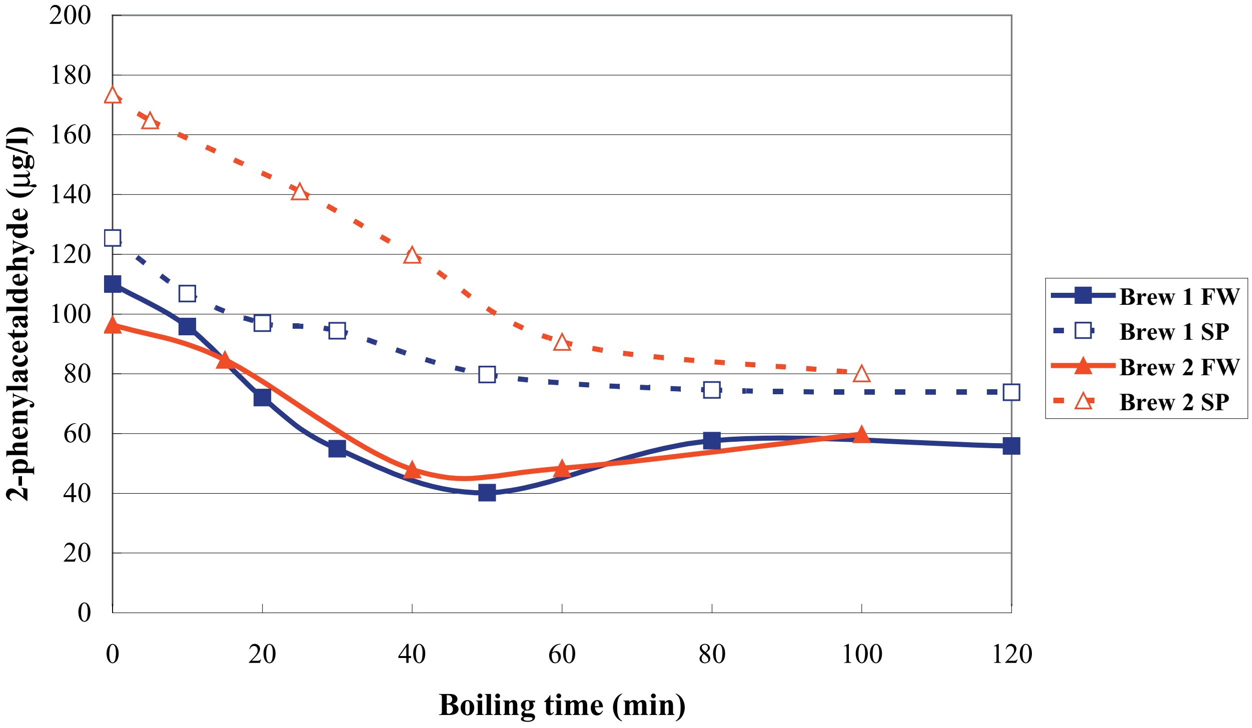

Fig. 9 Changes in the concentration of 2-phenylacetaldehyde during wort boiling; FW = first wort, SP = spargings, results from wort samples were adjusted to correspond to an original gravity of 12%

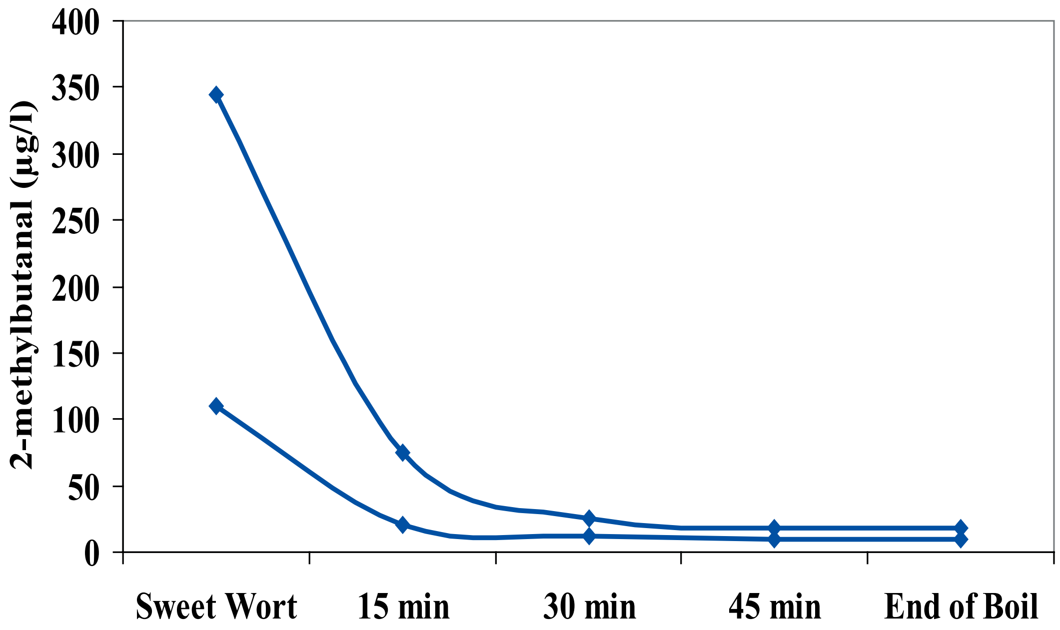

Fig. 10 Changes in the concentration of 2-methylbutanal during wort boiling done on an industrial scale, results from wort samples were adjusted to correspond to an original gravity of 12%

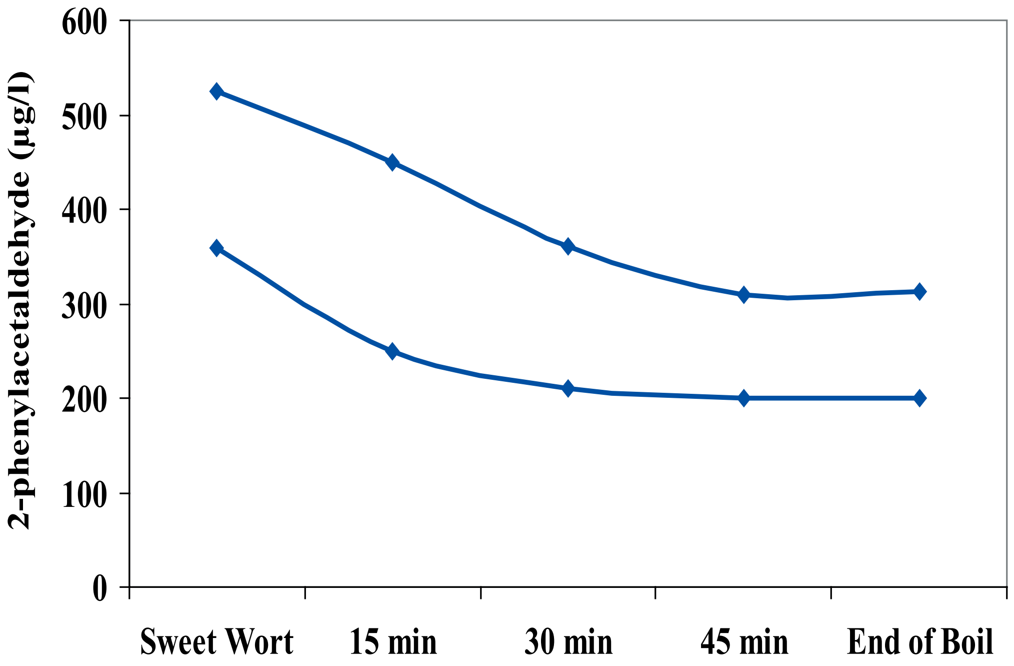

Fig. 11 Changes in the concentration of 2-phenylacetaldehyde during wort boiling done on an industrial scale, results from wort samples were adjusted to correspond to an original gravity of 12%

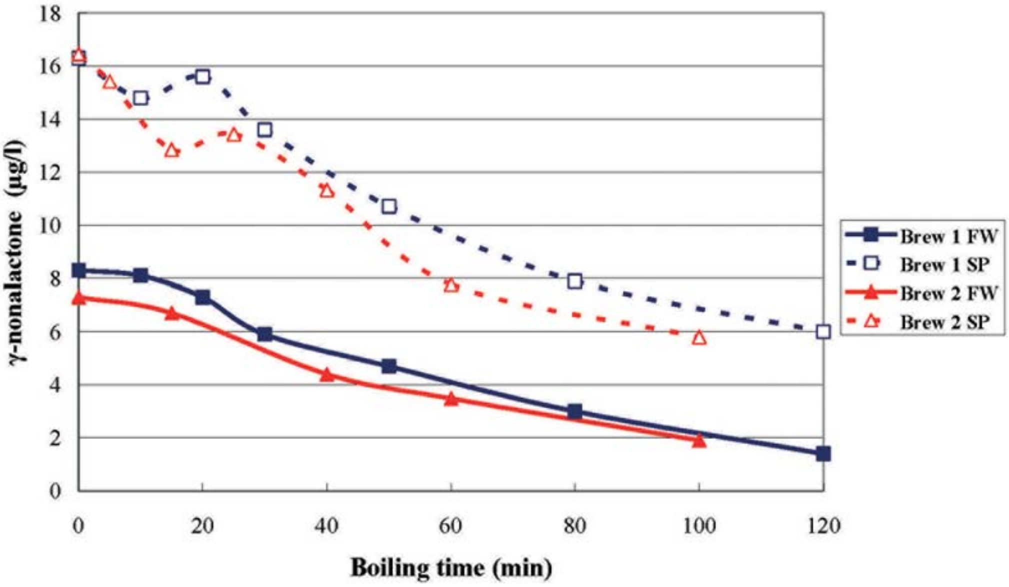

Fig. 12 Changes in the concentration of γ-nonalactone during wort boiling; FW = first wort, SP = spargings, results from wort samples were adjusted to correspond to an original gravity of 12%

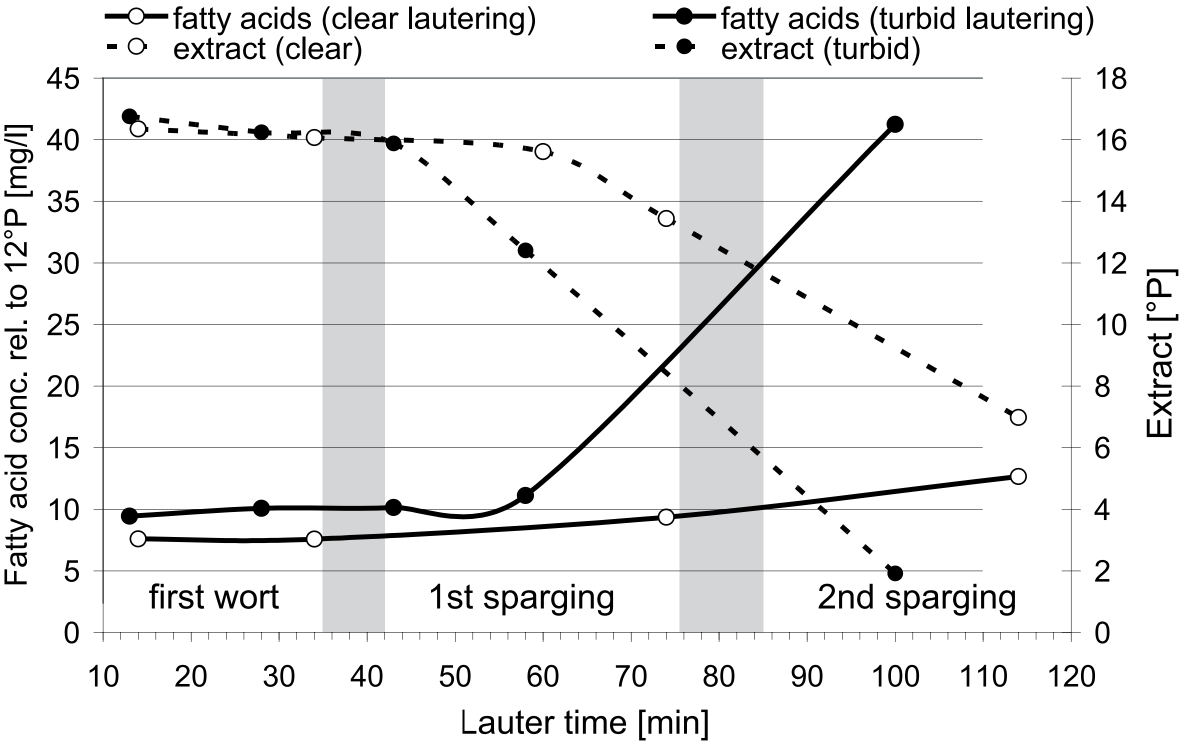

Fig. 13 Concentration of long-chain fatty acids (C14 – C18:3) adjusted to 12% original gravity (12°P) and the extract concentration in wort during clear and turbid lautering; clear lautering: average overall turbidity: 16 EBC, duration: 149 min; turbid lautering: 140 EBC, 110 min [54]

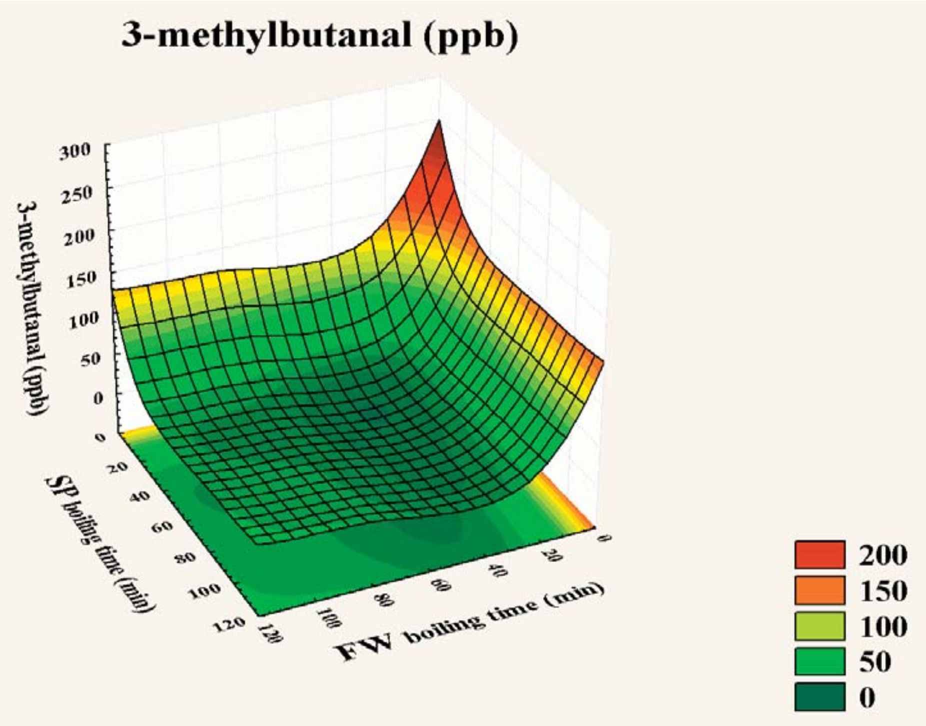

Fig. 14 Simulation with mixture of first wort and spargings and different boiling times (3-methylbutanal); FW = first wort, SP = spargings, results from wort samples were adjusted to correspond to an original gravity of 12%

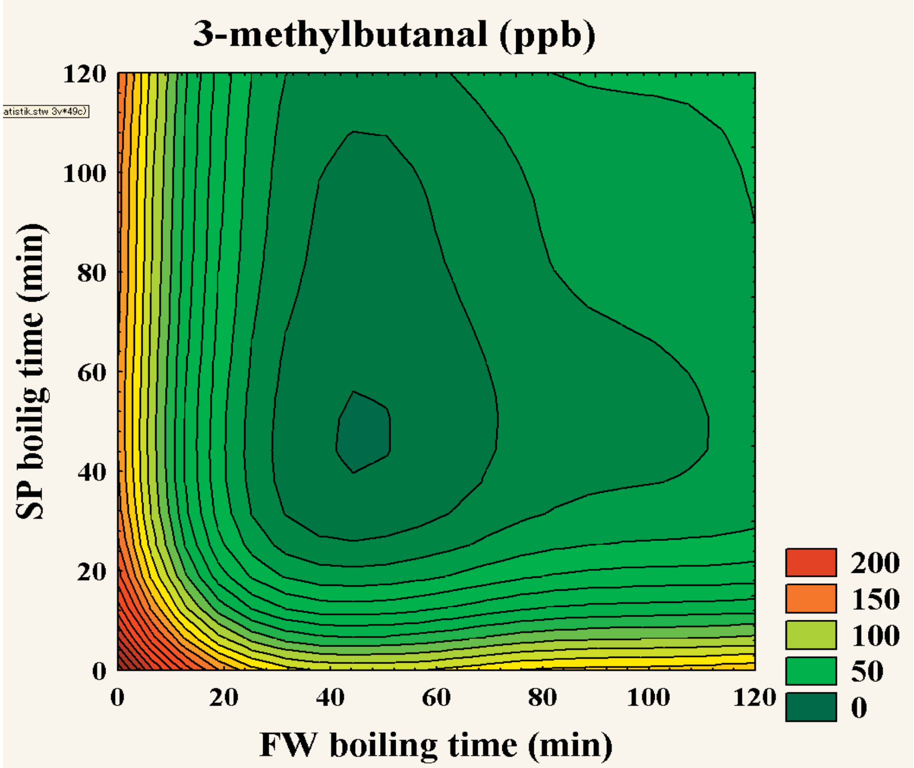

Fig. 15 Simulation with mixture of first wort and spargings and different boiling times (3-methylbutanal); FW = first wort, SP = spargings, results from wort samples were adjusted to correspond to an original gravity of 12%

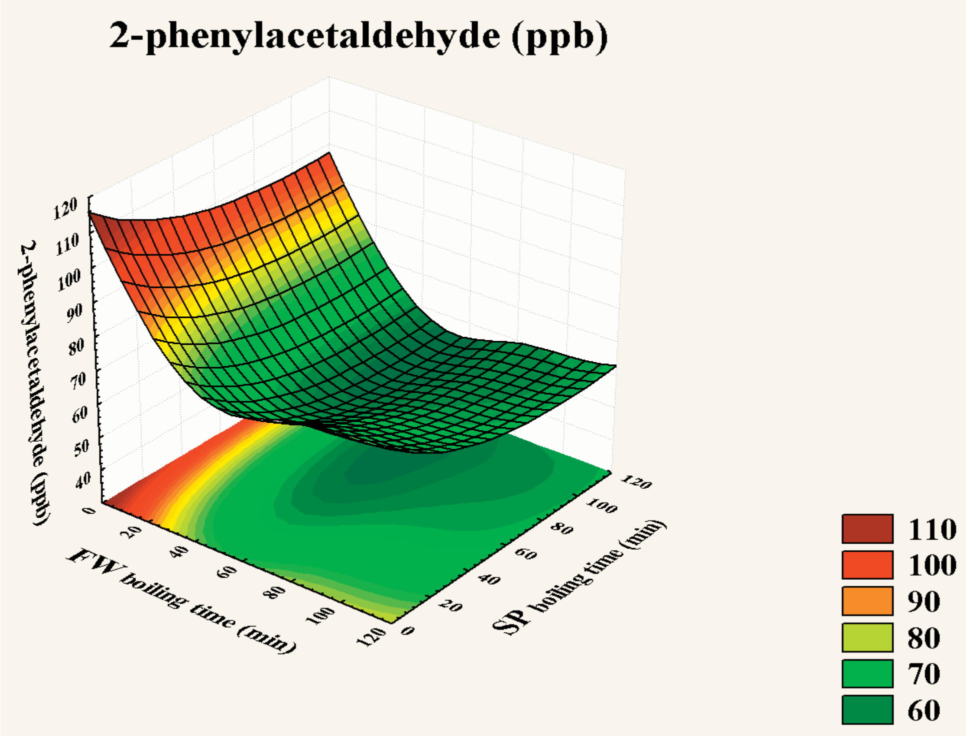

Fig. 16 Simulation with mixture of first wort and spargings and different boiling times (2-phenylacetaldehyde); FW = first wort, SP = spargings, results from wort samples were adjusted to correspond to an original gravity of 12%

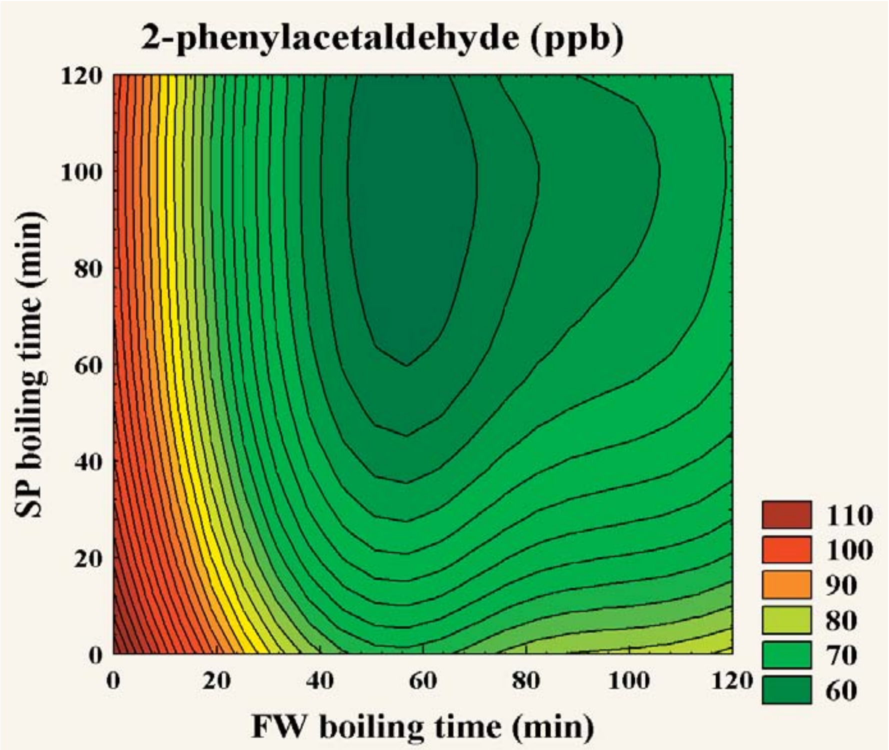

Fig. 17 Simulation with mixture of first wort and spargings and different boiling times (2- phenylacetaldehyde); FW = first wort, SP = spargings, results from wort samples were adjusted to correspond to an original gravity of 12%

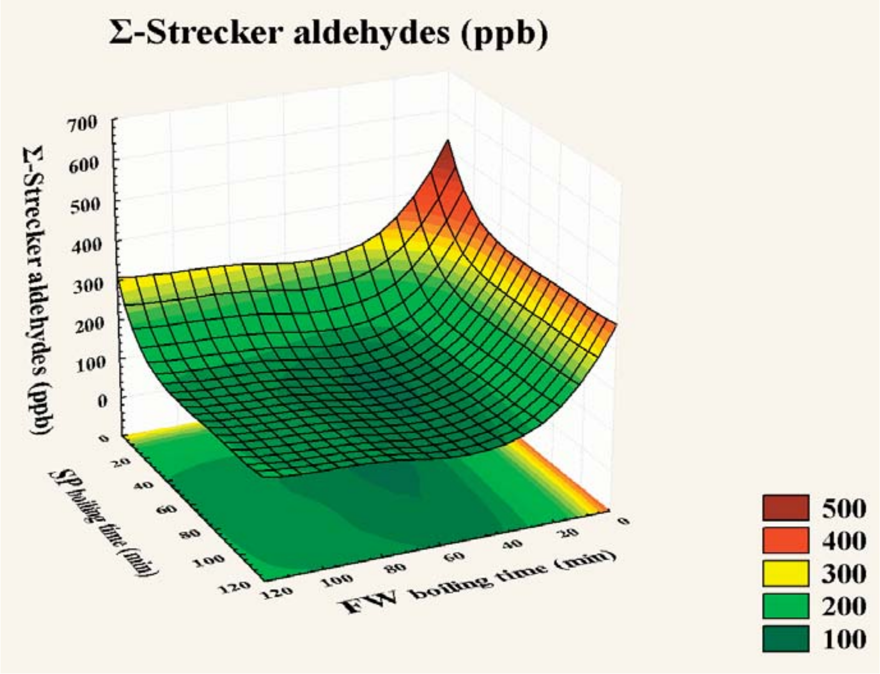

Fig. 18 Simulation with mixture of first wort and spargings and different boiling times (Σ-Strecker aldehydes); FW = first wort, SP = spargings, results from wort samples were adjusted to correspond to an original gravity of 12%

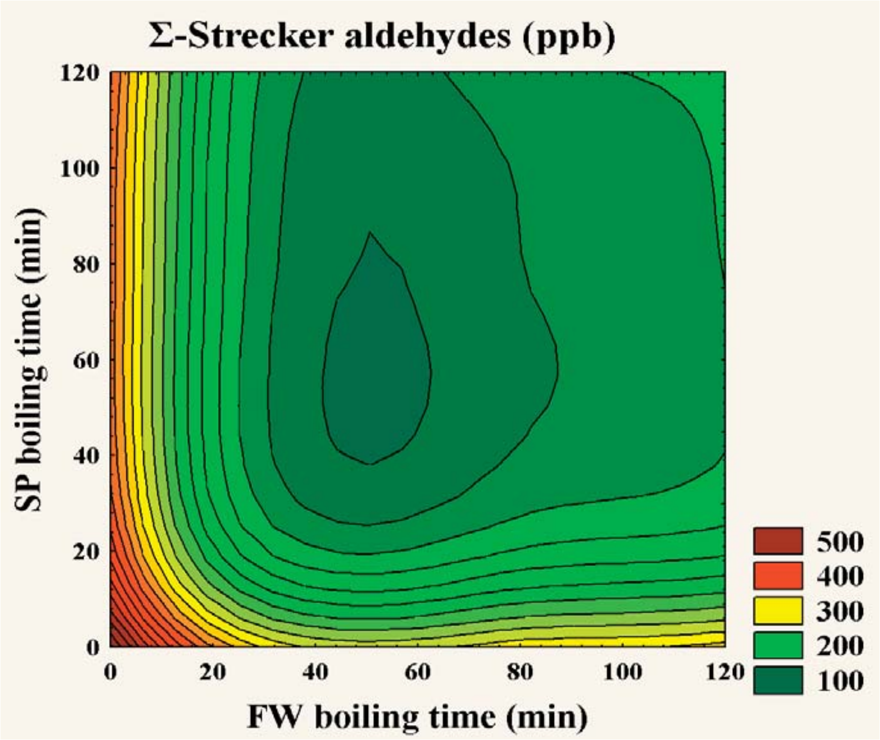

Fig. 19 Simulation with mixture of first wort and spargings and different boiling times (Σ-Strecker aldehydes); FW = first wort, SP = spargings, results from wort samples were adjusted to correspond to an original gravity of 12%