TECHNISCHE UNIVERSITÄT MÜNCHEN

TUM School of Life Sciences

Formal kinetic description of chemical reactions of important process markers at boiling processes in brewing technology for controlling and optimizing of boiling systems

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子曰：道亦所求

The route is the goal.

Confucius

for

my family

Acknowledgments

Life is dynamic and full of surprises. Following my medical studies in Shanghai, I began studying Germany's fascinating art of brewing. During my diploma studies I was inspired by the various types of beer and modern, ever-evolving brewing technologies. As a result, I agreed to do my doctorate at the Chair of Brewing and Beverage Technology (BGT).

At this point I would like to take the opportunity to thank the people who assisted me during my doctoral studies and gave me valuable tips. I thank my doctoral supervisor, Prof. Dr.-Ing. habil. Thomas Becker for making this doctorate possible and for his patient care.

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Peer reviewed publications

The results of this work were developed during my work as a research assistant at BGT at the Technical University of Munich from 2010 to 2015.

Review paper:

1. Huang, Y., Tippmann, J., Becker, T. (2017): Kinetic studies of main wort flavour compounds and iso-α-acids during wort boiling: a review. European Food Research and Technology. 243, 1485-1495
2. Huang, Y., Tippmann, J. and Becker, T. (2016): A Kinetic Study on the Formation of 2- and 3-Methylbutanal. Journal of Food Process Engineering, 40: e12375. doi:10.1111/jfpe.12375
3. Huang, Y., Tippmann, J., Becker, T. (2015): Non-Isothermal Kinetic Models of Degradation of S-Methylmethionine. Journal of Food Process Engineering, 39, 573–580
4. Huang, Y., Tippmann, J., Becker, T. (2013): Kinetic Modeling of Hop Acids during Wort Boiling. International Journal of Bioscience, Biochemistry and Bioinformatics, 3(1), 47-52

Paper without Review:

1. Huang, Y., Tippmann, J., Becker, T. (2015): Abnahme des koagulierbaren Stickstoffs während des Würzekochens, Brauwelt 10/155, 274-277

Scientific presentations:

1. Huang, Y., Tippmann, J., Becker, T.: Formalkinetische Beschreibung des Abbaus der Iso-α-Säure bei der Würzekochung, 45. Technologisches Seminar, Weihenstephan, 14.02.2012 und 20.02.2012
2. Huang, Y., Tippmann, J., Becker, T.: A kinetic study on the degradation of iso-α-acids, International Conference on Biotechnology and Food Engineering, Dubai, VAE, 05.08.2012 (Best Presentation)
3. Huang, Y., Tippmann, J., Becker, T.: Isothermal and non-isothermal decomposition kinetics of S-Methylmethionine, European Brewery Convention Congress, Luxemburg, 28.05.2013
4. Huang, Y., Tippmann, J., Becker, T.: Energetische und kinetische Betrachtungen zur Würzekochung im Brauprozess, 47. Technologisches Seminar, Weihenstephan, 19.02.2014 und 25.02.2014

5. Huang, Y., Tippmann, J., Becker, T.: Wie viel Zeit benötigen das Aufheizen und Kochen der Würze tatsächlich? Jahrestreffen der ProcessNet-Fachgruppen Lebensmittelverfahrenstechnik und Phytoextrakte, Weihenstephan, 27.02.2014
6. Huang, Y., Tippmann, J., Becker, T.: Influence of coagulate nitrogen concentration and kinetic chemistry during the wort boiling, Institute of Brewing and Distilling, 33rd Asia Pacific Convention, Ho Chi Minh City, Vietnam, 27.03.2014
7. Huang, Y., Tippmann, J., Becker, T.: Analytische Betrachtung und Wiederverwendungsmöglichkeiten von Heißtrub, 48. Technologisches Seminar, Weihenstephan, 11.02.2015 und 24.02.2015

Poster Presentation:

1. Huang, Y., Tippmann, J., Becker, T.: Influence of formation of hot break and kinetic chemist during wort boiling, Master Brewers Association of the Americas, Chicago, USA, 5.-7.06. 2014

Contents

Abbreviations

2- and 3-MB	2-and 3-methylbutanal
A	pre-exponential factor (s^{-1} for a 1st order reaction)
coag.-N	coagulable nitrogen
d	diameter (m)
DMS	dimethyl sulfide
E_a	activation energy (kJ/mol)
GC	gas chromatography
GI	glucose with isoleucin
GL	glucose with leucin
Hl	hundred liters
HPLC	high pressure liquid chromatography
HTWB	high temperature wort boiling
k	rate constant (s^{-1} for a1st order reaction)
MEBAK	Central European Commission for Brewing Analysis
MI	maltose with isoleucin
ML	maltose with leucin
N	rotation frequency
LPWB	low pressure wort boiling
Ne	Newton number (-)
NEB	non-enzymatic browning
P	stirring performance
R	universal gas constant (8.314 J $mol^{-1}K^{-1}$)
Re	Reynolds number
SMM	S-methylmethionine
T	absolute reaction temperature (K)
TBA	thiobarbituric acid
ρ	density (kg/m^3)

Zusammenfassung

Die Beurteilung und Optimierung von Würzekochsystemen sollten auf kinetischen Kenntnissen der chemischen, physikalischen und biologischen Reaktionen während des Kochprozesses basieren. Die kinetischen Kenntnisse dienen als Grundlage zur Simulation von Kochprozessen und der Vorhersage von Konzentrationen der wesentlichen Leitsubstanzen. Im Vergleich mit kinetischen Untersuchungen in der Chemie oder der Lebensmitteltechnologie, stecken derartige Untersuchung in der Brauwissenschaft noch in den Anfängen. Weitere und eingehendere Untersuchungen auf diesem Forschungsgebiet sind zwingend notwendig.

Ziel der vorliegenden Arbeit war das Erstellen einer übertragbaren, verfahrenstechnischen Beschreibung der Würzekochung in Abhängigkeit von den Prozessparametern Temperatur/Druck und Zeit mit Blick auf die Prozessmarker der Würze, für welche die formalkinetischen Zusammenhänge dargestellt werden. Des Weiteren ging es um die Frage, ob es möglich ist, die Kochdauer zu verkürzen, um dadurch die Belegungszeit des Kochgefäßes zu minimieren und den thermischen Energieverbrauch zu verringern. Hierfür bedarf es auch einer Wirtschaftlichkeitsbetrachtung dergestalt, dass zu prüfen ist, welche Glattwasser-Nutzschwelle unter Zugrundelegung von Malzkosten auf der einen Seite und Energiekosten für die Verdampfung auf der anderen Seite optimal ist. Im Rahmen dieser Arbeit wurden die Bildungskinetiken der wichtigsten Prozessmarker während des Würzekochens untersucht. Die Arbeit wurde in vier Teilschritten durchgeführt.

Im ersten Teil wurden die bisherigen Erkenntnisse der kinetischen Untersuchungen von Leitsubstanzen während des Würzekochens zusammengefasst. Ein Überblick über die Bildungskinetiken der Leitsubstanzen, wie sie auch in anderen Lebensmittelsystemen gegeben sind, vor allem in Bezug auf Maillard-Produkte, wurde in einem Review dargestellt und diskutiert.

Im zweiten Teil wurden der kinetische Mechanismus des Abbaus der Iso-α-Säuren bei verschiedenen pH-Werten (4,5; 5,2 5,5; 6,5), bei unterschiedlichen Temperaturen (90-130 °C) untersucht und die jeweilige Aktivierungsenergie und die Geschwindigkeitskonstanten des Abbaus berechnet. Die Isomerisierung der α-Säuren und der Abbau der Iso-α-Säuren sind beide als Reaktionen 1. Ordnung zu betrachten. Es wurde herausgefunden, dass mit steigenden pH-Werten nicht nur die Isomerisierung der α-Säuren, sondern auch der Abbau der Iso-α-Säuren beschleunigt werden.

Im dritten Teil wurde der DMS-Bildungsprozess in der Brautechnologie unter besonderer Berücksichtigung der Aufheizphase unter nicht-isothermen Bedingungen untersucht. Die Differential-Methode (nach Kissinger) und die Intergral-Methoden (nach Coats-Redfern und Ozawa-Flynn-Wall) wurden verwendet, um die Bildung von DMS genauer zu betrachten. Es wurde berechnet, welche Zeitdauern beim Aufheizen und beim Kochen mit unterschiedlichen Aufheizraten und Kochtemperaturen für das Erreichen einer optimalen DMS-Konzentration benötigt werden.

Im vierten Teil wurden die Reaktionsmechanismen für die Maillard-Produkte 2- und 3-Methylbutanal, aus ihren Vorläufern und Zwischenprodukten untersucht. Während der Kochung ist die Bildung von Aromastoffen, welche vorwiegend aus der Maillard-Reaktion stammen, von Bedeutung. Bisher wurden nur einzelne Schritte kinetischer Modelle in der Brauwissenschaft erforscht. Ein kinetisches Modell über mehrere Schritte wurde hier als bedeutsam angesehen und mathematisch erstellt.

Die Entwicklungstrends für die Kinetik der thermischen Reaktionen wurden von der einzelnen Schrittreaktion zur Multischritt-Modellierung, vom isothermen Zustand zum nicht-isothermen Zustand, untersucht. Auf Grundlage der erstellten kinetischen Modelle lassen sich optimale Kochdauern berechnen, auch mit dem Ziel, die Kochdauer zu verkürzen und um dadurch thermische Energie einzusparen. Die Ergebnisse werden sowohl für die Auslegung betrieblicher Kochprozesse als auch für die anlagentechnische Weiterentwicklung der Kochsysteme nützlich sein. Basierend auf ausgerechneten kinetischen Werten bei einem Würze-pH-Wert von 5,2, wurden die Kochdauern logarithmisch über die Kochtemperatur abgetragen. Die Darstellung von Kurvenscharen ermöglicht eine Übertragung der Erkenntnisse auf andere Kochprozesse. Die individuelle Kochdauer der Würze wird auch noch von weiteren Faktoren beeinflusst.

Der Hauptbeitrag dieser Arbeit wurde vom Doktoranden mit kritischer Überprüfung der vorhandenen Literatur sowie der Konzeption und Gestaltung der Arbeit gegeben. Sie führte den größten Teil der Experimente, die Analyse der Daten und die Interpretation der Ergebnisse sowie das Schreiben und Überarbeiten des Manuskripts durch.

Summary and contribution of the doctoral candidate

The assessment of wort boiling systems and optimization of the bill of materials should be based on the chemical, physical and biological kinetic information generated by reactions during wort boiling. This kinetic knowledge serves as a basis for the simulation of the boiling process and predicts the concentrations of dominant compounds. Compared with the kinetic studies in chemistry or food technology, brewing science kinetic investigations remain in the early stages. Further detailed studies are necessary.

The goal of this thesis is to develop a reproducible procedural description of the wort boiling process correlated to the applied process parameters (temperature/pressure, time and rate of evaporation) in order to present an insight into the resulting process markers of the produced wort. The work delves further into the question: is it possible to reduce the boil time, so the kettle cycle time and thermal energy usage can be reduced. The important process markers are amounts of free DMS and selected Strecker aldehydes, potential off flavours in finished beer. In the framework of this thesis, the formation kinetics of the most important process markers during wort boiling process were investigated. For this, an economic feasibility study was completed in such a way to determine optimal the cut off for last wort run-off in relationship to material costs on one side and energy costs for evaporation on the other. During the course of this work, the formation kinetics of the most important process markers during the wort boiling process were studied.

In the first part of this thesis, the existing kinetic modeling and kinetic parameters of evaporation and formation of main wort flavour components during wort boiling are summarized. A review of kinetic studies as they apply to other food systems in chemistry and food sciences is done. Kinetic investigations in brewing science remain rudimentary. An overview of the formation kinetics of similar substances in other food sciences, especially the formation of Maillard products is reviewed and discussed.

In the second section, the kinetic mechanism of the formation and degradation of the iso-α-acids at different pH values (4.5, 5.2, 5.5; 6.5) and at different temperatures (90-130 °C) was examined. The respective activation energy and the reaction rate constant for the formation and degradation of iso-α-acids were calculated. The isomerization of α-acids and degradation of iso-α-acids should be considered both as 1st order reactions. It was found that increasing pH values accelerated not only the isomerization of the α-acids, but also the degradation of iso-α-acids.

The third section the DMS development process was researched with special consideration given to the raise to boil period under non-isothermal conditions. The differential method (according to Kissinger) and the integral-method (according to Coats-Redfern and Ozawa-Flynn-Wall) were employed to better observe the development of DMS. The time duration needed for raise-to-boil and boiling was calculated employing various raise-to-boil rates and boiling temperatures to achieve an optimal DMS concentration.

In fourth section, the reaction mechanics for development of the Maillard 2- and 3-Methylbutanal, from of their precursors and intermediates were analyzed. Of importance during boiling is the development of aroma components, which occur mainly from Maillard reactions. Up to now, only individual kinetic modeling steps were researched in brewing science. A kinetic model covering multiple steps was considered of importance in this study and mathematically generated.

The development trends for the thermal reaction kinetics from the individual steps to multi-modeling, from isothermal conditions to non-isothermal conditions were studied. The optimal boil duration can be calculated based on the developed kinetic model with the goal of reducing the boil time and along with that, a saving in thermal energy. These results are useful not only for design of the boiling process but also for the further technical development of wort boiling systems. Based on calculated kinetic values, the boil duration at a pH of 5.2 was logarithmically plotted against the boil temperature. The array of curves makes it possible to transfer the findings to other boiling processes. The individual wort boiling times are influenced by further factors.

The main theme of this thesis was presented by the doctoral candidate with a critical review of the available literature as well as the conceptual design and layout of the work. She did the greatest number of the experiments, the analysis of data, and the interpretation of the results as well as the writing and editing of the manuscript.

1 Introduction and tasks

1.1 Explanation of the initial situation

In the field of wort boiling, there are a large number of established and proven process technologies. Nevertheless, there is still great potential for research into the kinetic behavior of process markers, which can ultimately improve the quality of the beer. Wort boiling plays a key role here. The aroma profile and beer stability as well as boiling process energy consumption are optimized employing the kinetic approach.

Saving energy and protecting the environment have long been regarded as a central theme within brewing technology (Schwill-Miedaner 2002, Hertel, 2007, Felgenträger 1993). Specific energy consumption of breweries in relation to the volume of beer sold has fallen significantly worldwide in the past 10 years. According to benchmark studies, the specific energy consumption of 225 examined breweries from 49 countries decreased on average from 271 MJ/hl in 2000 to 207 MJ/hl in 2012 (Leppin 2014). German breweries optimized for energy usage have achieved values of less than 100 MJ/hl (Hackensellner 2000).

Wort boiling is a central process step and of particular importance, as it alone requires approx. 40 % of the total thermal energy requirement in the brewhouse (Narziß 2005, Heyse 1995). The development of new wort boiling systems reduced boiling times in excess of two hours to less than one hour during the last 100 years (Mezger 2003, O'Rourke 2003, Parson 2002). However, many brew masters still adjust the boiling times according to their own experience, mostly solely on the basis of the desired original wort concentration and visual control of protein coagulation.

By reducing total evaporation during wort boiling, energy costs and CO_2 emissions are significantly reduced (Hackensellner 2000). A reduction in the thermal load (boiling time, total evaporation) leads to increased taste stability and thus an increase in product quality, which is an indispensable prerequisite for compliance with the “best-before date”. In addition, a targeted adjustment of the process markers can have a significant influence on the resulting beer quality (Narziß 2005, Scheuren 2010; Thum et al. 1995).

In order to calculate the optimal boiling time individually based on the raw materials used the following is required. Knowledge of the formation and evaporation kinetics of the important flavouring substances (Miedaner 1986, Felgenträger 1993, Hertel 2007, Scheuren 2010, Feilner 2013, Ditrych et al. 2019), the formation and degradation kinetics of the iso-α-acids (Askew 1964, Diffor et al. 1973, Intelmann 2010, Kappler 2010), the kinetic behavior of the hot trub and the denaturation and aggregation of the coagulable nitrogens is required (Felgenträger 1993, Kühbeck 2007).

Knowledge of important process parameters of formation kinetics is comparatively limited. Literature research has shown that the formal kinetic description of the wort flavouring substances to date has only been calculated as a zero or 1st order reaction, although Maillard reactions represent a complex network of parallel reactions and subsequent reactions (Zürcher 1979, Felgenträger 1993, Schwill-Miedaner 2001, Rübsam 2010). The sum parameter TBA, which comprises a large number of primary and secondary products of the Maillard reactions, represents a very simplified measure for determining the thermal load of the wort (Grigsby and Palamand 1975, Leong and Wedzicha 2000). However, individual aroma substances contained in the TBA have not yet been investigated in detail. Previous investigations have always been based on isothermal conditions, although many chemical reactions already take place during the heating phase up to boiling temperature.

The art of brewing aims to ensure consistent beer quality despite different raw materials and different brewhouse facilities. In order to ensure product quality, reduce energy costs, optimize vessel cycle time and thus brewhouse productivity, it is critical to optimize the boiling process as much as feasible.

1.2 Tasks of wort boiling

The wort obtained by the lautering process is boiled. During wort boiling chemical reactions (isomerization of α-acids, coagulation of proteins, formation of flavourings, deactivation of enzymes, separation of SMM to DMS), physical reactions (evaporation of volatile compounds, aggregation of hot break, achieving desired wort extract and extraction of hop flavours) and biological reactions (killing of microorganisms) take place. This also leads to an increase in wort colour and a decrease in pH values (Narziß 2005, Denk 2000, Goldammer 2008, O'Rourke 2002). It is important for beer taste shelf life and beer stability that the wort boiling tasks are performed flawlessly. If the wort boiling is presented as a building, the most important tasks can be presented as a supporting column (Fig. 1).

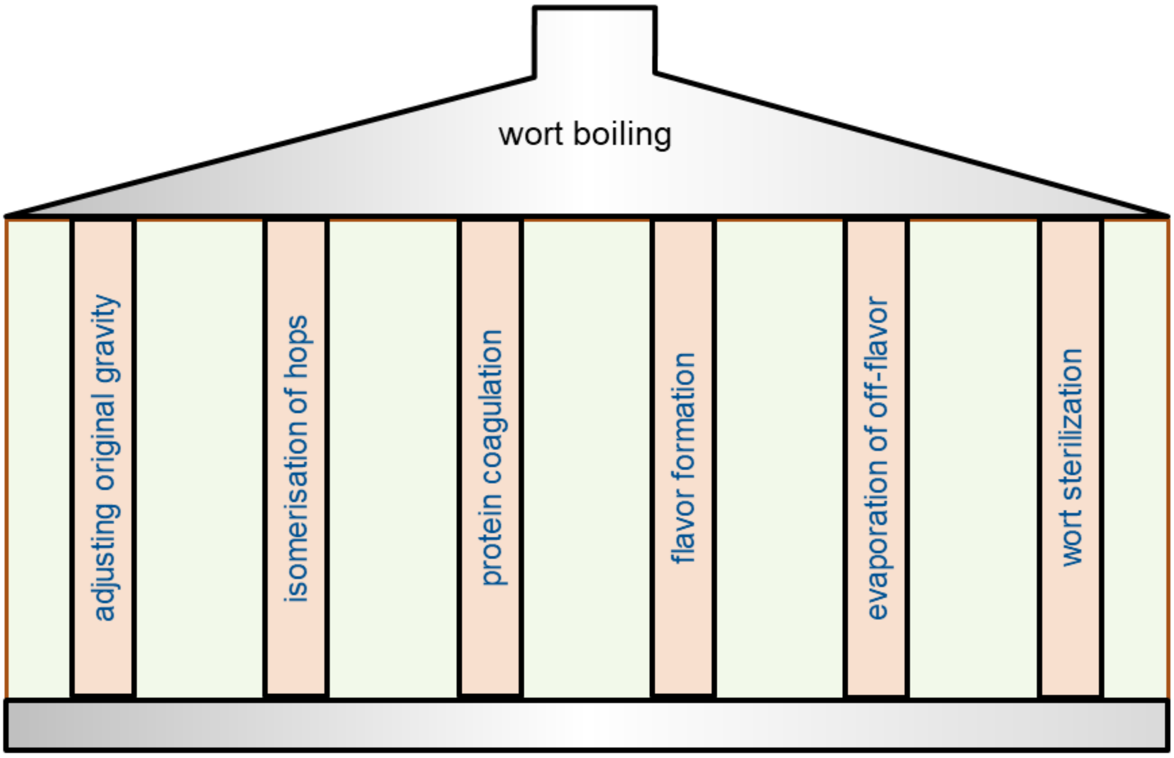


Fig. 1: The most important tasks in wort boiling

Physical, chemical and biological reactions during wort boiling are strongly dependent on the boiling temperature (Kessler 1996, Belitz 2001). Atmospheric boiling is the most common boiling process in breweries (Narziß 2009). The optimization of the boiling time is the most important method to guarantee the quality of the wort and to save energy. Through the development of new brewhouse technologies, such as wort stripping, the undesirable aroma substances are effectively expelled after the whirlpool rest. If employing LPWB and HTWB, wort is boiled under pressure (Feilner 2013a and 2013b, Andrews 2008).

Boiling can basically be divided into two relevant processes. The first involves evaporation, which removes numerous volatile substances from the wort. In the ideal system, the evaporation of a flavouring substance depends on the total evaporation, the initial concentration and the specific vapor/liquid equilibrium of the substance (Hertel et al. 2006, Emel'yanenko et al. 2007, Weinzierl 2005, Sun 2012). Secondly, numerous thermal induced reactions occur that are responsible for chemical conversion and formation of additional compounds. In addition to the specific reaction rate constant, the reaction rate is influenced by the temperature, any catalysts present, the concentrations of the reacting substances and the reaction time (Kessler 1996, Westphal et al. 1996).

In order to adjust the desired concentration of a flavouring substance through boiling Hertel et al. (2007) describe the dependence of the final concentration on the minimum required total evaporation. This is independent of the surface of the boiling wort and the wort composition. It is solely dependent on the substance-specific distribution factor determined in aqueous solution. During atmospheric boiling at 100 °C in a closed system, the concentration ratios between liquid and gaseous phase (aqueous solution) of hexanal, 2-MB, 3-MB and DMS were determined experimentally by means of GC. After adjusting the phase equilibrium (several hours boiling time), the distribution of the flavouring substances between the liquid and gaseous phases was determined in a closed boiling vessel. The experimental setup therefore was static and does not reflect the dynamic evaporation processes of the wort boiling process. In these experiments the distribution factors for hexanal, 2-MB, 3-MB and DMS could be determined. Hexanal was the lowest and DMS the highest (Hertel et al. 2007). Hertel concentrated its investigations on the relative total evaporation of the process markers, but without considering replication processes.

In his work, Scheuren examined only coupled expulsion and replication processes of the substance DMS. He analyzed and calculated the reaction rates in the temperature range between 80 and 99 °C in small steps (80, 85, 90, 98-99 °C) employing a rotary evaporator. As a result of analytical errors, no significant differences can be deduced applying such small temperature steps. The volatility of DMS was investigated for shears in the temperature range of 30 to 100 °C at stages of 10 °C. Studies at temperatures above 100 °C could not be carried out due to limitations of the test set-up.

In the context of the present work, the concentrations of the process markers formed were measured in several closed vessels (vials) having minimal headspace independent of the evaporation in the liquid phase. The samples were immediately cooled in an ice water bath after boiling to avoid further reactions. It was also possible to boil the samples under pressure, i.e. at boiling temperatures of over 100 °C. It was possible to take and analyze samples during the heating phase.

Currently, most breweries require total evaporation rates of 5-8 % at boiling times of 50-80 min in order to achieve the above targets. The possibilities of shortening the boiling time while maintaining wort and beer quality allow capacities to be increased. At the same time, costs and the formation of undesirable aroma-active substances (e.g. Strecker aldehydes) are reduced (Back 2008, Yamashita et al. 2006, Narziß and Back 1999, Noeddekaer 2007). This also improves wort and beer quality.

1.3 Adjust the original gravity

The target casting volume results from the desired original gravity concentration and the brewhouse yield. There are different opinions among brewing technologists about the boiling time of the wort. On the one hand, they want to achieve a high brewhouse yield sparging, but on the other hand, evaporation of sparging requires thermal energy to achieve the desired original gravity concentration. An economic consideration can show how the costs for the unused extract, i.e. malt loss, are distributed in comparison with the energy consumption required for boiling. If both cost shares are set equal, an economic use threshold can be calculated for the last wort concentration. The following calculation formula is used:

$$\left[\Delta t \cdot c \cdot 100 + \left(100 - \frac{100 \cdot p}{StW}\right) r\right] k = \frac{M \cdot p}{A}$$

Δt = difference raw wort temperature and boiling temperature
c = specific heat capacity of the wort (4.1868 kJ/(kg K)
p = extract concentration of the last running (kg/100 kg)
StW = extract concentration of original wort (kg/100 kg)
r = heat of evaporation of water at atmospheric pressure (approx. 2260 kJ/kg)
k = fuel costs per unit of heat. A heat loss of 16 % in the steam boiler and 10 % in the wort kettle was assumed (€/kJ)
M = malt costs in €/100 kg
A = brewhouse yield in kg/100 kg
The above equation converted to p gives:

$$p\left(\frac{100 \cdot r \cdot k}{StW} + \frac{M}{A}\right) = 100 \cdot k \cdot \Delta t \cdot c + 100 \cdot r \cdot k$$

$$p = \frac{100 \cdot k \cdot StW \cdot A(\Delta t \cdot c + r)}{100 \cdot r \cdot k \cdot A + M \cdot StW}$$

For malt costs between 30 and 50 €/100 kg and heating oil costs between 40 and 90 €/hl (density 0.84 kg/l) the last running thresholds are shown in Fig. 2. The average malt prices of the last 5 years are between 30 and 50 € per 100 kg.

In practice, the volume of secondary castings is already determined on the basis of the selected main casting volume. The temporal dosage of the after-cast volume is to be chosen in such a way that the extract still present in the spent grains can be obtained to a large extent and in a short time. Technologically, the control of the last running is done by determining the extract concentration. In the case of Pilsner-type beers or very light export beers with a comparatively low concentration of first wort, an increased concentration of last running are accepted (Narziß et al. 2005). Narziß recommends to monitor the volume of the after wort on the basis of the extract concentration of the last running (guideline value 0.5-0.8 %) and the concentration of the extract that can be washed out (guideline value 0.4-0.6 %). Approximately 15 years ago, the malt and energy costs at that time resulted in an economic last running usage threshold of about 2 g/100 g.

Fig. 2 shows that at current malt and energy costs the last running usage threshold is around 5 g/100 g. A higher evaporation is therefore not reasonable from an economic point of view. In addition, lower evaporation protects the wort with regard to its thermal load. For example, less Maillard products are produced, which are also responsible for beer ageing. However, the consideration of the last running usage threshold must always be based on the current malt and energy costs.

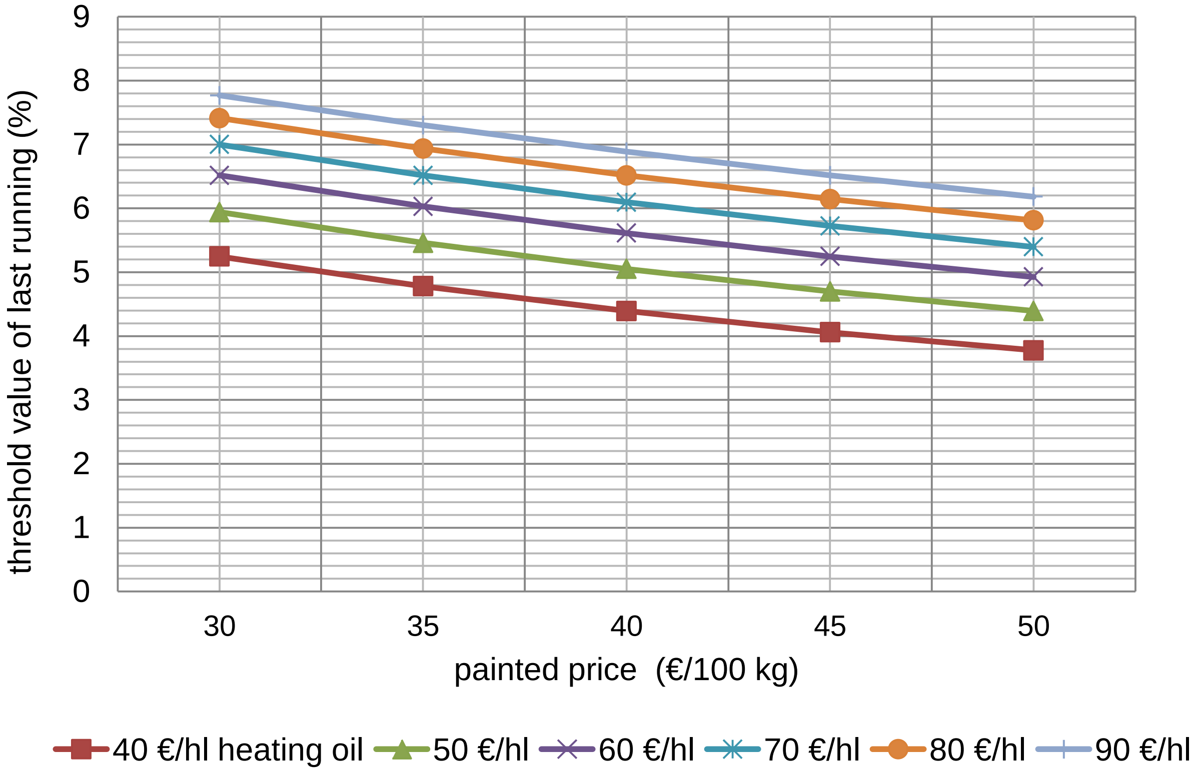


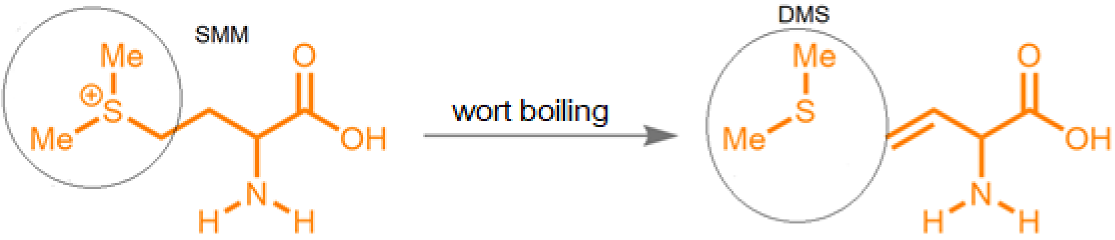
Fig. 2: Threshold value of last running in relation to heating oil price and malt price

1.4 Selected wort ingredients and their formation pathways

During wort boiling, the formation and evaporation of aroma substances is important. They have an influence on the taste and aroma profile of both fresh and aged beer. In the present work, important and analytically detectable aroma substances were selected and their formation kinetics investigated. However, there are also numerous substances in the wort for which a kinetic investigation is not possible. For example, there is a large number of substances in the wort which form different intermediate products during boiling, but which cannot be analytically determined. For some substances, the change in concentration that occurs under boiling conditions is very small, so that the measurement results are uncertain and cannot be interpreted. The selected substances are therefore DMS, 2- and 3-MB as well as the α-acids and iso-α-acids.

1.4.1 DMS

DMS is a sulfur-containing organic compound that forms during wort boiling (Narziß 1996). Malted grain contains a non-proteinogenic amino acid (SMM). During the wort boiling SMM degrades to DMS.



Scheme 1: Formation of DMS from SMM in heated wort

DMS has different concentrations in malt, wort and beer. Under atmospheric conditions, the boiling point of DMS is 37 °C. It is slightly volatile and is evaporated during wort boiling. The effect on the smell and taste of beer is controversial. While on the one hand 50-60 µg/l DMS is indicated as a taste threshold value, German beers, even with 100-120 µg/l, often exhibit no negative taste. Normally the guideline value for free DMS in light deflection wort or set wort is 20-80 µg/l. The total concentration of DMS and its precursor SMM in the wort should not exceed 100-120 µg/l (Fig. 3) (Narziß and Back 2005; Hertel 2007).

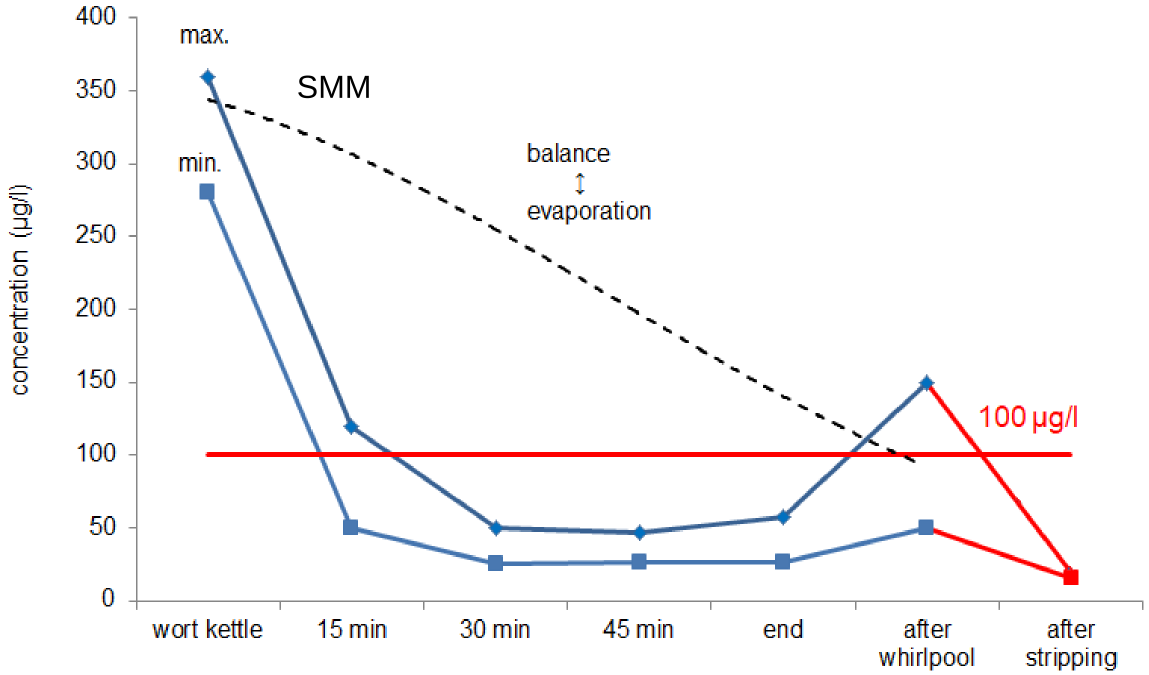


Fig. 3: Free DMS and SMM in the wort (Narziß and Back 2005)

In the wort boiling process it is of great importance to achieve the greatest possible splitting of the precursor SMM and to expel the released DMS. The boiling intensity and the homogeneity of the amount of wort in the wort kettle are decisive here. Malt texture and mashing work have a significant influence on the concentration of SMM in the wort before boiling. However, with a sufficient boiling time, it is possible to achieve concentration values of DMS that do not adversely affect further beer production processes (Heyse 1995, Krottenthaler 2010). Even after completion of the boiling process, further SMM can be split into DMS in the downstream whirlpool, but no more evaporation takes place here. Fig. 4 shows an overview of the factors influencing DMS formation.

Even if modern boiling systems operate in a well-defined range of elevated temperatures and controlled wort circulation, boiling times are often too short if protein precipitation is not too sufficient. For energy saving reasons, evaporation is often reduced, resulting in a risk that the threshold value for free DMS will be exceeded. Only HTWB always lowers the total DMS concentration to a large extent (Narziß and Back 2005).

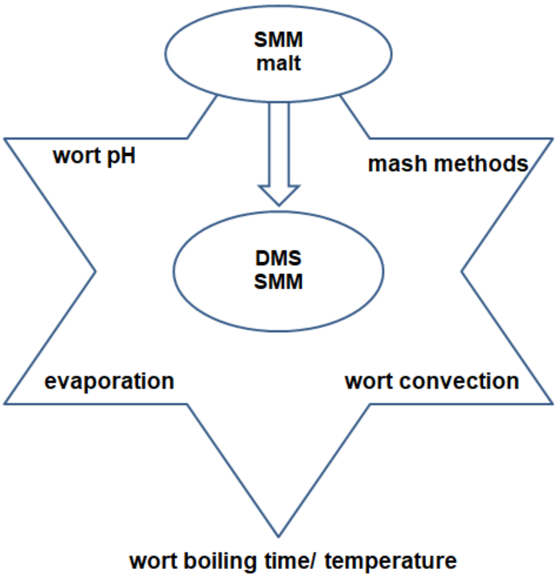


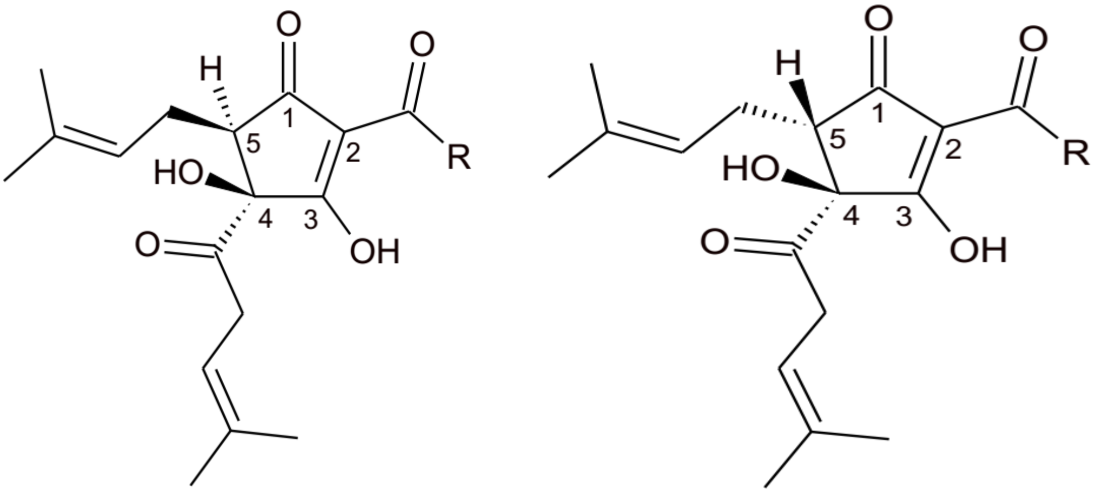
Fig. 4: Factors influencing DMS formation during wort boiling (Zürcher et al. 1979, Narziß 2009)

DMS is one of the most studied off-flavours in wort and beer. In the past, numerous investigations on the SMM reaction process were carried out using thermal analysis. DIN 8777 recommends a DMS concentration in cooled wort of < 100 µg/l in order not to exceed the taste threshold value of 100-130 µg/l in finished beer for all-malt beers (Mezger 2006, Scheuren 2014a and 2014b, Desobgo et al. 2015). The degradation of SMM to DMS is a single-stage reaction. It is a reaction of 1st order (Zürcher 1979, Felgenträger 1993, Scheuren 2010). Previous investigations were based on isothermal conditions, e.g. constant 80 °C, 90 °C or boiling temperature. The advantages were a simpler interpretation of the results and the possibility of differentiating between simple and accelerated reactions. However, the extended measurement times and the determination of the initial concentrations (C_0 cannot be well defined with fast reaction) are disadvantageous. The wort requires time to reach the boiling temperature during raise to boil phase. But during the heating phase a part of the SMM is converted into DMS. This can falsify the resultant calculation of the kinetic parameters. This problem is particularly relevant for investigations at higher temperatures.

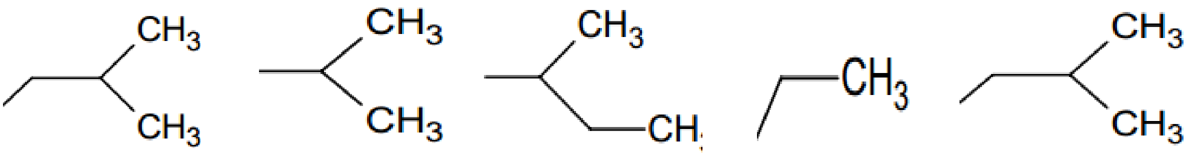
1.4.2 Formation and degradation of iso-alpha-acids

Hops contain many ingredients, but bitter substances, the so-called hop resins, are of particular interest for beer production. Three classes of hop resins can be defined as biochemical markers of the various hop varieties, namely α-acids (humulones), β-acids (lupulones) and hard resins, which are not defined in more detail. Especially the α-acids are the most important compounds for the bitterness of beer (Diffor et al. 1973). During wort boiling, the α-acids present in the hops isomerize to more soluble iso-α-acids, which are the essential bitter components in the finished beer. Iso-α-acids are simultaneously partially degraded to degradation products, especially to humulinic acids (Keukeleire and Verzele 1971, Intelmann 2010).

The following factors play an important role in the utilization of bitter substances: the solubility of the bitter substances, the conversions of the bitter substances, the properties of the conversion products and the affinity of the bitter substances to substances occurring in the wort during the brewing process, especially proteins (Brohult et al. 1955).

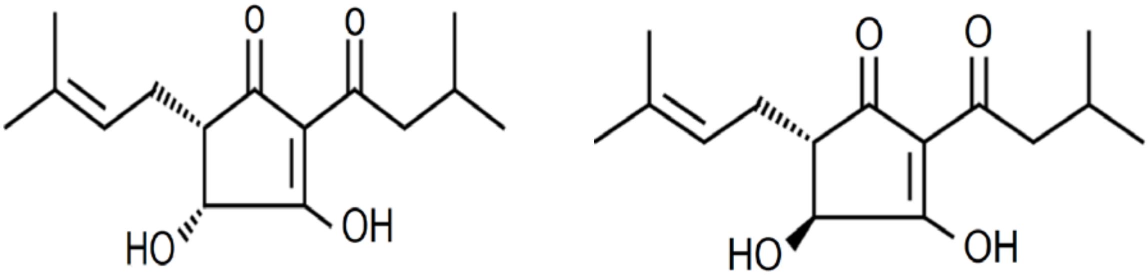


Scheme 2: Structural formula of cis-iso-α-acids and trans-iso-α-acids (Hertel 2007, Intelmann 2009)



iso-h. iso-co-h. iso-ad-h. iso-post-h. iso-pre-H.

Scheme 3: Structural formula of the radical R in the humulones (-h):



Scheme 4: Structural formula of cis-humulinic acids and trans-humulinic acids (Lewis and Young 2001):

In the past, numerous studies have been carried out on the utilization of α-acids under various boiling conditions. Up to now, the main focus has been on the utilization of bitter substances in beer in order to prescribe a corresponding hop dosage. A particular difficulty in the assessment of the bitter substance utilization lies in the fact that the formal kinetic description of the isomerization of α-acids has not been sufficiently clarified. In publications of various authors statements have been made in which the reaction kinetics of the isomerization of α-acids during thermal treatment is described as a 1^{st} order reaction (Jaskula et al. 2008). At the same time, some researchers have found that the assumption of a 1^{st} order reaction does not apply at the beginning of the reaction and that the loss of α-acids during wort boiling is higher than the loss of iso-α-acids (Mostek et al. 1978, Kappler et al. 2010).

The α-acids can give up a proton at C_3 by the application of heat and in this chiral center isomerizes to their cis- and trans-form, which provides bitterness. At the same time iso-α-acids hydrolysis to humulinic acids, loose the bitterness again. The knowledge of degradation of iso-α-acids is relatively limited. To simplify the calculation, back reaction will be here ignored.

$$\alpha - acids \overset{\overset{k_1}{\rightarrow}}{\leftarrow} iso - \alpha - acids \overset{k_2}{\rightarrow}\ humulinic\ acids$$

The results on the loss of beer bitterness during beer storage (Blanco et al., 2006; Caballero et al., 2012) could continue to be used for medical research on the degradation of α-acids and iso-α-acids in the human body (Hall et al., 2008).

1.4.3 Maillard products

Maillard products are not only responsible for a pleasant beer aroma, but also for the production of an off-flavour. The Maillard reactions are a complex network of parallel reactions and subsequent reactions by the reaction between reducing sugars and amino compounds. They are also responsible for the formation of colour and flavour in heated foods (Nursten 2005; Martins 2005; Brands et al. 2002; Maillard 1912). The control of the Maillard reactions is of particular technical and scientific interest. The Strecker aldehydes and the associated precursors are shown in Tab. 1.

Tab. 1 : Strecker aldehydes and their precursors
(Mezger 2005, Meilgaard et al. 1970)

precursors	flavour substances	boiling temperature (°C)	threshold value (mg/l)
leucine	3-MB	92-93	0.6
isoleucine	2-MB	91-93	1.2
phenylalanine	2-phenylethanol	219	1.6
methionine	methional	160-165	0.3
phenylglycine	benzaldehyde	179	1.5-3.0

2-MB and 3-MB are typical volatile beer aging components (Back 1996, Gernat 2020). Their boiling temperatures are 92 °C under atmospheric conditions. 2-MB and 3-MB are formed from the amino acid leucine and isoleucine respectively during Strecker degradation (Stephenson 1993). The aroma impression is described as green, pungent or bitter almond-like. The odour threshold in water is 3-4 ppb and in beer 0.6 mg/l for 3-MB and 1.2 mg/l for 2-MB (Meilgaard et al. 1970). One aim of this work was to model the aroma formation during wort boiling in order to investigate these two important aroma substances. The two important aroma substances are only moderately reactive and can form heterocyclic substances during condensation, thus impairing the taste properties of the beer.

Brands et al. (2002) published research results on aroma formation in the course of the Maillard reactions. They conclude that most kinetic studies have been carried out using model systems of amino acids and sugars and they have also confirmed the need for in-depth study of real foods. Although the chemistry of Strecker degradation is well known, the Strecker aldehyde's formation pathway is very complex.

2-MB 3-MB

Scheme 5: Chemical structure of 2-MB and 3-MB

In the present work, the kinetic mechanism under defined conditions of wort boiling was investigated. Quantitative control remains a major challenge for the future as many factors are involved, including reactant concentrations, time, temperature, pH, water activity, fat concentration, ionic strength, etc. One approach to quantitative control is the development of mathematical models on which the reaction kinetics are based, which can provide the determination of the corresponding speed-determining steps and a control of the processes by means of which the reactions can be controlled as required (Martins et al. 2001).

1.5 Kinetic principles

1.5.1 Isothermal conditions

The word kinetics comes from the Greek κινητική, i.e. to move and react (Martins 2005a). The reaction rate is a measure of the time sequence of a reaction. It is generally not constant, but depends on the temperature, any catalysts present, the concentration and number of reacting substances and the time itself (Kessler 1996). The reaction rate is equal to the change in concentration dC in the time dt:

$$\boldsymbol{\vartheta} = -\frac{\mathbf{dC}}{\mathbf{dt}} \tag{1}$$

dC: change of concentration
dt: time interval

The relationship between the velocity constant k and the temperature has been studied by several scientists in the past. The equations were first developed by Arrhenius and ten years later by Vant 'Hoff. A, B C, a and b are empirical constants. In Tab. 2

the used reaction rates are shown. The most commonly used equation is the Arrhenius equation.

Tab. 2 : Equations for the temperature dependence of the rate of reaction (Vyazokin 2000, Flynn 1977)

$d(lnk)/dT$	k=	Authors (year)
W/D	A_0T^B	Harecourt-Esson(1895)
A/T2	A_0exp(-A/T)	Arrhenius (1889)
(A+BT)/T2	A_0T^Bexp(-A/T)	Kooij(1893)
(A+CT2)/T2	A_0exp(CT)exp(-A/T)	Van't Hoff(1899)

The reaction order depends on the number of reacting substances and their concentrations. Reaction orders are divided as follows (Fitzer 1995; Westphal 1996; Kessler 1996):

The following relationship applies to a 0th-order reaction:

$$-\frac{\mathbf{dC}}{\mathbf{dt}} = \mathbf{k_{n=0}} \tag{2}$$

k: reaction rate constant

for a 1st order reaction:

$$-\frac{\mathbf{dC}}{\mathbf{dt}} = \mathbf{k_{n=1}} \cdot \mathbf{C_A} \tag{3}$$

C_A: concentration of substance A

for a 2nd order reaction:

$$-\frac{\mathbf{dC}}{\mathbf{dt}} = \mathbf{k_{n=2}} \cdot \mathbf{C_A} \cdot \mathbf{C_B} \tag{4}$$

C_B: concentration of substance B

The 0th order reaction is independent of the concentration of the reaction partners. Many chemical reactions can be described sufficiently precisely by this reaction. The 1st order reaction is used to describe decomposition processes in which one substance can give rise to one or more others. In the 2nd order reaction, the reaction rate is proportional to the product of two concentrations; this reaction occurs mainly in chemical reactions (Kessler 1996).

The reaction rates and the reaction rate constants k are dependent on the absolute temperature. The following relationship between k and the absolute temperature T results from the equations of Arrhenius. This connection is shown in eq. (5). Here the activation energy E_a can be determined from the slope of the straight line and the reaction rate constant A_0 from the ordinate section or by means of equations. Usually the application takes place $\ln k$ against $1/T$. This linear behaviour results from the following equation (Aktins and Paula 2006):

$$\ln k = \text{slope} \times \frac{1}{T} + \text{y axis intercept} \tag{5}$$

The gradient is $-E_a/R$ and the intersection point with the y-axis is equal to $\ln A$. This results in the Arrhenius equation:

$$\ln k = -\frac{E_a}{RT} + \ln A \tag{6}$$

One can also write this equation in the following form:

$$\mathbf{k = Ae^{-E_a/RT}} \tag{7}$$

A: pre-exponential factor or frequency factor

The importance of the activation energy is derived from the impact theory of the starting materials. It is defined as the minimum kinetic energy that the educts must have in order for a collision to lead to the formation of the products. Some reactions take place via the formation of an intermediate product, as in the successive reactions. The feedback reactions are not taken into account.

$$A \overset{k_1}{\rightleftarrows} B \overset{k_2}{\rightleftarrows} C$$

In order to recognize the properties of such subsequent reactions, the change in concentration of each substance must be determined from the reaction rate laws of the individual partial reactions during the course of the reaction. This results in the following initial conditions:

If t = 0, $[A] = [A]_0$
$[B] = 0$
$[C] = 0$
If t = t, $[A] + [B] + [C] = [A]_0$

This information can be used to create and solve the rate equations for the process to determine the concentrations of [A], [B], and [C] as a function of time. The speed of the unimolecular decay of A is:

$$\frac{d[A]}{dt} = -k_1[A] \tag{8}$$

There is no process by which A is formed, so the concentration must always decrease. The intermediate product B is formed from A (with a reaction rate of $k_1[A]$) and then decomposes to C (with the reaction rate $k_2[B]$). The net rate of formation is therefore:

$$\frac{d[B]}{dt} = k_1[A] - k_2[B] \tag{9}$$

The product C is finally produced by a unimolecular further reaction of B:

$$\frac{d[C]}{dt} = k_2[B] \tag{10}$$

The first law of speed, eq. (11), is simple first-order decay, and therefore applies to concentration:

$$[A] = [A]_0 e^{-k_1 t} \tag{11}$$

If this is inserted in eq. (9), the expression after transformation is obtained for [B]:

$$\frac{d[B]}{dt} = k_1[A]_0 e^{-k_1 t} - k_2[B] \tag{12}$$

This differential equation has a standard form. When $[B]_0$ set to 0, the following solution results:

$$[B] = \frac{k_1}{k_2 - k_1} \cdot \left(e^{-k_1 t} - e^{-k_2 t}\right) \cdot [A]_0 \tag{13}$$

At all times, [A] + [B] + [C] = $[A]_0$ so for [C]:

$$[C] = [A]_0 \cdot \left[1 - \frac{1}{k_2 - k_1} \cdot \left(k_2 e^{-k_1 t} - k_1 e^{-k_2 t}\right)\right] \tag{14}$$

After the start of the reaction, the concentration of the intermediate product B first rises to a maximum and then drops back to zero. The concentration of product C rises from zero to close to $[A]_0$ on.

When approximating quasi-stationary states, also known as the quasi-stationarity principle, we assume that the concentration of intermediate products initially increases from zero in an induction period, but hardly changes in the further course of the reaction.

$$\frac{d[B]}{dt} \approx 0 \tag{15}$$

This approximation considerably simplifies the treatment of multi-stage reactions. This approximation can be applied, for example, to the first-order follow-up reactions described above, by entering the following values in eq. (9) $d[B]/dt = 0$ is set:

$$k_1[A] - k_2[B] = 0 \tag{16}$$

and from it

$$[\mathbf{B}] \approx \left({}^{\mathbf{k_1}}/_{\mathbf{k_2}}\right)[\mathbf{A}] \tag{17}$$

For this relationship to be consistent with eq. (17), k1/k2 ≤ 1, this means that the time dependence of [B] can be neglected, even if [A] depends on time. This expression for [B] used in eq. (10) gives:

$$\frac{\mathbf{d[C]}}{\mathbf{dt}} = \mathbf{k_2}[\mathbf{B}] \approx \mathbf{k_1}[\mathbf{A}] \tag{18}$$

Apparently, C is now formed directly from A by a 1st -order reaction with a reaction rate constant of k_1 the speed constant of the slower (speed-determining) step. The solution of this equation results after inserting [A] from eq. (11):

$$[\mathbf{C}] = \mathbf{k_1}[\mathbf{A}]_\mathbf{0} \int_\mathbf{0}^\mathbf{t} \mathbf{e^{-k_1t}\,dt} = (\mathbf{1} - \mathbf{e^{-k_1t}})[\mathbf{A}]_\mathbf{0} \tag{19}$$

This (approximated) result corresponds to eq. (19), but can be won faster than before. In Fig. 5 the approximate solution is compared with the previously obtained exact solution. In order to for the approximation to be reasonable, k_2 does not now have to be much larger than k_1.

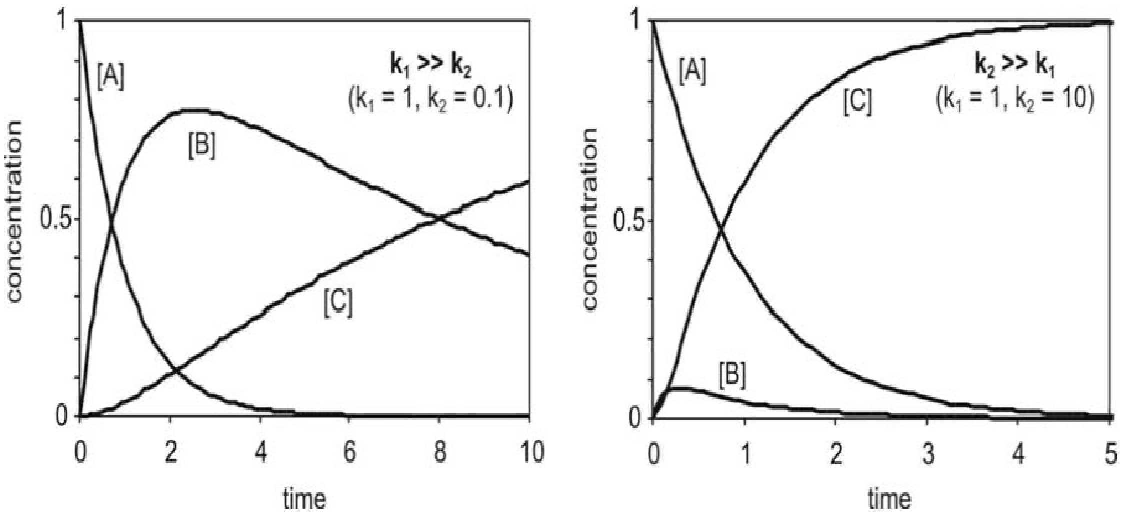


Fig. 5: Change in concentration of A, B and C (Atkins and Paula 2006)

The chemical reactions are classified into two types from the kinetic point of view. Homogeneous reactions take place only in one phase, it can be a gaseous or a liquid phase. Heterogeneous reactions take place in two or more phases, for example in gaseous form on the surface of a solid catalyst or on the walls of a container. Lewis' (1905) pioneering work was based on the kinetics of autocatalytic thermal decomposition of silver oxide. Since the concentration C could not be used to characterize the state of conversion from the solid, α was replaced with the extent of conversion (Vyazovkin 2000):

$$\frac{\mathbf{da}}{\mathbf{dt}} = \mathbf{ka}(\mathbf{1} - a) \qquad (20)$$

The concept of the single-stage reaction was investigated experimentally (Helfferich 2004). The kinetics of a multi-stage reaction is described in full by a series of simultaneous rate equations r_i and one for each participant (educt, intermediate, product, catalyst, dormant partner). The mathematical description is simple, but cumbersome, because the masses involved are large when the reaction has more than a few steps.

1.5.2 Non-isothermal conditions

The selection of the appropriate kinetic function has a great influence on the parameters E_a and A. Therefore, scientists have long searched for kinetic functions that correspond as closely as possible to the actual course of the reaction. Early kinetic research was carried out under isothermal conditions. Until the beginning of the 20th century kinetic investigations were calculated using non-isothermal methods. Since heating processes are often carried out at constant heating rates, i.e. $\beta = dT/dt$, the non-isothermal kinetic equation becomes the form shown below after conversion (α: reaction percentage). The non-isothermal method commonly used in thermal analysis is much more complicated than the isothermal method. According to the form of the equations they are divided into the differential method and the integral method. The integral method cannot offer an exact solution due to the complicated temperature integration p(u). Therefore, many approximation functions were developed in the 1950s to 1970s (e.g. Freeman-Carroll equation 1958; Coats-Redfern 1964; Zsaco equation 1975). The differential method does not include the difficult temperature integration, but requires an accurate dα/dT or dα/dt (Rong et al. 2001; Hu et al. 2001; Hammam et al. 2017).

Tab. 3 : Comparison of kinetic equations

	Isotherm		Non-Isotherm
Differential	$\frac{dc}{dt} = k(T)f(c)$	$c \rightarrow \alpha$; $\beta = dT/dt$	$d\alpha/\,dT = (\frac{1}{\beta})\ k(T)f(\alpha)$
Differential	$d\alpha/dT = Aexp(-\frac{E}{RT})\, f(\alpha)$	$k = A\exp(-RT/E)$	$d\alpha/dT = (\frac{A}{\beta})\ \exp(-\frac{E}{RT})\, f(\alpha)$

Integral	$G(\alpha) = \int_0^t A\exp\left(-\frac{E}{RT}\right)dt = kt$	$G(\alpha) = \int_{T_0}^T \left(\frac{A}{\beta}\right)\exp\left(-\frac{E}{RT}\right)dT$
Temperature integral		$u = \frac{E}{RT}$ $P(u) = \int_\infty^u -\left(\frac{e^{-u}}{u^2}\right)du$

There are different approaches to integrate the temperature curve (Rong, et al. 2001; Órfão 2007) or by applying the methods of Frank-Kameneskii, Doyle, Gorbachev and Zsako. In the following only the Coats-Redfern method is presented eq. (21-28).

$$\mathbf{P(u) = \int_\infty^u -\left(\frac{e^{-u}}{u^2}\right)du} \quad (21)$$

$$\mathbf{P(u) = \int_\infty^u \frac{1}{u^2}de^u} \quad (22)$$

$$\mathbf{P(u) = \frac{e^{-u}}{u^2} - \int_\infty^u e^u(-2)u^3du} \quad (23)$$

$$\mathbf{P(u) = \frac{e^{-u}}{u^2} - \int_\infty^u 2u^{-3}de^{-u}} \quad (24)$$

$$\mathbf{P(u) = \frac{e^{-u}}{u^2} - \frac{2}{u^3}e^{-u} + \int_\infty^u 6u^{-4}de^{-u}} \quad (25)$$

$$\mathbf{P(u) = \frac{e^{-u}}{u^2} - \frac{2}{u^3}e^{-u} + \frac{6}{u^4}e^{-u} - \int_\infty^u 24u^{-5}de^{-u}} \quad (26)$$

$$\mathbf{P(u) = \frac{e^{-u}}{u^2}(1 - \frac{2!}{u} + \frac{3!}{u^2} - \frac{4!}{u^3} + \cdots)} \quad (27)$$

$$u = \frac{E}{RT} \qquad T = E/Ru \qquad dT = -\frac{E}{Ru^2}du$$

$$\mathbf{\int_0^T e^{E/RT}dT = \frac{E}{R}\frac{e^{-u}}{u^2}(\frac{1}{u^0} - \frac{2!}{u} + \frac{3!}{u^2} - \frac{4!}{u^3} + \cdots)} \quad (28)$$

In the Coats-Redfern approach function only the first two terms $(1 - \frac{2!}{u})$ considers eq. (29-32).

$$\mathbf{P(u) = \frac{e^{-u}}{u^2}(1 - \frac{2!}{u} + \frac{3!}{u^2} - \frac{4!}{u^3} + \cdots)} \quad (29)$$

$$\mathbf{P_{CR}(u) = \frac{e^{-u}}{u^2}(1 - \frac{2}{u})} \quad (30)$$

$$\boldsymbol{\int_0^T e^{E/RT}dT = \frac{E}{R}\frac{e^{-u}}{u^2}(1 - \frac{2}{u})} \quad (31)$$

$$\boldsymbol{\int_0^T e^{E/RT}dT = \frac{RT^2}{E}(1 - \frac{2RT}{uE})e^{E/RT}} \quad (32)$$

In the present work a differential method (Kissinger) and two integral methods (Coats-Redfern, Ozawa-Flynn-Wall) were used to investigate the kinetics of degradation from SMM to DMS. The formulae can be found in the results section under 2.4.

2 Results

2.1 Summary of results (peer-reviewed publications)

The four publications are summarized in this chapter. The copies of the individual publications are inserted. Figures, which have been not published in the Journals, are added here.

Part I	Kinetic studies of main wort flavour compounds and iso-α-acids during wort boiling: a review

Wort boiling is a central process step and of particular importance. During wort boiling a multitude of physical, chemical and biological reactions take place. In this thesis we concentrate on the existing kinetic modelling and kinetic parameters of the individual reaction functions of wort boiling. The kinetic methods used so far will be summarized and discussed in a review. DMS, Maillard products (such as 2- and 3-MB, methional, phenylacetaldhyde, TBA and NEB), iso-α-acids were selected as lead substances. These substances are further investigated in this thesis under the conditions of wort boiling.

Part II	A kinetic study on the formation of 2- and 3-MB

Using formal kinetics and the *Athena Visual Studio* software, speed constants, activation energies and pre-exponential factors were calculated. Temperature and time are the most important influencing factors. The sugar (glucose and maltose) and amino acid concentrations (leucine and isoleucine) were analyzed by HPLC and the changes in concentrations were statistically checked and compared. The result shows that the decreases of the two sugars and amino acids do not differ statistically significantly within a 95 % confidence interval. Due to the high concentrations of sugar, the reactions of maltose and glucose have no quantitative influence on the formation of 2- and 3-MB. At the same concentrations of amino acids and sugars, 3-MB forms a higher concentration than 2-MB. This means that the isomers of leucine and isoleucine have a marked influence on the formation of 2- and 3-MB. The order of reaction rates in this thesis are GL>ML>GI>MI.

2- and 3-MB have a relatively high concentration in the wort and a comparatively low taste threshold in the beer. As Fig. 6-9 show, the formation of 2- and 3-MB is strongly dependent on the boiling temperature. At 100 °C less than 1 % isoleucine/leucine was converted to 2- and 3-MB after 60 min boiling time. Compared to the importance of boiling temperature and boiling time, the concentration of the starting substances isoleucine/leucine on the formation of 2- and 3-MB can only be influenced to a limited extent. At a boiling temperature of 110 °C, the concentration of 2- and 3-MB increases significantly.

Fig. 6 and 7 show 2-MB in mole percent of initial concentration of isoleucine with glucose or maltose. Fig. 8 and 9 show 3-MB in percent of initial concentration of leucine with glucose or maltose, logarithmic application of boiling time over temperature.

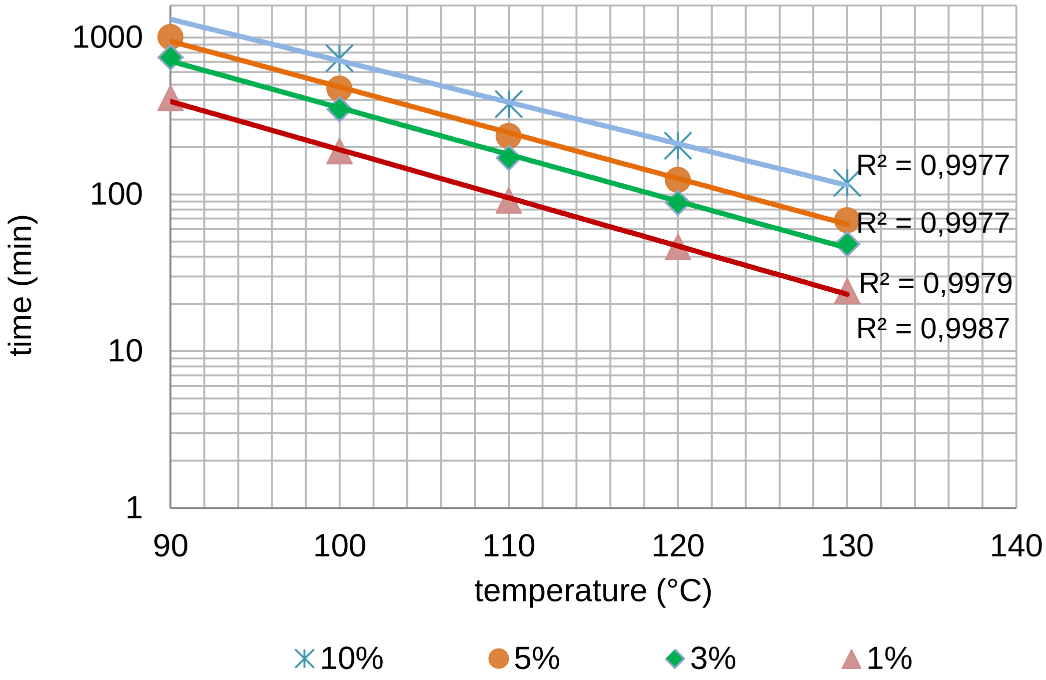


Fig. 6: 2-MB in mole percent of initial concentration of isoleucine (glucose and isoleucine), logarithmic application of boiling time over temperature

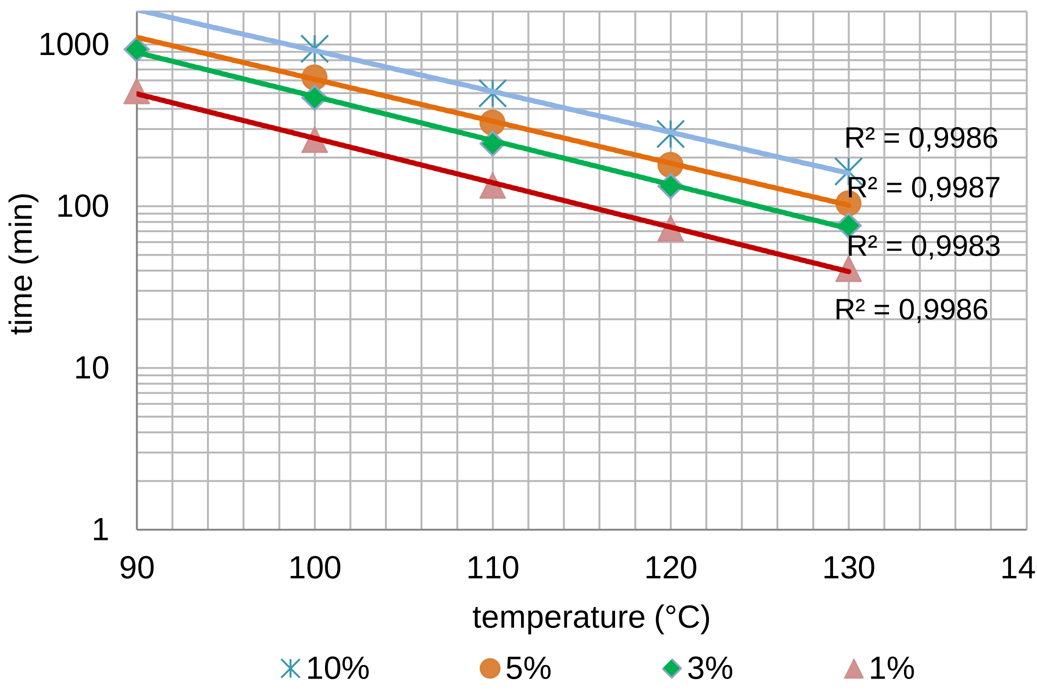


Fig. 7: 2-MB in mole percent of initial concentration of isoleucine (maltose and isoleucine), logarithmic application of boiling time over temperature

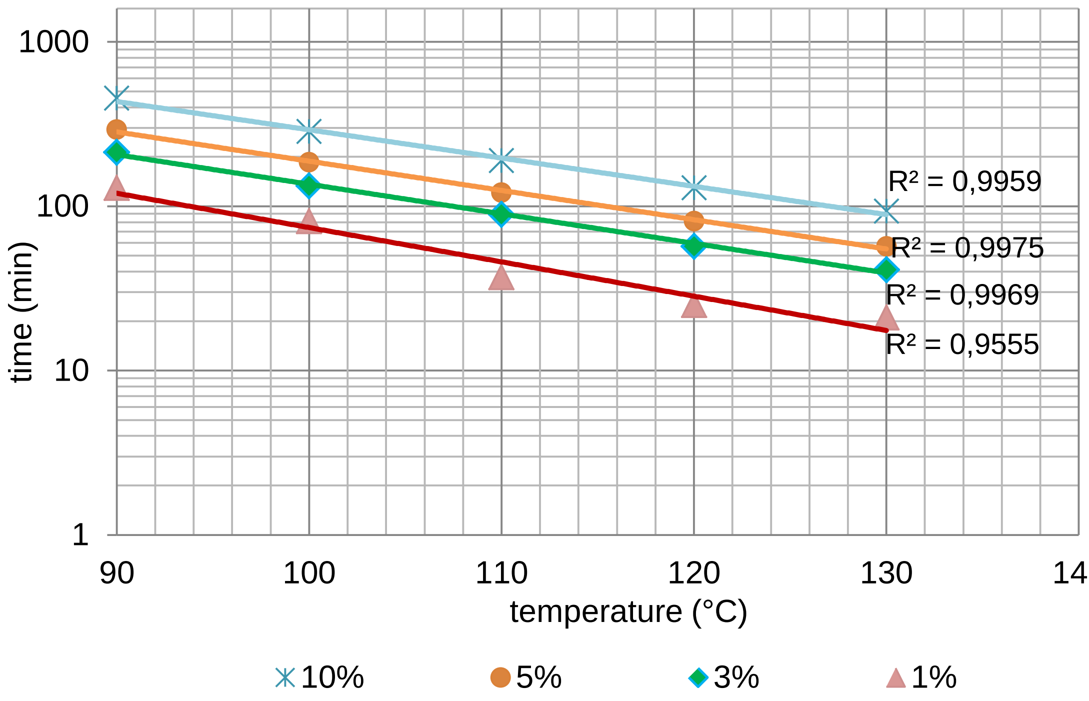


Fig. 8: 3-MB in mole percent of initial concentration of isoleucine (glucose and leucine), logarithmic application of boiling time over temperature

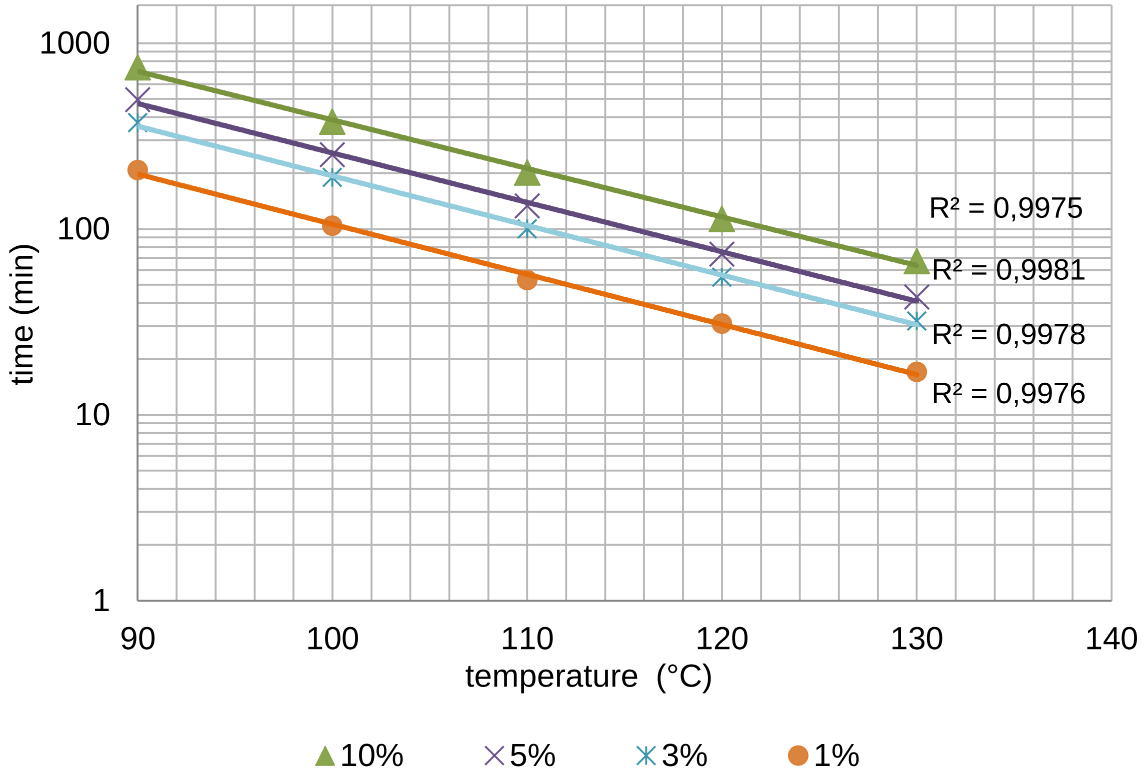


Fig. 9: 3-MB in mole percent of initial concentration of isoleucine (glucose and leucine), logarithmic application of boiling time over temperature

Part III	Non-isothermal kinetic models of degradation of SMM

The DMS formation process was examined here for the first time under non-isothermal conditions. Three approximation methods were selected for the calculation: Coat-Redfern, Ozawa and Kissinger. All three methods led to similar results. Under non-isothermal conditions, an activation energy of about 82 kJ/mol is calculated, which is thus lower than the values from investigations under isothermal conditions. With the aid of the calculated kinetic parameters, it was determined which time periods are required for heating up and boiling at different heating rates and boiling temperatures in order to achieve an optimum DMS concentration. The investigations and calculations also led to the conclusion that the calculation formula for the SMM concentration according to MEBAK had to be corrected by a factor of 2.64. The following results have been corrected.

Part IV	Kinetic modeling of hop acids during wort boiling

The isomerization of α-acids is one of the most important tasks of wort boiling. The results of this work can be used to calculate the time required to achieve the maximum concentration of iso-α-acids in the brewhouse. The chemical kinetics of the isomerization of α-acids and the degradation of iso-α-acids to humulinic acids under different pH values and temperatures can be described as a 1st order reaction. The reaction rates of the degradation of total isohumulones, co-isohumulones and N+Ad isohumulones are almost identical. At 100 °C and pH 5.2, the maximum concentration of iso-α-acids is only available after a theoretical boiling time of 140 min. In order to shorten the boiling time, pre-isomerized hop products could be added on the one hand and, on the other hand, if an appropriate dosing device is present, the first hop could also be added in an underback vessel. The aroma-intensive hop oils could also be partially dissolved outside the brewhouse by adding hops in downstream fermentation or storage vessels. In addition, pH values also play an important role in the isomerization and degradation of iso-α-acids. At higher pH-values not only the isomerization of the α-acids, but also the degradation of the iso-α-acids is favored. The maximum concentration of iso-α-acids cannot be reached during a 60 min boiling period. If the pH value is optimally adjusted, the concentration of iso-α-acids in the wort may increase. Fig. 9 and 10 (see capital 2.5) show the correlation between the time until the maximum concentration of the iso-α-acids is reached and the boiling temperatures of the wort.

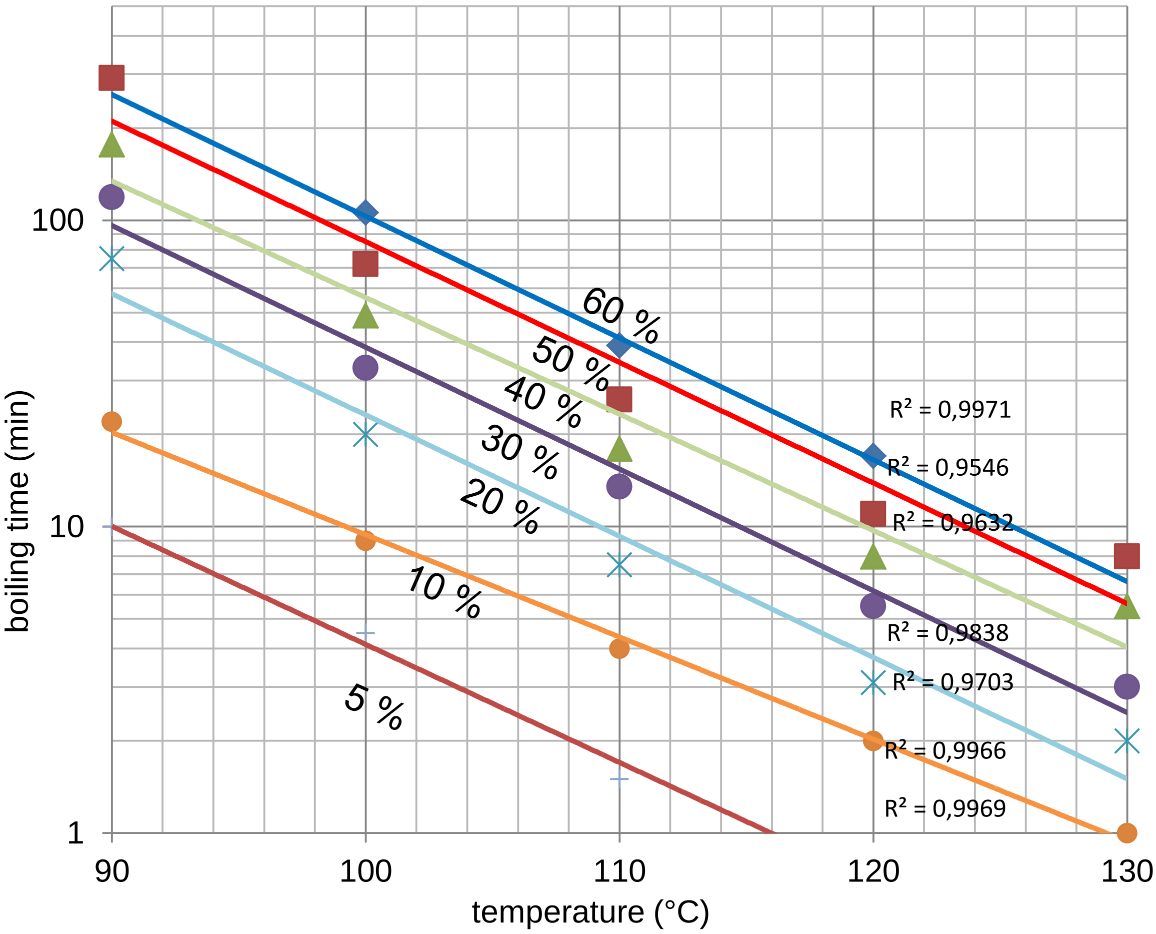


Fig. 10: Logarithmic application of the degrees of isomerization of α-acids in percent at pH 5.2 above the boiling time with consideration of the degradation of iso-α-acids

Fig. 10 shows that the boiling time can be considerably reduced by increasing the temperature achieving the same degree of isomerization. At 130 °C, 60 % isomerization of is achieved within a few min. Pre-isomerization in an underback vessel can further improve the degree of isomerization.

2.2 Kinetic studies of main wort flavour compounds and iso-α-acids during wort boiling: a review

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REVIEW ARTICLE

Kinetic studies of main wort flavor compounds and iso-α-acids during wort boiling: a review

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Abstract During wort boiling, the biochemical and physical reactions in wort give beer its characteristics flavors, color, and bitterness. These reactions are affected by many process factors, such as temperature, boiling time, and concentrations of substances in raw materials. The concentrations of the wort flavor compounds influence not only the quality of wort and finished beer, but also the shelf life. Therefore, it is necessary to control the process parameters as well as the main wort flavor components in wort and beer. Kinetic knowledge provides a basis for the simulation of the boiling process and prediction of the formation and evaporation rate of main wort flavor components. Using the kinetic modelling can achieve a decrease of the boiling time, save energy and produce good beer quality. By comparison with the advanced kinetic studies in chemistry or other food sciences, in brewing science the kinetic investigation remains in its initial stage. This paper summarizes the existing kinetic modelling and kinetic parameters of evaporation and formation of main wort flavor components (e.g., 2-methylbutanal, 3-methylbutanal, DMS, hexanal, 2-phenylethanol, 2-phenylethanal) and the formation and degradation of iso-α-acids.

Keywords Kinetic modelling · Wort boiling · Strecker aldehydes · Dimethyl sulfide (DMS) · α-Acid · Coagulable nitrogen

Introduction

Wort boiling is not only at the midpoint of the brewhouse, but also is one of the most important steps in producing an excellent beer [1–5]. To assure the product's quality, decrease energy costs and optimize the occupation time of the facilities and thereby the commercial efficiency, it is necessary to optimize the boiling process as much as possible [6, 7].

Due to the different purposes and numerous parameters, the kinetic study in brewing science faces a multifactorial problem [8, 9]. On the one hand, substances are formed by thermal processes (e.g., dimethyl sulfide, Maillard reaction products, isohumulones) [10, 11], and on the other hand, existing or formed substances are evaporated (e.g., dimethyl sulfide, Strecker aldehydes) [2, 11–14]. Some of these substances are desired but others should be almost completely evaporated. Finally, at the end of the boiling process, the content of certain substances should be within defined ranges [13, 15, 16]. Whether the values of the main wort flavour components is within the defined range, belongs to one important criterion for brewing apparatus manufacturing. Until today, basic knowledge and understanding of the wort boiling process is available in the related literature [1, 17–19]. However, this complex process and the direct relation between what is known and the independent process parameters are not yet consistently described [20, 21]. None of the previous studies determine the dynamical performance of important process markers, which are consisting of generation, conversion and evaporation.

Obviously, there is still a lack of a simple, intuitive and effective on demand method to help brewers to produce the same good beer and at the same time save boiling time as well as thermal energy. A general description of kinetic studies of the main wort flavour components and

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iso-α-acids during boiling connected to the used process markers is urgently needed.

Kinetic modelling

In general, simple reaction orders like zero and first order have been used to describe changes in most reactions during the wort boiling [11, 19, 22–24]. The kinetics of chemical reactions and biochemical or biological processes has been primarily studied in systems in which the reactant and product concentrations decrease or increase monotonically [3, 4, 11].

The kinetic models of the formation of dimethyl sulfide (DMS), thiobarbituric acid (TBA), non-enzymatic browning (NEB), furfural, isomerization of α-acids, and precipitation of coagulable nitrogen (coag. N) were determined using knowledge about the single step reaction $A \rightarrow B$. For a single reactant the decomposition of the reactant A can be written in a differential equation (Eq. 1) [25, 26].

$$-\frac{dC_A}{dt} = kC_A^n \tag{1}$$

where t is the time, C_A is the concentration of reactant A, k is the reaction rate constant and n is the number of molecules involved in the reaction, otherwise known as, the reaction order. The unite for k for a reaction with order n is $(\mathrm{dm}^3/\mathrm{mol})^{\mathrm{n}-1}/\mathrm{s}$.

Using multiresponse kinetic modelling, the kinetic modelling of Maillard reaction can be established [27].

$$\mathrm{A} + \mathrm{S} \overset{\mathrm{k}_1}{\rightarrow} [\mathrm{AS}]_{\mathrm{sb}} \overset{\mathrm{k}_2}{\rightarrow} \mathrm{AR} \rightarrow \begin{cases} \overset{\mathrm{k}_2}{\rightarrow} Str \\ \overset{\mathrm{k}_2}{\rightarrow} Mel \\ \overset{\mathrm{k}_2}{\rightarrow} others \end{cases}$$

Wort flavors are volatile organic chemicals. During the wort boiling, an amount of flavors have been developed, unfolded and evaporated. The evaporation of wort flavor is a thermodynamic separation process. Relative volatility (α) of compounds (i and j) in different wort boiling systems is an important factor pertaining to flavor retention in the wort and in the finished beer (Eq. **2**) [28]

$$\alpha = \frac{(y_i/x_i)}{(y_j/x_j)} = {}^{K_i}\!/_{K_j} \tag{2}$$

where K vapor–liquid distribution ratio of compounds, y the vapor–liquid equilibrium concentration of component in the vapor phase, and x the vapor–liquid equilibrium concentration of component in the liquid phase is. If the wort flavor is relative volatile, it has a bigger K value. The evaporation of this flavor in an atmosphere boiler system can be calculated based on Rayleigh function.

$$\frac{x_1}{x_0} = \frac{L_1^{(K-1)}}{L_0} \tag{3}$$

It is important to distinguish between an atmosphere boiler and a closed boiler. In a closed boiler system the wort flavor evaporate slower than in an atmosphere boiler. In a closed boiler, all the flavors vapor is in relation to the volatility of the entire liquid (Eq. 4). In an atmosphere boiler system, the flavors vapor takes place with respect to the just vaporized molecules. Depending on the total evaporation, the flavors vapor can deviate from each other [16, 29].

$$\frac{x_1}{x_0} = \frac{L_0}{L_1 + K \cdot (L_0 - L_1)} \tag{4}$$

It is always a big challenge to control the wort flavors, which is significant influenced on raw materials and many process parameters.

Hertel [16, 23, 30–32] measured activity coefficients of flavors in his study at infinite dilution. The limiting separation factor (K^{∞}) is directly obtained as a ratio of absorbance of the equilibrium vapor- and liquid-phase samples (Eq. 4). Limiting activity coefficient γ^{∞} were calculated from the measured K^{∞} values (Eq. 5). The K^{∞} and γ^{∞} of hexanal, 2-methylbutanal, 3-methylbutanal, DMS, 2-phenylethanol, 2-phenylethanal are summarized in Table 1.

$$\mathbf{K}^{\infty} = \lim_{\mathbf{x_x} \to 0} \left(\frac{\mathbf{y_x}}{\mathbf{x_x}} \right) \tag{5}$$

where y/x is the ratio of the vapor and the liquid equilibrium mole fractions; K^{∞} the limiting separation.

$$\gamma^{\infty} = \frac{P}{P^0} \frac{y_x}{x_x} \tag{6}$$

Table 1 Pure components vapor pressures p^0, limiting separation factors K^{∞}, and limiting activity coefficients γ^{∞} of hexanal, 2-methylbutanal, 3-methylbutanal, dimethylsulfide, 2-phenylethanol, 2-phenylethanal

Selected flavors	T/°C	p^s /kPa	K^{∞}	γ^{∞}	Source
Hexanal	98.55	40.40^a	48.3 ± 3.9	121.1 ± 9.7	[33]
2-Methylbutanal	98.55	128.4^b	57.7 ± 6.6	45.4 ± 5.2	[33]
3-Methylbutanal	98.55	121.1^c	55.5 ± 4.8	46.4 ± 4.0	[33]
DMS	98.55	572.4^a	75.6 ± 5.1	13.4 ± 0.9	[33]
2-Phenylethanol	100	1385.7^a	2.28 ± 0.05	167 ± 4	[34]
2-Phenylethanal	100	2300^c	5.32 ± 0.13	234 ± 6	[34]
2-Furfural	100	13.51^a	7.54 ± 0.2	56.5 ± 1.5	[35]
γ-Nonalactone	100	55.8^b	3.10 ± 0.06	5.6 ± 0.1	[35]
Benzaldehyde	100	8.38^a	20.76 ± 0.96	251 ± 12	[35]
Linalool	100	3.49^c	35.46 ± 1.28	1029 ± 37	[35]

[a] Calculated with component plus (Pure components properties server; Editor: ProSim)

[b] Estimated with the method of Marrero and Gani

[c] Calculated with the Antoine equation at the Dortmund data bank

where γ^∞ limiting activity coefficient, P 1013 bar and P^0 vapor pressure of pure component at the same temperature.

Feilner's major research focus is wort stripping after the whirlpool process to reduce the amount of undesired volatile substances. He investigated the evaporation behavior of single undesired wort flavor also at infinite dilution. The limiting factors of the leading substances in wort at 100 and 90–95 °C are listed in Table 2. Except for phenylethanal, the rest results are similar to Hertel's results [36–41].

The evaporation of main wort flavor components (e.g., 2-methylbutanal, 3-methylbutanal, DMS, 2-phenylethanol, 2-phenylethanal), have been investigated as pure substances in water or sugar solutions. The knowledge of the formation of the main wort flavor in wort is still limited.

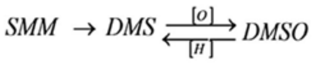
Studies on formation of flavor compounds

The wort flavors usually are divided into three main groups, which are (1) Strecker aldehyde, (2) indicator of thermal stress, and (3) indicators of evaporation. The Strecker aldehyde, which are formed in the Maillard reaction, such as 2- and 3-methylbutanal, and 2-phenylethanal, are indicators of thermal stress during whirlpool dwell. Due to the low evaporation rate, furfural is considered as an indicator of thermal stress during wort boiling and whirlpool dwell. Aldehyde such as pentanal, hexanal, heptanal and alcohols such as 2-, 3-methylbutanol, 1-pentanol, phenylethanol are indicators of evaporation during wort boiling [42]. Up to now, the brewing scientists have investigated just the kinetics of formation of DMS, furfural and non-enzymatic browning (NEB). Because DMS has the odor of fresh corn and is not compatible with beer flavor, to achieve the DMS concentration in beer under taste threshold, a free DMS concentration of <100 µg/l by middle of the cooling wort is recommended in the German standard DIN 8777 [15, 43]. The kinetic studies on formation of residual off-flavors, for instance, Strecker aldehydes, are described general as kinetic of formation of TBA, NEB, and furfural. About the kinetic studies on formation of single Strecker aldehydes in wort, we focused on the results from other experiment conditions in other food science area.

Dimethyl sulfide (DMS)

DMS is a double-carbon sulfur containing small molecule that is formed by cleavage of the side chain of S-methylmethionine. DMS has the odor of fresh corn and is not compatible with beer flavor [44]. For many years, efforts have been made to develop a useful, realistic simulation kinetic model to control the concentration of DMS during the wort boiling (Fig. 1). Despite the many studies of the kinetic mechanism of DMS formation, and of DMS evaporation, the excess of DMS in finished beer is still a common problem in breweries. The reaction kinetics of the DMS concentration decrease or increase in malt, wort and beer was first examined in the 1970s [45]. Since then, many scientists repeated this experiment with different wort boilers. The half-life method was used to determine the rate of degradation of S-methylmethionine. The half-life of S-methylmethionine is independent of the initial concentration of S-methylmethionine, because this reaction follows first-order kinetics. However, the results are different from each other. This may be due to the inhomogeneous temperature distribution in the wort boiler and different surface temperature. The pH of wort plays also an important role in this reaction. Dickson found, the time of the half-life degradation of S-methylmethionine at pH 5.2 is about 28.0 min. At pH 5.5 the half-life time increases to 32.5 min. The half-life time doubles with every 6 K temperature reduction [45]. Zücher [46] found that the S-methylmethionine has a half-life of approximately 59.5 min at 100 °C. Rübsam [47] confirmed the result and calculated the first-order equation $(A_t = A_0 e^{-[\ln(2)/5.9\times10^6 e^{-0.11T}]t})$.

Although more than 200 volatile flavors have been identified in beer, up to now in brewing science just only one flavor—(DMS)—has been investigated on its formation and evaporation rate during wort boiling [49–51]. Evaporation



$$SMM \rightarrow DMS \underset{[H]}{\overset{[O]}{\rightleftarrows}} DMSO$$

Fig. 1 Thermal decomposition of SMM to DMS. Due to oxidation, DMS can further convert to DMSO [48]

Table 2 Relative volatility of selected flavors at 100 °C and 90–95 °C [41]

Selected flavors	Molecular weight	Limiting separation factors K^∞ at 100 °C	Limiting separation factors K^∞at 90–95 °C
3-Methylbutanal	86.13	62.43 ± 1.26	65.55 ± 11.1
2-Methylbutanal	86.13	64.69 ± 2.21	69.47 ± 10.9
Furfural	96.09	7.72 ± 0.34	9.43 ± 2.65
Benzaldehyde	106.12	21.58 ± 1.98	27.35 ± 1.98
Phenylethanal	154.24	13.67 ± 0.54	16.64 ± 0.52

and formation of DMS during wort boiling take place mostly parallel, but occur in variable degrees. Scheuren combined the kinetic knowledge from evaporation and formation of DMS and described the change of DMS with kinetic model in direct kettle boiler (Eq. 6) and external boiler (Eq. 6–9). The modelling based on the assumption that evaporation and formation of DMS represent equilibrium processes in wort. Mass transport does not take place between gas and liquid. The pressure and temperature in the gas and liquid phases are identical for a closed boiler. The concentration of gas and liquid of DMS are also in equilibrium with each other [29, 51].

$$\frac{dx}{dt} = -\frac{\dot{D}}{L_0}\left(K \cdot x - x - c_0\right) + k \cdot c_0 \cdot e^{-kt} \tag{7}$$

$$\frac{dx}{dt} = -\frac{\dot{D}}{L_0} \cdot \left(\omega \cdot K \cdot x - x - c_0\right) + n(t) \tag{8}$$

$$\omega = \frac{\dot{L}_0}{\dot{L}_0 - \dot{D} + \alpha^\infty \cdot \dot{D}} \tag{9}$$

$$\begin{aligned} n(t) = \frac{c_0}{u} \cdot \ln \left(\frac{F}{L} \cdot \frac{\dot{L}}{\dot{L} - \dot{D}} \cdot e^{-k_A \cdot u} + \frac{L - F}{L} \cdot e^{-k_A \cdot u} \right) \\ \cdot \left(\frac{F}{L} \cdot \frac{\dot{L}}{\dot{L} - \dot{D}} \cdot e^{-k_A \cdot u} + \frac{L - F}{L} \cdot e^{-k_A \cdot u} \right)^{t/u} \end{aligned} \tag{10}$$

where D is the evaporation rate, L_0 time-dependent total evaporation, K temperature-dependent volatility, c(t) after formation of the disulfide, ω correction factor, k formation reaction rate of DMS, α^∞ relative volatility, u specific internal energy [51, 52].

Felgentraeger [19] developed the models for the kinetic of thermal decomposition of S-methylmethionine to DMS as a function of pH, and temperature (Eq. 10): The constants of the exponential function for other indicators are summarized in Table 3.

$$\begin{aligned} C_{\mathrm{SMM}} = C_{0(\mathrm{SMM})} \cdot \exp(-1.358 \cdot 10^{-8} \cdot t \cdot \exp(0.115 \cdot T) \\ \cdot \exp(0.478 \cdot pH)) \end{aligned} \tag{11}$$

Maillard reactions

Maillard reactions are involved in flavor, color and nutritional value [54, 55]. The Strecker degradation and the formation of heterocyclic compounds as is known have high to be of much significance in the development of wort flavors [56]. The concentration of corresponding proteins, pH values, and evaporation of Strecker aldehydes can strongly influence the concentration of Strecker aldehydes in finished beer. The intermediates lead to the development of off-flavors during shelf life [57, 58].

On the one side, the Maillard reaction is responsible for formation of the wort flavors, on the other side the accumulation of Maillard intermediated cause thermal processing of wort, which might lead to the development of off-flavors during shelf time [59, 60]. Narziss found that the beer contained low concentration of beer aging compounds, if it was brewed by relative low mash-pH, high mash temperature (>62 °C) and low concentration of oxygen during the brewing process [61]. Although the kinetic behaviors of Maillard reactions are very complicated, some scientists used relatively simple kinetic models [23, 62]. In brewing science, the kinetic behavior of TBA, and NEB are often used to present thermal load during wort boiling. In this paper, the results of the formation of Strecker aldehydes studies were summarized from studies with other foods in last 30 years, which match the same Strecker aldehydes as wort. However, in brewing science there is just one off-flavor, 5-hydroxy-methyl-furfural (HMF), has been investigated with reference to its formation kinetic.

Thiobarbituric acid (TBA)

Grigsby et al. [63] first used the thiobarbituric acid method (or TBN/TBI) to present intermediates of Maillard reactions. Schwill-Miedaner [64] examined a pseudo 0th order with an activation energy of 107 kJ/mol and $k = 0.19 \pm 0.021$ to describe thermal load in wort during the wort boiling. The TBA value increases linearly with time

Table 3 Kinetic parameters obtained from different authors

Authors	Zürcher [53]	Rübsam [47]	Scheuren [51]	Hertel [23]	Schwill-Miedaner[22]	Felgentraeger [19]
Ea (kJ/mol)	121.779	115.015	182 ± 12	–	–	–
A (min^{-1})	2.88E+15	3.09E+14	(4 ± 0.3)E+24	–	–	–
R^2	0.9937	0.9900	–	0.9930	0.9795	–
k (100 °C)	0.0258	0.02450	0.0367	0.0231 ± 0.0022	0.0165 ± 0.0022	0.115
Arrhenius-function	$k = 2.88 \cdot 10^{15} \cdot e^{-1012470,6/T}$	$k = 3.09 \cdot 10^{14} \cdot e^{-956,234}/T$	$k = (4 \pm 0.3) \cdot 10^{24} \cdot e^{-1,513,148}/T$	–	–	–

(dTBA/*dt* = *k*). De Schutter and coworkers [59] have investigated the kinetic behavior with laboratory wort and two industrial worts (1 and 2). The *k* value of laboratory wort can be written as:

$$k = 3.9E8 \cdot x \cdot e^{\left(-\frac{79269\left(\frac{J}{\mathrm{mol}}\right)}{RT_0}\right)} \tag{12}$$

The *k* value of the industrial wort (1) is follows:

$$k = 4.86E10 \cdot x \cdot e^{(-\frac{93496\left(\frac{J}{\mathrm{mol}}\right)}{RT_0})} \tag{13}$$

The *k* value of industrial wort (2) is:

$$k = 9.64E8 \cdot x \cdot e^{(-\frac{81849\left(\frac{J}{\mathrm{mol}}\right)}{RT_0})} \tag{14}$$

They found that the development of TBA also follows a pseudo 0th order reaction and the pH value and wort density influences TBA definitely. The lowest increase of TBA was obtained in a range between pH 5 and 6. In general, the concentration of TBS increases with increasing density. Felgentraeger [19] has developed a kinetic model for TBA and color with respect to pH value.

$$\begin{aligned} C_{\mathrm{TBA}} = C_{0(\mathrm{TBA})} &\cdot \exp(5.998 \cdot 10^{-7} \cdot t \cdot \exp(0.105 \cdot T) \\ &\cdot \exp(-0.338 \cdot pH)) \end{aligned} \tag{15}$$

$$\begin{aligned} C_{\mathrm{color}} = C_{0(\mathrm{color})} &\cdot \exp(6.090 \cdot 10^{-10} \cdot t \cdot \exp(0.115 \cdot T) \\ &\cdot \exp(0.807 \cdot pH)) \end{aligned} \tag{16}$$

NEB

Different beer types have different colors. The color of beer is principally determined by the quality of solution and kilning of the malt. Apart from this, wort color increasing depends on oxygen, which from pump or while filling enter into vessel, and Maillard browning during the wort boiling, which belongs to NEB [61]. However, the kinetic of NEB during the heating and long storing food has been thoroughly studied during the last decades [65]. At pH 5.5, the major pathway in browning is the 3-deoxyosulose [66]. Rapusas et al. [67] determined that NEB in onion slices could be modeled as a zero-order reaction. The reaction follows the Arrhenius relation, and the reaction rate varies as a quadratic of water activity. Ajandouz and Puigserver [68] investigated the interaction between glucose and essential amino acids at 100 °C at pH values ranging from 4.0 to 12.0. They found out, that the NEB follows pseudo-zero-order kinetics and lysine is the most strongly destroyed amino acid, followed by threonine. The Maillard reaction rate might be related to the degree of hydrolysis of the peptide bond and the stability of the peptide bond as the heating time increased. The stability of the peptide bond in triglycine was higher than that in diglycine [69]. Additionally, kinetic modelling of NEB of apple juice concentrate [70] and honey [71] have been investigated under isothermal and dynamic heating conditions. They considered that NEB and the degradation of some antioxidant compounds do not follow a zero- or first-order kinetic. The kinetic model (Fig. 2) developed by Davies et al. [72] is based on the production of melanoidins whose molar extinction coefficient remains constant throughout the observation period. The reaction of sulphite species S (IV) with a key reactive intermediate in the NEB reaction to trap this intermediate and hence deduce the rate of its formation. This model is based on the assumption that, in the presence or absence of S (IV), the first two reaction rates cannot be influenced. They investigated the kinetic dependence on concentration of the individual steps in glucose-glycine reaction. (Eq. 16–18).

$$\frac{d[DH]}{dt} = k_1 \left[\text{glucose}\right] \left[\text{glycine}\right] \tag{17}$$

$$\frac{d[I]}{dt} = k_2 [DH] \left(\left[\text{glycine}\right] + 5.1 \left[\text{glycine}\right]^2 \right) \tag{18}$$

$$\frac{dA_{450}}{dt} = k_3 [I] \tag{19}$$

Leong et al. [73] did also the kinetic analysis for the NEB of glucose-glycine. They found that the 3-step model for the browning of glucose/glycine is accurate. The modified rate equations are written as (Eq. 19, 20), where *E* is the molar extinction coefficient of the melanoidins.

$$[S(IV)]_t = [S(IV)]_0 - k_1 t + \frac{k_1}{k_2}(1 - e^{-k_2 t}) \tag{20}$$

$$(A_{470})_t = E\left\{ k_1 t - \frac{k_1}{k_2} - \frac{k_1}{k_3} + \frac{k_1 k_3}{k_2(k_3 - k_2)} e^{-k_2 t} - \frac{k_1 k_2}{k_3(k_3 - k_2)} e^{-k_3 t} \right\} \tag{21}$$

Formation of Strecker aldehydes

In the Maillard reaction, degradation of amino acids play an important role in the generation of amino-acid-specific products, such as Strecker aldehydes [56]. The formation of

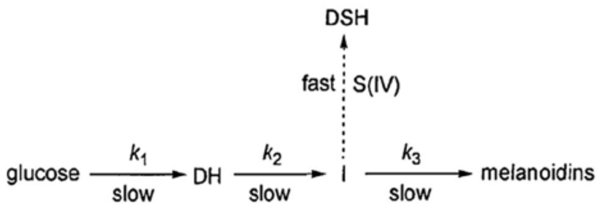


Fig. 2 A kinetic model for the glucos/glycine browning reaction, showing the point at which the reaction is inhibited by sulfite species, S (IV). DH is 3-deoxyhexosulose, DSH is 3, 4-dideoxy-4-sulphohexosulose, and I is an unspecified intermediate [72, 74]

Strecker aldehydes is strongly influenced by many factors, for example reactant concentrations, time, temperature, pH, water activity, fat content, ionic strength, filling technology, the type of beer, respective containers, etc. [75]. 3-methylbutanal, 2-methylbutanal, phenylacetaldehyde, methional are typical wort flavors and key staling flavors [76, 77]. Amino acids, their corresponding aldehydes, threshold, and flavor expression are summarized in Table 4.

Since Hodge in the 1950s published his consolidated scheme, a huge amount of research on the Maillard reaction have been performed. They established other important pathways, which improve the Hodge scheme [78]. Brands et al. [79–82] published a series of papers describing kinetic models of Maillard reaction pathways. Ketoses seemed to be more reactive in the sugar degradation reactions than their aldose isomers, and whereas the Amadori product was detected as a Maillard reaction intermediate in the aldose-casein system, no such intermediate could be found in the ketose-casein system [79]. The fate of Amadori *N*-(1-1-doxy-D-fructose-1-yl)-glycine degradation pathways in the aqueous model system was described as a function of pH and temperature [83]. Using the law of mass action, they wrote the differential equations for each reaction step. They also found out that the initial pH value had a different effect on the various rate constants; a mixture between specific acid and base catalysis. The initial concentrations of glucose and glycine have no effect on the estimated rate constants [74]. The rate constants were calculated by the software (the software package Athena Visual Workbench). The relationship between the rate constant (k) and temperature (T) is indicated by the Arrhenius equation. In addition to this, they investigated the melanoidins extinction coefficient in the glucose/glycine Maillard reaction.

2-, 3-Methylbutanal

2-Methylbutanal and 3-methylbutanal are highly volatile compounds and typical beer aging compounds and formed in the Strecker degradation of leucine and isoleucine, respectively. Although the chemistry of the Strecker degradation is well defined, it is still complex and involves a number of intermediates of unknown concentration, some of which are very short-lived. Tressel et al. investigated the intermediated of formation of isoleucine-specific Maillard products from [1-13C]-D-glucose and [1-13C]-D-fructose. The observed distribution of the label support 3-deoxyaldoketoses as intermediates of 2-formylpyrroles, and disqualifies 4-deoxy- and 1-deoxydiketose routes to 2-acetylpyrroles [66]. Parker et al. developed the kinetic model that described the formation of 2-, 3-methylbutanal in heated extract of beef liver. The Strecker degradation is limited by the availability of glucose. They found that there was no correlation between Strecker aldehydes formation and the concentration of amino acids. The radio of leucine and isoleucine to the total amino acid content remained constant during the whole heating period. The Maillard reaction was simplified to a relative simple kinetic models with four steps (Fig. 3) [84].

Methional and phenylacetaldehyde

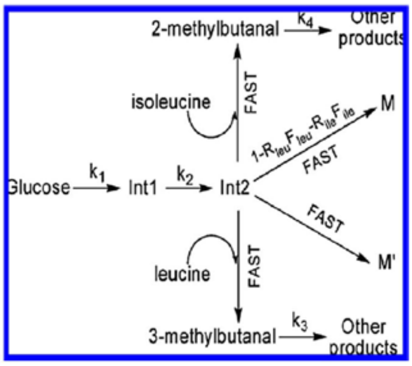
Methional and phenylacetaldehyde, derived from methionine and phenylalanine degradation, are also important Strecker aldehydes on the flavor stability of beer as direct participants or as indicators of aroma deterioration. Methional was for the first time reported by Patton as a flavor compound in relation to the development of the "sunlight" defect in milk [12]. Since that time it has reported as the typical flavors of aged beer like "cooked potato" [85–87]. Phenylacetaldehyde have a distinctive flowery/rose-like scent. The contribution of methional and phenylacetaldehyde to aged flavor was first to be insignificant, but seemed of remarkable importance later on [88].

One source of methional is the Strecker degradation mechanism. Chan et al. described the kinetics of compound formation through Strecker degradation are a zero-order reaction [89]. A second mechanism is involved in the development of the sunlight flavor [90]. After exposing milk and aqueous solutions of methionine to sunlight, Patton found that riboflavin was a critical agent in the conversion of methionine to methional in milk [91].

Table 4 Amino acids and their corresponding aldehydes in beer [50]

Amino acid	Corresponding aldehyde	Threshold (mg/l)	Flavor in beer
Alanine	Acetaldehyde	25	Green leaves, green apple
Valine	2-Methylpropanal	1.0	Fruity, sweet
Leucine	3-Methylbutanal	0.6	Grainy, sweet
Isoleucine	2-Methylbutanal	1.2	Grainy, sweet
Phenylalanine	Phenylacetaldehyde	1.6	Flowery
Phenylalanine	Benzaldehyde	1.5–3.0	Almond
Methionine	Methional	0.25	Cooked potatoes

Fig. 3 Proposed mechanism for the formation of 3-methylbutanal, 2-methylbutanal and other Maillard reaction products with (M) or without (M') the incorporation of free amino acids, respectively



Maillard products (M), no Maillard products (M'), intermediates 1(Int1); intermediates 2(Int2)

parameter	optimal estimates
$k_1(min^{-1})$	$1.36\cdot10^{-2}\pm9.22\cdot10^{-4}$ (7%)
$k_2(min^{-1})$	$5.77\cdot10^{-2}\pm2.88\cdot10^{-2}$ (50%)
$k_3(min^{-1})$	$2.72\cdot10^{-3}\pm1.01\cdot10^{-3}$ (37%)
$k_4(min^{-1})$	$2.56\cdot10^{-4}\pm8.75\cdot10^{-4}$ (342%)
Fleu	$2.33\cdot 10^{-2}\pm2.45\cdot10^{-3}$ (11%)
File	$3.83\cdot10^{-2}\pm4.28\cdot10^{-3}$ (11%)
$Ea_1(kJ\ mol^{-1})$	$1.37\cdot10^{2}\pm1.52\cdot10^{-3}$ (11%)
$Ea_2(kJ\ mol^{-1})$	$4.87\cdot10^{1}\pm8.40\cdot10^{1}$ (173%)
$Ea_3(kJ\ mol^{-1})$	$7.82\cdot10^{1}\pm3.90\cdot10^{1}$ (50%)
$Ea_4(kJ\ mol^{-1})$	not determined

$$\text{methionine} + \left\{\begin{array}{c}\text{light}\\ \text{riboflavin} \rightarrow \text{methional}\\ \text{oxygen}\end{array}\right.$$

Methionine may be methylated from S-methylmethionine which is heat labile and readily degrades to dimethyl sulfide and homoserine [82]. Balance has demonstrated that methional decomposes to yield methyl mercaptan. It was observed that the methional content decreased and the methyl mercaptan content increased. Prolonged holding of the extracts resulted in the complete disappearance of methional [92].

methional → methylmercaptan + acrolein

Mevissen et al. investigated the volatile compounds which arise from the reaction of D-glucose with L-phenylalanine in a buffered, aqueous medium. 37 substances were determined, 29 of which were structurally identified [93]. Westphal et al. have done the similar experiment and measured the concentration of D-glucose and L-phenylalanine. They found that the curve of the decrease in D-glucose shows two minima ($t=0$ and $t=70$ min), and the curve of the decrease in phenylalanine exhibits one minimum ($t=70$ min) during the induction phase of the reaction. The kinetics of the Maillard browning just fitted first reaction models $\lg E=0.027t\text{-}1.95$ [94]. Da Costa et al. reported a zero-order model for the formation of Strecker aldehydes during beer aging [62]. Table 5 shows the activation energies (Ea) and rate constants (Kref) at the reference temperature (Tref) of 40 °C (Table 5).

Studies on formation and degradation of iso-α-acids

The extraction and isomerisation of compounds derived from hops belongs to one of the important functions of wort boiling. During the boiling the α-acids are isomerized to iso-α-acids. The degree of isomerisation of the insoluble α-acids determines the degree of bitterness in beer [95]. The concentrations of iso-α-acids are mostly responsible for the characteristic, fine bitter taste and the foam properties of beer. In addition to boiling time und boiling temperature, pH value of the wort has a great influence on the isomerisation reaction of α-acids and hydrolysis process of iso-α-acids to humulinic acids, which has no bitterness (Fig. 4) [61, 96–100].

Under conventional wort boiling conditions (60 min) at atmospheric pressure about one-third of the α-acids isomerize to iso-α-acids, about one-third α-acid remains in the wort, and residual rest disappears [97]. Up to now the kinetic studies on the isomerisation of α-acids was investigated without taking into account the degradation of iso-α-acids [101]. Intelmann investigated the kinetic of degradation of iso-α-acids during the beer aging by half-life methods. He found, that the degradation of iso-α-acids follows a 1.27-order reaction.

Table 5 One-step nonlinear regression modeling parameters, Ea, Kref, and C_0, for methional and phenylacetaldehyde [62]

$c=c_0\left\{tk_{\text{ref}}\times\exp^{-(E_a/R)[1/T)-(1-T_{\text{ref}})]}\right\}$	Model order	Ea (kJ/mol)	Kref (μg/L)·h^{-1}	C_0 (μg/L)
Methional	Zero	62.2 ± 13.6	0.0031 ± 0.0011	0.51 ± 0.11
Phenylacetaldehyde	Zero	76.7 ± 33.2	0.0051 ± 0.0042	0.73 ± 0.37

$$\propto-\text{acids (humulones)}\xrightarrow{\text{isomerization}}\begin{cases}\text{cis}-\text{form}\\ \text{trans}-\text{form}\end{cases}\quad \text{iso}-\alpha-\text{acids}\xrightarrow{\text{hydrolysis}}\text{humulinic acid (no bitter)}$$

Fig. 4 Hops acid structures and chemical significance [99, 100]

Trans-isocohumulon is more instable than cis-isocohumulon [102, 103]. The combination of kinetic parameters for the formation and degradation of iso-α-acids with respect to boiling temperature, time and pH values supports the knowledge of beer bitterness and better predicts the concentration of iso-α-acids. It is necessary that a diagram should be generated, in which boiling time can be read with given variables (pH-values and boiling temperature) [96].

The kinetic knowledge of degradation of iso-α-acids during the wort boiling is relatively limited. Schellhammer studied the kinetic behavior using the losses of α-acids during wort boiling [104]. The differences between the reducing of concentration of α-acids and formation of concentration iso-α-acids were assumed equal to the quantity of degradation produced. The rate constant and activation energy for the total iso-α-acid were calculated as $(7.9\cdot10^{11})e^{(-11858/T)}$ min^{-1} and 98.6 kJmol^{-1}. Huang et al. [105] studied the kinetic behavior of degradation of iso-α-acids using iso-extract, which contains 30% w/v aqueous solution of iso-α-acids solution. It was found that the reaction rate of the degradation of α-acids follows also the first-order reaction and Arrhenius equation. Increasing the pH value decreases the free energy of activation. The relative proportion of isocohumulone stays almost constant.

Conclusion

The development trends for kinetics of thermal reactions are from simple step reaction to multi-response modelling, from isothermal condition to non-isothermal condition, and from homogenous phase to inhomogeneous phase. However, in brewing science, generally, simple reaction orders like zero and first order are used to describe changes in most reactions, such as flavor, color, thiobarbituric acids value (TBA), etcetera. The developed kinetic modeling is strictly speaking only valid for simple reactions with simple reactants. This review provides the present state of development of chemical kinetic during the wort boiling and summarizes some kinetic modelling of chemical and physical, changes with focus on evaporation and formation of flavors as well as isomerisation of α-acids and degradation of iso-α-acids. The process leading substances, such as DMS, Maillard reaction, are discussed in detail. The kinetic of formation of hot trub in wort should be investigated in the near future.

In food technology, the boiling process plays an important role in many production processes. This is no exception for the breweries. Up to now the kinetics studied on the leading substances in wort has been studied in small scales or under laboratory conditions. The known kinetic values of evaporation and formation of process parameters alone are not easy to simulate the development of the leading substances in different modern boiling systems.

In the literature, we found experimental dates only under isothermal conditions, although during the “heating up” process many reactions also take place. However, this complex process and its relation to knowledge the independent process parameters are not consistently described. None of the previous studies involve the dynamic performance of critical process markers, which are dependent on the formation and conversion of the main wort flavor components. Multi-response modelling has been used to estimate kinetic parameters of Maillard reactions in model solution. In addition to Maillard reactions, the kinetic researches of lipid oxidation, interaction between Maillard reaction and lipid oxidation, interactions in the whole wort matrix, shelf life time, nutrient content, etc., should also use multi-response modelling. Due to the complex wort matrix, the kinetic studies are a big challenge for brewing scientists. This paper should be considered as a global presentation on kinetic studies in the wort.

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Compliance with ethical standards

Conflict of interest There is no conflict of interest.

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2.3 A Kinetic Study on the Formation of 2- and 3-Methylbutanal

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A KINETIC STUDY ON THE FORMATION OF 2- AND 3-METHYLBUTANAL

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ABSTRACT

2-Methylbutanal (2-MB) and 3-methylbutanal (3-MB) are highly volatile compounds and typical beer aging compounds. The development of a kinetic model of the formation of 2- and 3-MB is useful to estimate the wort flavor during the wort boiling and the flavor stability of beer. A model experiment was performed, using D-glucose/D-maltose and L-leucine/isoleucine to study Maillard reaction in a buffer solution of a pH value of 5.2. The simulated solution was heated at different time (0–360 min) and temperature (90–130 °C). The concentrations of D-glucose/D-maltose, L-leucine/isoleucine and 2- and 3-MB were determined by HPLC and GC. This kinetic model is based on the correlation between the concentration of 2- and 3-MB and the concentration of L-leucine/isoleucine. In this paper, the multistep formation method of 2-MB/3-MB is simplified to a three-step reaction. The kinetic parameters E_a, A and k were calculated for each reaction step. It was found that the formation of 2- and 3-MB has a sequence like this: GL > ML > GI > MI. The total activation energies for the formation of 2- and 3-MB are ranging from 107.87 to 178.88 kJ/mol. The developed kinetic model gives a good fit with the experimental data. This study is useful for the control of the wort quality during the boiling process and could also be interesting for food and beverage industries.

PRACTICAL APPLICATIONS

Maillard reactions are responsible for the formation of most wort flavors. In addition to this there exist intermediates, which lead to the development of off-flavors during shelf life. The 2- and 3-MB are the Strecker aldehydes and have typical wort flavors. Wort boiling is not only the midpoint of the brewhouse, it is also one of the most important steps in producing an excellent beer. The kinetic study of the formation of 2- and 3-MB is very useful for the control of wort boiling parameters. This result is helpful to calculate a suitable wort boiling time for different raw materials under brewing process conditions.

INTRODUCTION

Heat processing is one of the most important processes in food industry. It is widely used to preserve foods and to develop texture, flavor, color and nutritional value. In the brewery, during wort boiling it is also an important part of the brewing process. The main purposes of wort boiling are the adjustment of final density, the hop isomerization, the destruction of enzyme activity and the sterilization of wort. The elimination of off-flavors and the formation of desired flavors ensure a good tasting beer. Monitoring of aroma formation in brewing process is of great scientific and industrial interest. 2-Methylbutanal (2-MB) and 3-methylbutanal (3-MB) are important wort off-flavors and ageing compounds, which lead to a typical sweet, bread like off-flavor. The study of the off-flavor is based on the study of the formation and evaporation of the off-flavors. Hertel *et al.* (2007) have investigated the limiting separation factors and the limiting activity coefficients of 2- and 3-MB in water. Feilner (2013) has calculated the relative volatility of 2- and

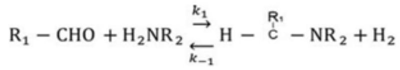
3-MB in wort at 100 °C and 90–95 °C. However, the study of formation of Strecker aldehyde in brewing science is limited.

Maillard reactions, a matrix of consecutive, parallel and reversible reactions, are responsible for the formation of the flavors and color. Reducing sugar and different amino acids via a pathway of the complex network and Strecker degradation can form a series of many volatile off-flavors, for instance, acetaldehyde, 2-methylpropanal, 2-methylbutanal (2-MB), 3-methylbutanal (3-MB) and phenyl acetaldehyde. Brand and van Boekel (2001) published a series of papers describing kinetic modeling of Maillard reaction pathways. Ketoses seem to be more reactive in the sugar degradation reactions than their aldose isomers. Whereas the Amadori product was detected as a Maillard reaction intermediate in the aldose–casein system, no such intermediate could be found in the ketose–casein system. The fate of Amadori N-(1-1-doxy-D-fructose-1-yl)-glycine degradation pathways in aqueous model systems was described as a function of pH and temperature (Martins 2003). Using multiresponse kinetic modeling, they established two proposed kinetic models of Amadori N-(1-1-doxy-D-fructose-1-yl)-glycine degradation pathways (Martins and van Boekel 2003) and four proposed kinetic models of the glucose/glycine Maillard reaction pathways (Martins 2003). Using the law of mass action, they wrote the differential equations for each reaction step. They also found out that the initial pH has a different effect on the various rate constants, a mixture between specific acid and base catalysis. The initial concentrations of glucose and glycine have no effect on the estimated rate constants (Martin and van Boekel 2005).

Up to now, the most experiments were studied in model systems consisting of deionized water, amino acids like alanine and sugars, especially glucose, a simple monosaccharide (Gallardo *et al.* 2008). The results cannot present the real process of the kinetics in the complicated Maillard reaction. The Maillard reaction gets even more complicated by a series of factors, such as pH, fat content, temperature and ionic strength. Balagiannis *et al.* (2009) chose an aqueous extract of beef liver as a simplified food matrix for studying the kinetic of the Maillard reaction in a meat system. They found out that the proportion concentration of leucine, isoleucine and the concentration of the total amino acid is maintained constant in raw and heated liver extract. Depending on these results, they assumed the Strecker degradation is limited by the availability of glucose. There was no correlation between the formation of Strecker aldehydes and the concentration of amino acids.

In the 1970s, Grigsby and Palamand (1975) were the first in using the thiobarbituric acid (TBA) method (or TBN/TBI) to present intermediates of Maillard reactions. Since then TBA methods are used to describe the thermal stress in wort during wort boiling with different boiling systems. Single reaction steps and individual Strecker aldehydes have never been studied under wort boiling conditions. Schwill-Miedaner (2002) has resumed her researches and other scientists published results and considered the TBA as a summary of primary and secondary products of the Maillard reaction. She concluded that the TBA value at temperatures of 80, 90, 95 and 98.5 °C follows a zero reaction kinetics order. De Schutter *et al.* (2008) examined a pseudo-zeroth order to describe thermal load in wort.

There are three major stages during Maillard reaction including early, advanced and final stages. Some studies concentrated on the early stage of Maillard reactions (including the formation of N-glucosides, Amadori rearrangement and the decomposition of Amadori compounds) and melanoidines. Haugaard *et al.* (1951) attempted to follow the kinetic of Schiff base in a various amino acid aldose system under alkaline conditions (Eq. 1).



$$R_1 - CHO + H_2NR_2 \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} H - \overset{R_1}{\overset{|}{C}} - NR_2 + H_2$$

$$\frac{\mathbf{dC_N}}{\mathbf{dt}} = k_1\mathbf{C_C C_A} - k_2\mathbf{C_{AC}} \tag{1}$$

where C_N is the concentration of compounds containing nitrogen, C_C is the concentration of carbohydrate, C_A is the concentration of amino acid anion and C_{AC} is the concentration of reaction of Schiff base.

In the 1970s, Hashiba (1976) investigated the oxygen-dependent browning of Amadori compounds in soya sauce. He found out that the browning of all Amadori compounds except fructose-arginine was accelerated notably by oxygen and Fe^{2+} (40 ppm) and the presence of Mn^{2+} showed an inhibitory effect on the browning of fructose-serine, fructose-glutamic acid and fructose-leucine. Ge and Lee (1997) studied the kinetic significance of the Schiff base reversion in the early-stage Maillard reaction of a phenylalanine–glucose aqueous model system (Eqs. 2–4)

$$\mathbf{A+S} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} [\mathbf{AS}]^*_{\mathbf{sb}} \xrightarrow{k_2} \mathbf{AR}$$

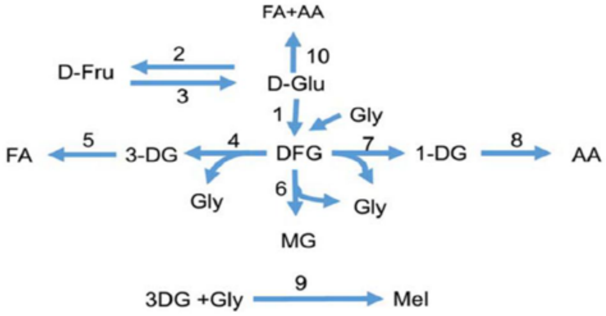
where A is the amino acid, S is the sugar, $[AS]^*_{sb}$ is the Schiff base complex intermediate and AR is the Amadori compound:

$$-\mathbf{d}[\mathbf{A}]/\mathbf{dt} = \boldsymbol{k}_1[\mathbf{A}][\mathbf{S}] - \boldsymbol{k}_{-1}[\mathbf{AS}] \tag{2}$$

$$\mathbf{d}[\mathbf{AS}]/\mathbf{dt} = \boldsymbol{k}_1[\mathbf{A}][\mathbf{S}] - (\mathbf{k}_{-1} + \mathbf{k}_2)[\mathbf{AS}] \tag{3}$$

$$\mathbf{d}[\mathbf{AR}]/\mathbf{dt} = \boldsymbol{k}_2[\mathbf{AS}] \tag{4}$$

The equilibrium constant *K* among the Schiff base complex intermediate $[AS]^*_{sb}$, amino acids and glucose can be represented by



SCHEME 1. KINETIC MODEL NO. 4 FOR THE GLUCOSE/GLYCINE MAILLARD REACTION. GLYCINE (GLY), METHYLGLYOXAL (MG), GLUCOSE (GLU), MANNOSE (MAN), FRUCTOSE (FRU), ACETIC ACID (AA), FORMIC ACID (FA), 3-DEOXYOSONE (3-DG), 1-DEOXYOSONE (1-DG) AND MELANOIDINES (MEL) (MARTINS, 2003)

$$\boldsymbol{K}=[\mathbf{AS}]/([\mathbf{A}][\mathbf{S}]) \tag{5}$$

$$[\mathbf{AS}]=[\mathbf{A}]_0-[\mathbf{A}]-[\mathbf{AR}] \tag{6}$$

where $[A]_0$ is the initial concentration of the amino acid.

If $[A] = [S]$, then Eq. (4) can be written as

$$\boldsymbol{k}=\frac{[\mathbf{A}]_0-[\mathbf{A}]-[\mathbf{AR}]}{[\mathbf{A}][\mathbf{A}]} \tag{7}$$

The production of blue pigments in the early stage of a Maillard reaction between D-xylose and glycine was kinetically analyzed to be associated with the mechanism for polymerization (Gomyo *et al.* 1989). Based on Arrhenius plots, the activation energy was found to be 73 kJ/mol

$$v=\boldsymbol{k}(\mathrm{d}-\mathbf{xylose})^2\cdot(\mathbf{glycine})^2 \tag{8}$$

The Model

The kinetic data related to series of reactions are manifested by superpositions of exponential decays that are often difficult to dissect. (1) Reliable and unbiased determination of the number of reaction steps and (2) stable reconstruction of the distribution of kinetic rate constants are two major challenges, which have hampered the kinetic analysis of multistep chemical reactions (Zhou and Zhuang 2007). Some studies (Haugaard *et al.* 1951) concentrated on the early stage of Maillard reaction (including the formation of N-glucosides, Amadori rearrangement and the decomposition of Amadori compounds) and melanoidines. Some studies (Wedzicha 1995; Wedzicha and Kedward 1995) focused on the kinetic of nonenzymatic browning (NEB) during the heating of different fluid food media. They found that the NEB follows a pseudo-zero-order kinetic or a zero-order kinetic. Van Boekel in his critical review mentioned a major difficulty is that principles from chemical kinetics are strictly speaking only valid for simple elementary reactions, and foods are all but simple (van Boekel 2008). Using multiresponse modeling, he and his team have developed up to 10 steps modeling of Maillard reaction (Scheme 1).

$$\mathrm{A}_1 \overset{\mathrm{k}_1}{\rightarrow} \mathrm{A}_2 \overset{\mathrm{k}_2}{\rightarrow} \cdots \mathrm{A}_\mathrm{m} \overset{\mathrm{k}_\mathrm{m}}{\rightarrow} \mathrm{B}$$

$$\mathrm{A}_1 \overset{\mathrm{k}_1}{\rightarrow} \mathrm{A}_\mathrm{m} \overset{\mathrm{k}_2}{\rightarrow} \mathrm{B}$$

SCHEME 2. SIMPLIFY A M-STEP REACTION TO A TWO-STEP REACTION

Multiresponse modeling is often criticized to dissect the multistep reaction kinetics quantitatively from experimental data, to determine the rate constant distribution of each step and to investigate the dependence of these rate constants on external conditions. It is a challenging task, if the total number of reaction steps is unknown a priori or some of the reaction steps cannot be described by a single rate constant. Zhou and Zhuang (2007) found, biological processes may not only consist of multiple steps, but a seemingly two-step reaction is better described by a distribution of kinetic rate constants instead of a single one (Scheme 2).

In this study, the kinetic of the formation of 2- and 3-MB is studied. The multistep formation way of Strecker flavor is simplified to a three-step reaction (Scheme 3). Intermediates consist of all substances which take part in the formation of 2- and 3-MB, e.g., Schiff base, Amadori-like products and/or their degradation products and reactive dicarbonyl compounds.

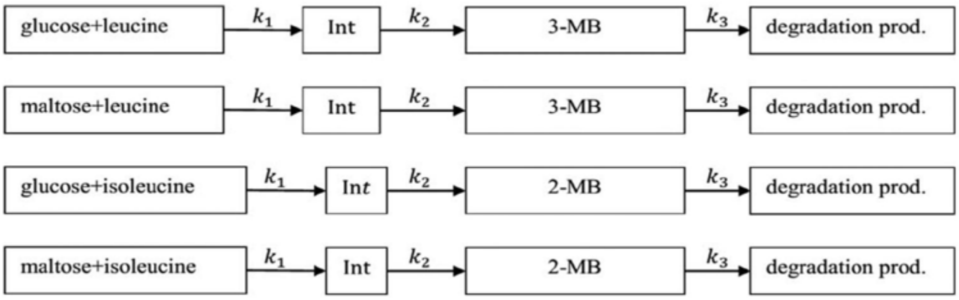
MATERIALS AND METHODS

Materials

All experiments were realized in a model system. Glucose, maltose, leucine and isoleucine were purchased from Sigma-Aldrich (Taufkirchen, Germany). Bi-distilled water used for HPLC was purified by means of a Milli-Q Gradient A 10 system (Millipore, Billerica, MA). Methanol (HPLC LiChrosolv, gradient quality), phosphoric acids (85%) monohydrogenphosphat, dihydrogenphosphat and hydrochloric acid were obtained from Merck (Darmstadt, Germany).

Preparation of Reaction Mixtures

Disaccharide sugar (maltose) or monosaccharide sugar (glucose) and amino acid (leucine or isoleucine), mol ratio 10:1,



SCHEME 3. KINETIC MODEL FOR THE FORMATION OF 2- AND 3-MB

were dissolved in a phosphate buffer (0.1 mol/L; pH 5.2), because the formation of 2- and 3-MB depends on pH and the molar ratio between sugar and the amino components (Cremer and Eichner 2000a,b). The rate of the Maillard reaction, sugar isomerisation and subsequent degradation decreases with decreasing pH (Brands and van Boekel 2002). Maillard reaction velocity can be increased by raising the molar ratio between glucose and the amino component until a threefold glucose excess was reached. The recommended value for the pH of wort is 5.2 and the molar ratio between sugar and amino compound of wort is more than 3:1 (Fox *et al.* 1983). The samples were heated for various times (0–360 min) at different temperatures (90–130 °C) in a heating block in closed glass tubes. After a planned time interval, the glass tube was taken out and cooled immediately in ice water. The tests were repeated three times.

Analyses

The analyses were following the method of MEBAK with major modification (Pfenninger 1993).

Analyses of Sugars. Glucose and maltose were monitored using an ICS-1000 ion chromatography system (Dionex Corp., Sunnyvale, CA), which includes an AS 50 autosampler, AS 50 thermal compartment with amperometric detection cell, ED50 electrochemical detector and GP50 gradient pump. A sample (100 μL) was mixed with a solution of internal standard (900 μL) and injected onto a Carbopac PA10 column (Dionex). Two different buffer solutions were prepared: solvent A (H_2O) and solvent B (400 mmol/L NaOH) were prepared for analysis of monosaccharide. 96% of solvent A and 4% of solvent B were used for the first 30 min to separate sugars. For the next 10 min, a solution of 40% of solvent A and 60% of solvent B was used to wash the column. Dionex Chromeleon software version 6.0 was used for data processing.

Analyses of Amino Acids. Leucine and isoleucine were derivatized and measured by a HPLC (Dionex UltiMate 3000 system, Dionex, Sunnyvale, CA) which was equipped with an LPG-3400 M pump, an FLM-3300 flow management system, a VWP-3400 detector, a WPS-3000 autosampler module and a NAN 1000 and CAP 100 flow splitter cassette. All samples were diluted 1:10 on an analytical laboratory balance. Used reagents were OPA-reagent (10 mg o-phthaldialdehyde), 6.5 µL mercaptopropionsäure (MCP), 500 µL methanol, 500 µL Boratbuffer), Capping-reagent (6.5 mg iodine acetic acid in 1 mL Boratbuffer), FMOC-reagent (Agilent Technology P/N: 5061-3337) and reducing agent (2.5 µL MCP in 1 mL Boratbuffer).

Analyses of 2- and 3-MB. Maillard-flavors were measured by GC (Hewlett-Packard Model 5890), which was equipped with a Chrompack Purge and Trap Injector (PTI), two flame ionization detectors (FID) and a Shimadzu CR3A integrator. Capillary column I (HP Innowax; polyethylene glycol) 50 m × 0.20 mm × 0.40 µm and capillary column II (HP Ultra 2; 5% Ph–95% Me-Si; 50 m × 0.20 mm × 0.33 µm) were used. The pressure of the column was set to 190 kPa. Four microliters of samples was injected in the chromatographic column and purged with hydrogen gas at 1.0 ml/min. The temperature of the purge vessel was set to 50 °C. The capillary was heated to 210 °C for 4 min and remained 210 °C for 36 min. Hydrogen gas was used at a flow rate of 1.9 ml/min. Detection temperatures were 250 °C.

Modeling

The kinetic of the formation of 2- and 3-MB was investigated with the multiresponse modeling using the Athena Visual Studio software package (Athena Visual Software Inc., Naperville, IL) (Eqs. (8–11)).

$$\frac{\mathbf{d[Leu]}}{\mathbf{dt}} = -\boldsymbol{k_1}[\mathbf{Leu}] \tag{9}$$

$$\frac{\mathbf{d[ZP]}}{\mathbf{dt}} = \boldsymbol{k_1}[\mathbf{Leu}] - \boldsymbol{k_2}[\mathbf{ZP}] \tag{10}$$

$$\frac{\mathbf{d[3MB]}}{\mathbf{dt}} = \boldsymbol{k_2}[\mathbf{ZP}] - \boldsymbol{k_3}\ [\mathbf{3MB}] \tag{11}$$

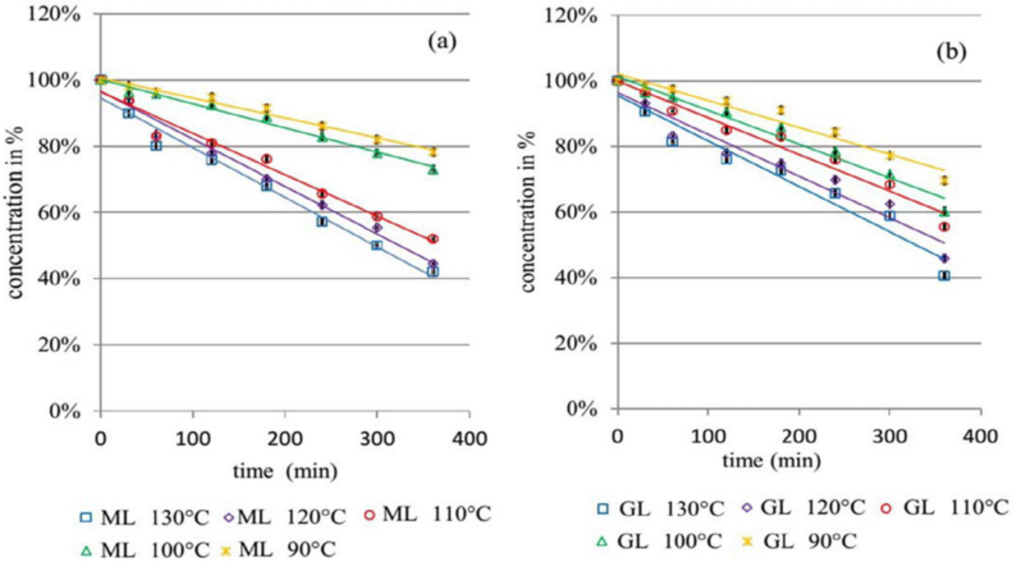


FIG. 1. CONCENTRATION OF LEUCINE DURING THE REACTION WITH MALTOSE (A) OR GLUCOSE (B) AT DIFFERENT TEMPERATURES

$$\frac{\mathbf{d[AP]}}{\mathbf{dt}} = \boldsymbol{k}_3[\mathbf{3MB}] \qquad (12)$$

Statistical Analysis

Analysis between two combinations (glucose/maltose and leucine/isoleucine) was determined using the Scheffe's F test. A significance level of $p < 0.05$ was selected.

RESULTS AND DISCUSSION

Amino Acids

The concentration of amino acids (leucine or isoleucine) in each sample was detected. At 130 °C more than 60% of leucine in samples with glucose/leucine is degraded after 360 min boiling time. But at 90 °C about 20% leucine is degraded by the same heating time (Fig. 1a). In Fig. 1b, similar trend lines are shown. The difference between the change in concentrations of samples with maltose/leucine and samples with glucose/leucine is that glucose already at low temperature, e.g., 90 °C, reacts preferred with leucine. With increasing temperature, the speed of loss of leucine in all reactions with glucose is slower than in the reaction with maltose.

Figure 2a shows that 35% isoleucine in the reaction with maltose is degraded after 360 min boiling time at 130 °C. The concentration of isoleucine is constant at the beginning or even increased slightly at 90 °C. Only 10% isoleucine is degraded after the same heating time. Isoleucine in the reaction with glucose does not decrease linearly with heating time (Fig. 2b). At 90 °C and 100 °C, the losses of isoleucine in reaction with glucose/isoleucine show similar trends like in reaction with maltose/isoleucine. The losses of isoleucine seem to occur in three steps in the reaction between 110 and 130 °C. At the beginning, isoleucine is degraded fast, and then gradually slows down. After that the losses of isoleucine accelerate again.

Spatial structure, bond lengths and angles for the side chains affect the speed of reaction. Compared to isoleucine, leucine is degraded about two times faster than isoleucine. The side chains of leucine have a little bit shorter bond lengths, for instants, N-Cα, Cα-C$'$, and obvious larger angles than the side chains of isoleucine (Gould *et al.* 1985). Therefore, leucine reacted with glucose or maltose more active than isoleucine.

Sugar

The concentration of glucose decreases linearly with heating time. Results were similar to that reported by Renn and Sathe (1997). They observed glucose loss (glucose/leucine) appears to occur in three stages. During the first 360 min, the concentration of glucose has a linear reduction between 360 and 700 min there is a stationary or increased phase and finally the glucose concentration decreases again. The losses of glucose increase with raising temperature (Fig. 3). Figure 3a shows samples with glucose/leucine at 130 °C have about 40% debatement of glucose after 360 min heating time. Samples with glucose/isoleucine (Fig. 3b) at 130 °C have a debatement of glucose of about 30%. A statistical F test was

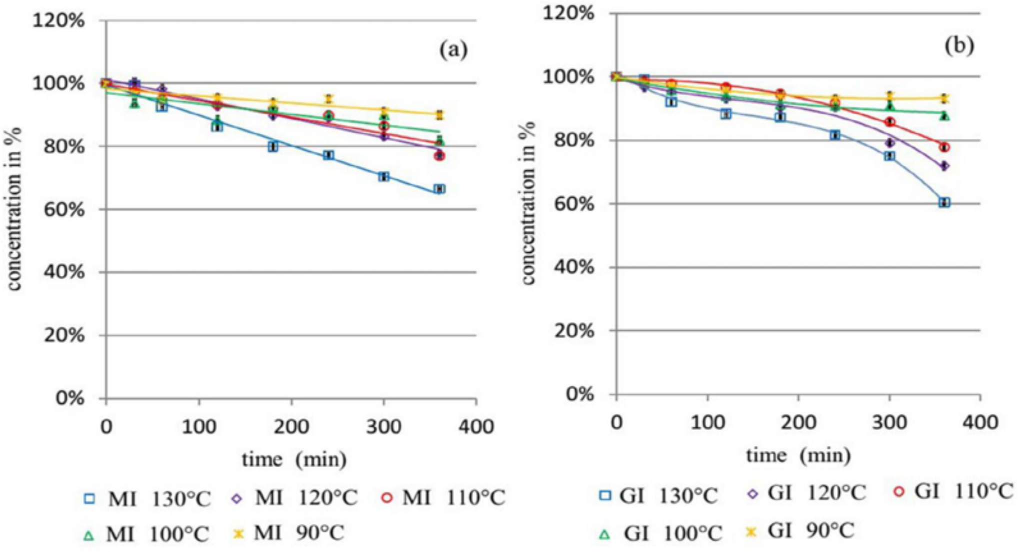


FIG. 2. CONCENTRATION OF ISOLEUCINE DURING THE REACTION WITH MALTOSE (A) OR GLUCOSE (B) AT DIFFERENT TEMPERATURES

used to compare the losses of glucose in the two groups. Differences were considered significant when $p < 0.05$. Apart from the results at 130 °C, there were no significant differences between the losses of glucose, which reacted with leucine or isoleucine at 90–120 °C. A fraction of the glucose can be inverted to mannose and fructose. maltose, maltotriose and sucrose can also be detected in all samples with glucose/leucine or glucose/isoleucine.

Maltose, also known as maltobiose or malt sugar, comprehends over 50% fermentable sugar in wort. In this paper, the

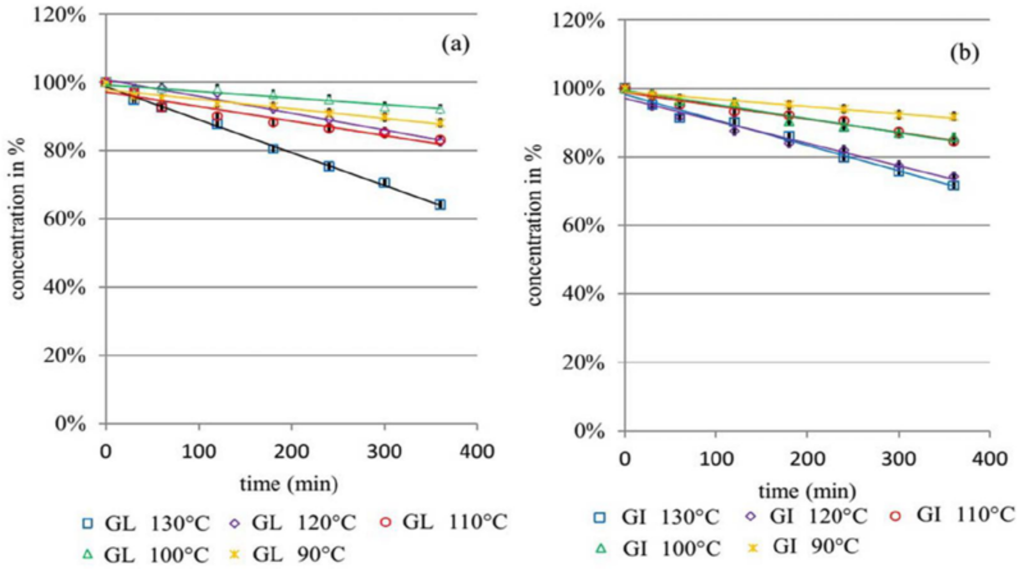


FIG. 3. CONCENTRATION OF GLUCOSE DURING THE REACTION WITH LEUCINE (A) OR ISO-LEUCINE (B) AT DIFFERENT TEMPERATURES

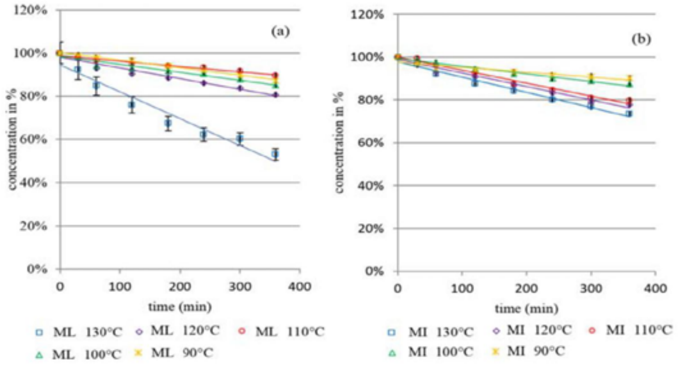


FIG. 4. CONCENTRATION OF MALTOSE DURING THE REACTION WITH LEUCINE (A) OR ISO-LEUCINE (B) AT DIFFERENT TEMPERATURES

reaction occurring in disaccharide-leucine/isoleucine was studied. Several investigations have shown that the reaction mechanisms of monosaccharides and disaccharides (having glucose at the reducing end) differ and that reaction products obtained from monosaccharides are different from those obtained from disaccharides (Brands and van Boekel 2003). Kato *et al.* (1988) compared the reaction mechanisms in a glucose/disaccharides and ovalbumin system. They found, browning dependents on sugar structure. Maltose with a glycosidic bond at the C4 hydroxyl group of glucopyranose is difficult to cleave.

The concentration of maltose decreases linearly with heating time, except samples with maltose/leucine at 130 °C (Fig. 4). The loss of maltose in samples with maltose/isoleucine occurs more rapid than in samples with maltose/leucine. A statistical *F* test was also used to compare the loss of maltose in the two groups. There were significant differences between the losses of maltose, which react with leucine or isoleucine at 90–130 °C. Compared with the loss of glucose, the loss of maltose occurs a little bit slower.

2-MB and 3-MB

The concentrations of 2- and 3-MB can drastically be increased by raising the temperature. Raising the temperature from 90 °C to 130 °C increases the concentrations of 3-MB about 100 times (Fig. 5a). Figures 5b and 6 show similar trend lines. The concentrations at 90 °C in Figs. 5 and 6 look

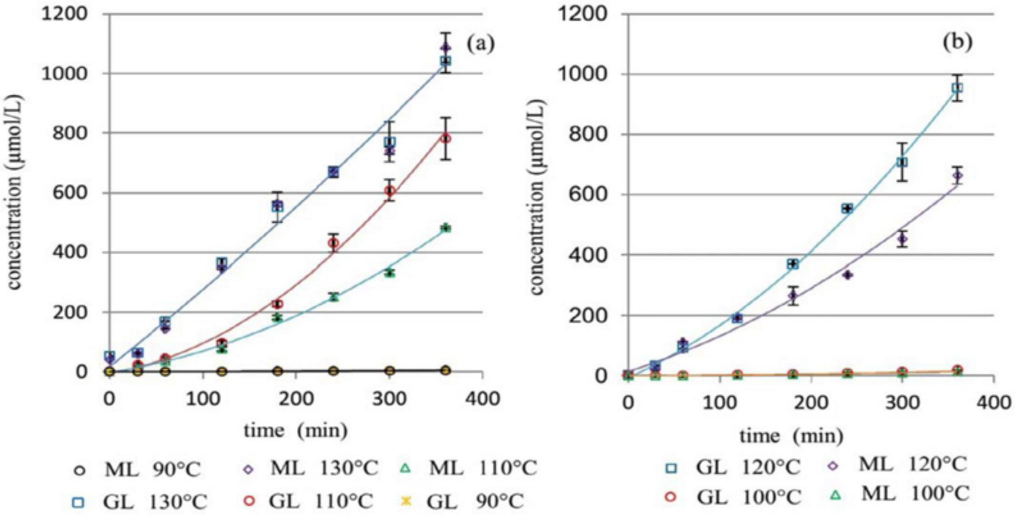


FIG. 5. CONCENTRATION OF 3-MB DURING THE REACTION LEUCINE WITH GLUCOSE (A) OR MALTOSE (B)

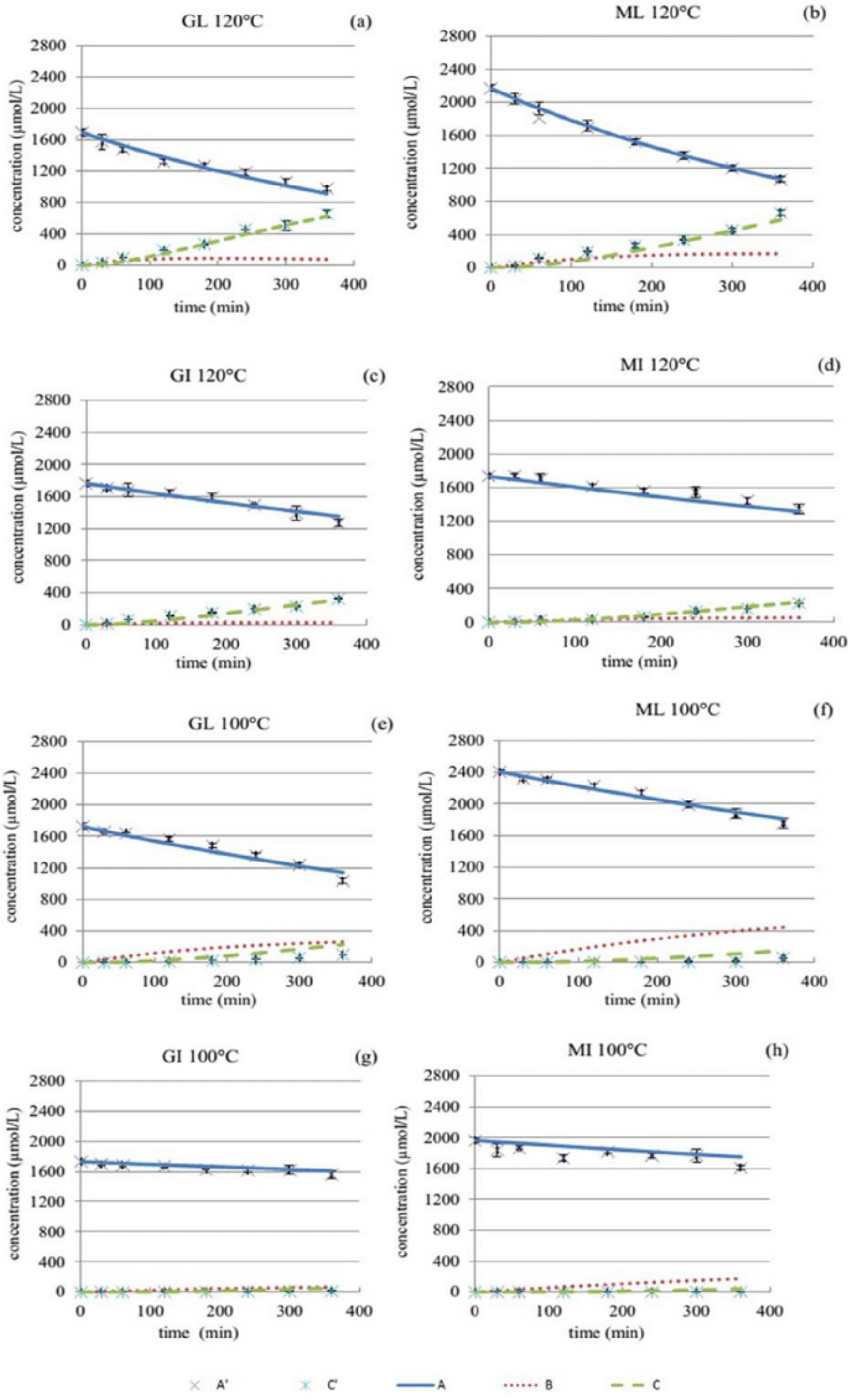


FIG. 7. COMPARISON OF THE CONCENTRATIONS OF LEUCINE/ ISOLEUCINE, INTERMEDIATES AND 2-MB/3-MB ON THE MODEL AND THE MEASUREMENT POINT AT 120 °C AND 100 °C ([A] THE THEORETICAL CONCENTRATION OF LEUCINE, [B] THE THEORETICAL CONCENTRATION OF THE INTERMEDIATE PRODUCT, [C] THE THEORETICAL CONCENTRATION OF 3-MB; [A] THE EXPERIMENTAL CONCENTRATION OF LEUCINE AND [C] THE EXPERIMENTAL CONCENTRATION OF 3-MB)

formation, 2-MB/3-MB is continuously reduced to degradation products. This is the step 3 with a rate constant k_3, which is the smallest and has the largest corresponding interval. The reaction rates of the reaction (k_1 and k_2) between glucose/maltose and leucine/isoleucine at 100 °C are summarized in Table 1.

The Arrhenius equation and reaction energy were calculated with constant reaction rates at different temperatures. The results are showed in Table 2. The comparison of the concentrations of leucine/isoleucine, intermediate and 2-MB/3-MB on the model and the measurement points at 100 °C and 120 °C is illustrated in Fig. 7. The kinetic parameters of simplified model give a good fit with the experimental data. The intermediate increases faster than 2-MB/3-MB during the wort boiling. 2-MB/3-MB is considered not only as a typical wort flavor, but also an aging indicator. The boiling point of 2-MB/3-MB is 91 °C. A part of 2-MB/3-MB could be evaporated during the wort boiling. Furthermore, a part of 2-MB/3MB can be reduced to the corresponding alcohols during the fermentation. Straight after bottling 2-MB/3-MB has a much lower concentration (Lustig 1994). During the storage, the concentration of 2-MB/3-MB increases continuously, which depends on the storage temperature and storage time. We believe the intermediates may be responsible for the formation of aging components like 2-and 3-MB during storage.

CONCLUSION

Flavors are the soul of the beer. For a quantitatively estimate, the formation of wort flavors during the wort boiling is one of the most important tasks for beer brewing. In this study, the development of a kinetic modeling of the formation of 2- and 3-MB with two amino acids (leucine and isoleucine), monosaccharide (glucose) and disaccharide (maltose) under wort boiling conditions in a buffer solution of a pH value of 5.2 was aimed. The multistep formation way of 2-MB/3-MB was simplified to a three-step reaction. The free energies of activation and the reaction rate constants with reference to the formation of 2- and 3-MB were studied and simulated and show the change of concentration of 2- and 3-MB during the wort boiling. The total activation energies for formation of 2- and 3-MB are between 107.87 and 178.88 kJ/mol. It was found that the type of sugar (glucose or maltose) influences greater the amount of Strecker flavors than the type of amino acids. Leucine reacted with glucose or maltose more active than isoleucine. As can be seen, the formation of 2- and 3-MB has a sequence like this: GL > ML > GI > MI. Thus it can be seen that the proportion of amino acids and sugars in wort plays an important role for the formation of 2- and 3-MB. With these results, it will be possible to monitor the reactions occurring during the wort boiling stage in a more transparent manner. These results are very useful to calculate a suitable wort boiling time for individual raw materials and brewing process conditions.

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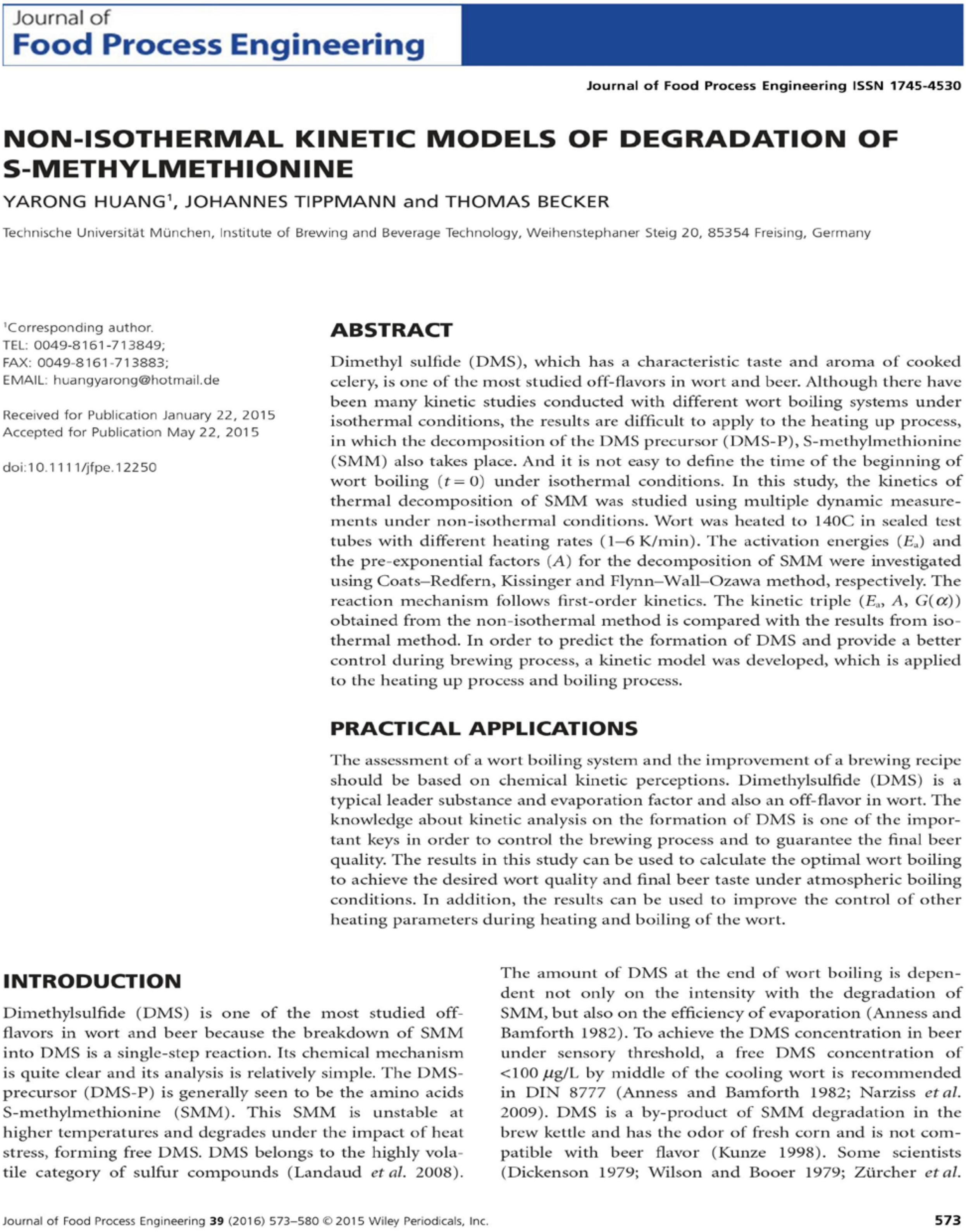
2.4 Non-Isothermal Kinetic Models of Degradation of S-Methylmethionine



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NON-ISOTHERMAL KINETIC MODELS OF DEGRADATION OF S-METHYLMETHIONINE

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ABSTRACT

Dimethyl sulfide (DMS), which has a characteristic taste and aroma of cooked celery, is one of the most studied off-flavors in wort and beer. Although there have been many kinetic studies conducted with different wort boiling systems under isothermal conditions, the results are difficult to apply to the heating up process, in which the decomposition of the DMS precursor (DMS-P), S-methylmethionine (SMM) also takes place. And it is not easy to define the time of the beginning of wort boiling ($t = 0$) under isothermal conditions. In this study, the kinetics of thermal decomposition of SMM was studied using multiple dynamic measurements under non-isothermal conditions. Wort was heated to 140C in sealed test tubes with different heating rates (1–6 K/min). The activation energies (E_a) and the pre-exponential factors (A) for the decomposition of SMM were investigated using Coats–Redfern, Kissinger and Flynn–Wall–Ozawa method, respectively. The reaction mechanism follows first-order kinetics. The kinetic triple (E_a, A, $G(\alpha)$) obtained from the non-isothermal method is compared with the results from isothermal method. In order to predict the formation of DMS and provide a better control during brewing process, a kinetic model was developed, which is applied to the heating up process and boiling process.

PRACTICAL APPLICATIONS

The assessment of a wort boiling system and the improvement of a brewing recipe should be based on chemical kinetic perceptions. Dimethylsulfide (DMS) is a typical leader substance and evaporation factor and also an off-flavor in wort. The knowledge about kinetic analysis on the formation of DMS is one of the important keys in order to control the brewing process and to guarantee the final beer quality. The results in this study can be used to calculate the optimal wort boiling to achieve the desired wort quality and final beer taste under atmospheric boiling conditions. In addition, the results can be used to improve the control of other heating parameters during heating and boiling of the wort.

INTRODUCTION

Dimethylsulfide (DMS) is one of the most studied off-flavors in wort and beer because the breakdown of SMM into DMS is a single-step reaction. Its chemical mechanism is quite clear and its analysis is relatively simple. The DMS-precursor (DMS-P) is generally seen to be the amino acids S-methylmethionine (SMM). This SMM is unstable at higher temperatures and degrades under the impact of heat stress, forming free DMS. DMS belongs to the highly volatile category of sulfur compounds (Landaud *et al.* 2008). The amount of DMS at the end of wort boiling is dependent not only on the intensity with the degradation of SMM, but also on the efficiency of evaporation (Anness and Bamforth 1982). To achieve the DMS concentration in beer under sensory threshold, a free DMS concentration of <100 $\mu g/L$ by middle of the cooling wort is recommended in DIN 8777 (Anness and Bamforth 1982; Narziss *et al.* 2009). DMS is a by-product of SMM degradation in the brew kettle and has the odor of fresh corn and is not compatible with beer flavor (Kunze 1998). Some scientists (Dickenson 1979; Wilson and Booer 1979; Zürcher *et al.*

TABLE 1. KINETIC PARAMETERS OBTAINED FROM THE AUTHORS

Authors	Zürcher *et al.* (1979)	Rübsam and Krottenthaler (2010)	Scheuren (2010)	Hertel *et al.* (2006)	Schwill-Miedaner (2002)	Felgenträger ((1993)
Ea (kJ/mol)	121.779	115.015	182 ± 12	–	–	–
A	2.88E+15	3.09E+14	(4 ± 0.3)E+24	–	–	–
R^2	0.9937	0.9900	–	0.9930	0.9795	–
k (min^{-1}) (100C)	0.0258	0.02450	0.0367	0.0231 ± 0.0022	0.0165 ± 0.0022	0.115
Arrhenius function	$k = 2.88\cdot10^{15}\cdot e^{-1012470,6/T}$	$k = 3.09\cdot10^{14}\cdot e^{-956234/T}$	$k = (4 \pm 0.3)\cdot10^{24}\cdot e^{-1513148/T}$	–	–	–

1979; Rübsam and Krottenthaler 2010) have tried in their investigations to predict the concentration of DMS and his precursor SMM during each brewing process step. In 1970s, Dickenson examined for first time the reaction kinetics of the DMS concentrations decrease or increase in malt, wort and beer. He used a temporal exponential function, which was derived from variation of different process parameters (pH, boiling temperature in the range 85.5 to 101C). He did the experiments in laboratory under atmospheric conditions. He noted that the half-life of degradation of SMM at pH 5.2 is about 28.0 min. At pH 5.5 the half-life increases to 32.5 min. This half-life doubles every 6C reduction in temperature. This information is very useful to predict the DMS content of wort throughout the brewing process, until the end of wort cooling. In 1979, Wilson and Booer (1979) examined that SMM has a half-life of approximately 35 min at pH 5.4. Zürcher *et al.* (1979) did a very similar experiment using a pilot boiler and an external boiler by boiling temperature in the range 70 to 100C. He found that the SMM have a half-life of approximately 59.5 min at 100C. At 110C need the decomposition of SMM only 23.6 min. The half-life trebles about every 10C reduction in temperature. Rübsam and Krottenthaler (2010) repeated Zürcher's study by using an external boiler and calculated the first-order equation. The relation between the concentrations of SMM during boiling (A_t) depends on the temperature (T) in degree kelvins (Eq. 1). Because of the fact that the temperature of the wort in an external boiler cannot accurately be measured, a correction factor is used to describe the real results of decomposition of SMM.

$$A_t = A_0 e^{-[\ln(2)/5.9\times10^6 e^{-0.11T}]t} \tag{1}$$

All above-mentioned kinetic models (Table 1) were determined using knowledge about the single-step reaction under isothermal conditions. Isothermal methods have the advantage of simplicity and easier interpretation when used for the wort boiling process. But, there is a major problem in the isothermal experiment, which is that wort requires some time to reach the boiling temperature. During the heating period, some SMM has already transformed into DMS what likely affects the results of the following kinetic analysis. This problem especially restricts the use of high temperatures in isothermal experiments (Vyazovkin and Wight 1997). Different authors determined different kinetic parameters in the last 30 years. Because of different experiment conditions, wort boiler types und evaluation methods, some values of reaction rate (k) deviate from each other. The part of SMM, which has been transformed into DMS during the heating phase, was ignored. These methods may cause errors in the final results.

Non-isothermal methods have been widely used for studies of kinetics of the single-step reaction. The first kinetic evaluations of non-isothermal data involved samples that were heated at a constant rate:

$$\beta = dT/dt \tag{2}$$

$$T = T_0 + \beta t \tag{3}$$

Where T_0 is the starting temperature. To determine kinetic constants, Vyazovkin *et al.* suggested the replacement of the temporal differential by $dt = dT/\beta$ (Vyazovkin and Wight 1998). In the 1960s, Freidman *et al.* developed isoconversional methods under non-isothermal conditions. Friedman and Ozawa found the activation energy to vary with according to the extent the decomposition of a phenolic plastic and of polyamide occurred, respectively. Friedman also demonstrated that the observed variation significantly exceeded the confidence intervals for the activation energy. Using simulated multiple-step kinetics, Flynn and Wall explained the phenomenon of variable activation energy and thereby "legalized" its existence (Vyazovkin 2000). Dollimore *et al.* (1996) believed that the activation energy is dependent on both, α and temperature, and the relationship is linear with temperature.

KINETIC METHODS

For the degradation of SMM, its reaction equation can be expressed as:

$$DMS - P \xrightarrow{heating} DMS$$

The classical isothermal reaction rate, derived from a batch reactor model, can be expressed in two different forms of equations.

$$\text{Differential form: } \frac{d\alpha}{dt} = k(T)f(\alpha) \tag{4}$$

$$\text{and integral form: } G(\alpha) = k(T)t \tag{5}$$

Where α is the extent of reaction, k is the reaction rate constant which depends on the temperature. $f(\alpha)$ is a function that represents the reaction model.

If the sample temperature is changed by a controlled and constant heating rate $\beta = dT/dt$, the variation in the degree of conversion can be analyzed as a function of temperature, this temperature being dependent on the time of heating. Therefore, the reaction rate can be written together with Eq. (2) as (Nunez 2000).

$$\frac{d\alpha}{dT} = \frac{k(T)}{\beta} f(\alpha) \tag{6}$$

The relationships between rate constant k and temperature T were proposed almost at the same time, at the end of the 19th century. The most commonly used equation for that is the Arrhenius equation.

$$k = A\exp(-E_a/RT) \tag{7}$$

Where A is the pre-exponential factor, E_a the activation energy and R the molar gas constant (8.314 J $mol^{-1}K^{-1}$). But, some scientists criticized the temperature-independent pre-exponential factor A used in this equation has no theoretical justification (Vyazovkin 2000).

Combining Eq. (6) with Arrhenius equation (Eq. 7), Eq. (8) was obtained:

$$\ln\left[\frac{\left(\frac{d\alpha}{dT}\right)\beta}{f(\alpha)}\right] = \ln A - \frac{E_a}{RT} \tag{8}$$

As well as its integral form

$$G(\alpha) = \int_0^\alpha \frac{d\alpha}{f(\alpha)} = \frac{A}{\beta}\int_{T_0}^{T} e^{-E_{\alpha}/RT}\,dT \tag{9}$$

Where T_0 is the initial temperature of the reaction.

Two kinds of methods for evaluating kinetic parameters at non-isothermal methods were used in this work: one is the single-heating-rate method, and the other is the multi-heating-rate method. In both, there are two routines that are most often used, namely the differential method (e.g. Kissinger method) and the integral method (e.g. Coats–Redfern method, Ozawa–Flynn–Wall method; Chen 1993). It is also considered that the integral methods are less subjected to experimental errors (noise of the device, etc.) as they evaluate the whole experimental data set and are based on the primary experimentally acquired data, α and T (Popescu 1996).

In this study, the three methods were used to calculate the reaction rates and activation energies. These three methods belong to the most popular methods for calculating the values of the non-isothermal kinetic parameters.

DIFFERENTIAL METHOD

Kissinger method

The Kissinger method is based on the change in the position of the peak maxima (T_p) with the heating rate being used. Kissinger assumed the reaction mechanism function is

$$f(\alpha) = (1-\alpha)^n \tag{10}$$

and the kinetic equation is expressed as

$$\frac{d\alpha}{dt} = Ae^{-E_a/RT}(1-\alpha)^n \tag{11}$$

A differentiation on both sides of Eq. (11)

$$\begin{aligned}\frac{d}{dt}\left[\frac{d\alpha}{dt}\right] &= \left[A(1-\alpha)^n\frac{de^{-E_a/RT}}{dt} + Ae^{-E_a/RT}\frac{d(1-\alpha)^n}{dt}\right]\\ &= A(1-\alpha)^n e^{-E_a/RT}\frac{(-E_a)}{RT^2}(-1)\frac{dT}{dt}\\ &\quad - Ae^{-E_a/RT}n(1-\alpha)^{n-1}\frac{d\alpha}{dt}\\ &= \frac{d\alpha}{dt}\frac{E_a}{RT^2}\frac{dT}{dt} - Ae^{-E_a/RT}n(1-\alpha)^{n-1}\frac{d\alpha}{dt}\\ &= \frac{d\alpha}{dt}\left[\frac{E_a\frac{dT}{dt}}{RT^2} - An(1-\alpha)^{n-1}e^{-E_a/RT}\right]\end{aligned} \tag{12}$$

If the first derivative is zero,

$$\frac{E_a\frac{dT}{dt}}{RT_p^2} = An(1-\alpha_p)^{n-1}e^{-E_a/RT} \tag{13}$$

Take log of both sides

$$\ln\left(\frac{\beta_i}{T_{pi}^2}\right) = \ln\frac{AR}{E_a} + \ln[n(1-\alpha_p)^{n-1}] - \frac{E_a}{R}\frac{1}{T_{pi}} \tag{14}$$

Kissinger believed $n(1-\alpha_p)^{n-1}$ is unrelated with β. Its value is approximately equal to 1.

So, Kissinger equation could be written as

$$\ln\left(\frac{\beta_i}{T_{pi}^2}\right) = \ln\frac{AR}{E_a} - \frac{E_a}{R}\frac{1}{T_{pi}},\ i = 1, 2, \ldots, 4 \tag{15}$$

$\ln\left(\frac{\beta_i}{T_{pi}^2}\right)$ and $\frac{1}{T_{pi}}$ express a linear function with intercept $\ln\frac{AR}{E_a}$ and slope E_a/R.

Integral Methods

The integral methods can be expressed as

$$G(\alpha)=kt \tag{5}$$

For non-isothermal data gives

$$\begin{aligned} G(\alpha)&=\int_0^\alpha \frac{d\alpha}{f(\alpha)}=\frac{A}{\beta}\int_{T_0}^{T}\exp(-E_a/RT)dT\\ &=\frac{A}{\beta}\int_0^T \exp(-E_a/RT)dT \qquad (16)\\ &=\frac{AE}{\beta R}\int_\infty^u \frac{-e^{-u}}{u^2}du=\frac{AE_a}{\beta R}p(u)\\ p(u)&=\int_u^\infty \frac{\exp(-u)}{u^2}d(u); \quad u=\frac{E_a}{RT} \end{aligned}$$

The $p(u)$ has no analytical solution but has many approximations. For $p(u)$, the different treatment constitutes a series of integral equation method. Although many methods have been applied, most notably the methods and equations are as following.

Coats–Redfern Method

The Coats–Redfern method requires only one heating rate to calculate the reaction order n, reaction activation E_a and pre-exponential factor A. This method utilizes a similar approach to calculate $p(u)$ (Eq. 17).

$$\begin{aligned} p(u)&=\int_u^\infty \frac{\exp(-u)}{u^2}d(u)\\ &=\frac{\exp(-u)}{u^2}\left(1-\frac{2!}{u}+\frac{3!}{u^2}-\frac{4!}{u^3}+\dots\right) \qquad (17)\\ &\cong \frac{\exp(-u)}{u^2}\left(1-\frac{2}{u}\right) \end{aligned}$$

The combination of Eqs. (10) and (16) leads to the following equations:

$$\int_0^\alpha \frac{d\alpha}{(1-\alpha)^n}=\frac{A}{\beta}\frac{RT^2}{E_a}\left(1-\frac{2RT}{E_a}\right)e^{-E_a/RT} \tag{18}$$

$$\text{If } n\neq 1, \ln\left[\frac{1-(1-\alpha)^{1-n}}{T^2(1-n)}\right]=\ln\left[\frac{AR}{\beta E_a}\left(1-\frac{2RT}{E_a}\right)\right]-\frac{E_a}{RT} \tag{19}$$

$$\text{If } n=1, \ln\left[\frac{-\ln(1-\alpha)}{T^2}\right]=\ln\left[\frac{AR}{\beta E_a}\left(1-\frac{2RT}{E_a}\right)\right]-\frac{E_a}{RT} \tag{20}$$

The change in experimental conditions from isothermal to non-isothermal has no effect on the reaction kinetics. Therefore, non-isothermal kinetic parameters can agree with the isothermal values. A series of researches have studied und proved that the degradation of SMM follows the first reaction order: ($n=1$; Rübsam and Krottenthaler 2010, Schwill-Miedaner 2002). And therefore $\ln\left[\frac{-\ln(1-\alpha)}{T^2}\right]$ and $\frac{1}{T}$ expresses a linear function with intercept $\ln\left[\frac{AR}{\beta E_a}\left(1-\frac{2RT}{E_a}\right)\right]$ and slope $-E_a/R$ (Chen and Wang 2007).

Ozawa–Flynn–Wall Method

Ozawa–Flynn–Wall method is written as following expression:

$$\log\beta=\log\left(\frac{AE_a}{RG(\alpha)}\right)-2.315-0.4567\frac{E_a}{RT} \tag{21}$$

Plot of logß versus $1/T$ with a given $G(\alpha)$ expresses a linear function with intercept $0.457E_a/R$. The calculated E_a of this method is independent of the thermal decomposition reaction mechanism (Chen and Wang 2007).

MATERIALS AND METHOD

Wort was obtained from the research brewery of Chair of Brewing and Beverage Technology at the Technical University of Munich. The concentrations of SMM were analyzed according to MEBAK vol. II 1.3.2.

Sample Preparation and Heating Condition

20 mL wort was placed in each 22-mL glass test tube with closure and heated in a glycerin bath. Heating rates were set from 1.1 to 5.1 K/min. The degradation of SMM takes place at temperatures above 75C. The sampling was performed every temperature rise of 15–20 K in the range to 70C. When wort temperature rises above 70C, sampling was done every 8–10 K step by low heating rates (1.10, 1.85, 2.40 K/min) and every 4–6 K by high heating rates (3.48 and 5.12 K/min). After a settled time interval, the test tube was taken out and cooled immediately to 0C in an ice water bath. Different heating time was chose for different heating rates. The heat transfer problems can be minimized using small amounts of wort. The experiment was replicated three times.

RESULTS AND DISCUSSION

Thermal degradation of SMM was studied at five different heating rates. The experimental data were calculated with three above-mentioned methods. One of the important calculation steps for all three methods dependents on plotting one mathematical formula (e.g. $\ln\left[\frac{-\ln(1-\alpha)}{T^2}\right]$, ln ß, or ln$ß/T_p^2$ against $1/T$). Therefore, the following figures are plotted as a function of temperature instead of time. The

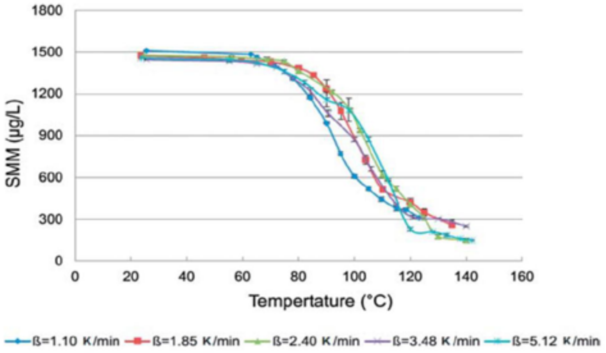

FIG. 1. THE CONCENTRATIONS OF SMM IN THE WORT AGAINST THE TEMPERATURE AT THE HEATING RATES OF 1.10, 1.85, 2.40, 3.48 AND 5.12 K/MIN

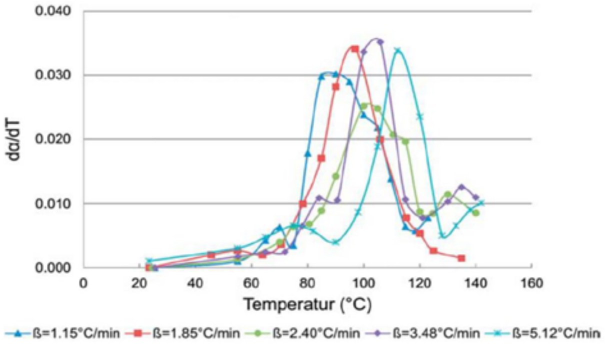

FIG. 2. *DA/DT* AGAINST TEMPERATURE AT THE HEATING RATE OF 1.10, 1.85, 2.40, 3.48 AND 5.12 K/MIN

concentrations of SMM at the heating rate of 1.10, 1.85, 2.40, 3.48 and 5.12 K/min are plotted against the temperature. And the first derivative $d\alpha/dT$ against the temperature shown in Figs. 1 and 2.

T_p from Fig. 2 were calculated by using software Origin (version 7.5, OriginLab Corp., Northampton, MA, U.S.A). Table 2 shows the values of T_p, $1/T_p$ and other characteristic parameters, e.g. $\ln\beta$, $\ln(\beta/T_p^2)$, which are necessary when plotting data on figures. The higher the heating rate applied, the higher T_p values are for degradation of SMM.

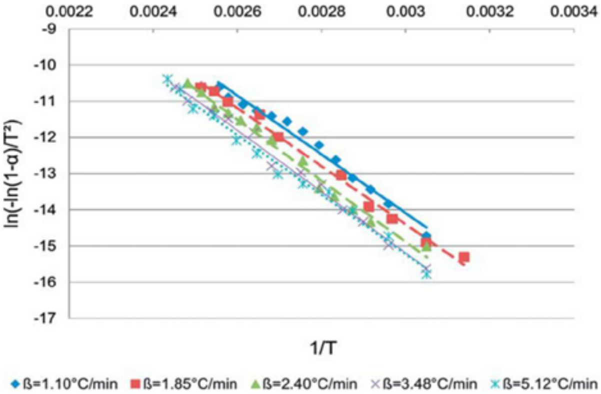

FIG. 3. PLOT LN(-LN(1-A)/T^2) VERSUS 1/T FOR VARIOUS HEATING RATES

Kinetic Analysis Using Coats–Redfern Method

Based on a similar approach, $\ln\left[\frac{-\ln(1-\alpha)}{T^2}\right]$ and $\frac{1}{T}$ express a linear function with intercept $\ln\left[\frac{AR}{\beta E}\left(1-\frac{2RT}{E}\right)\right]$ and slope $-\frac{E}{R}$ (Eq. 20). It is considered that multi-heating-rate methods give more reliable results than those based on data from single-heating-rate methods. Although the Coats–Redfern method requires only one heating rate to calculate the kinetic parameters, to further analyze, experiments were done also three times with five different heating rates. The plot of $\ln\left[\frac{-\ln(1-\alpha)}{T^2}\right]$ versus $1/T$ is shown in Fig. 3.

The fitted results including activation energy (E_a), pre-exponential factor (A), ln A and correlation coefficients (R^2) are summarized in Table 3. The values of kinetic parameter do not differ much from each other.

Kinetic Analysis Using Ozawa–Flynn–Wall Method

Ozawa–Flynn–Wall method makes a model-free evaluation of the activation energy from a series of non-isothermal

TABLE 2. TP AND OTHER CHARACTERISTIC PARAMETERS OF SMM AT THE DIFFERENT HEATING RATES

Heating rate (K/min)	1.15	1.85	2.40	3.48	5.12
T_p(°C)	91.6	96.7	102.2	104.8	113
T_p (K)	364.6	369.7	375.2	377.8	386
$1/T_p$	0.002743	0.002705	0.002665	0.002647	0.002591
$1/T_p^2$	7.52E-06	7.31E-06	7.10E-06	7.00E-06	6.71E-06
Lnβ	0.139	0.615	0.875	1.247	1.633
$\ln(\beta/T_p^2)$	−11.65	−11.21	−10.97	−10.62	−10.27

TABLE 3. KINETIC PARAMETERS AND CORRELATION COEFFICIENTS ACCORDING TO COATS–REDFERN METHOD

β(K/min)	E_a (kJ/mol)	A	ln$A$	R^2
1.15	81.25	2.96E+9	21.80	0.9712
1.85	81.05	1.422E+9	21.07	0.9695
2.40	80.93	9.26E+9	22.95	0.9891
3.48	82.09	1.47E+9	21.11	0.9806
5.12	82.45	2.20E+9	21.51	0.9629

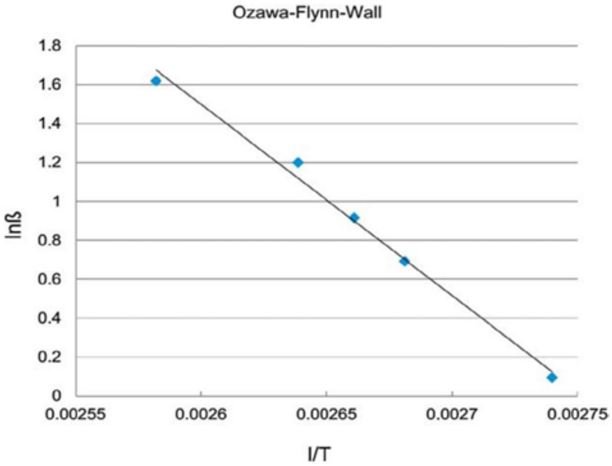

FIG. 4. PLOTS OF LNS VERSUS 1/T AT CONVERSION VALUES (*A*) OF 62 % ACCORDING TO OZAWA–FLYNNWALL METHOD ($R^2 = 0.976$)

measurements carried out at different heating rates. This method can determine the activation energy without knowledge of the special kinetic model. It is considered that the resulting errors are often greater than generally expected (Opfermann 1992) Figure 4 shows a plot of lnβ versus $1/T$ at conversion values (α) of 62 %. Here, the activation energy and pre-exponential can be calculated from the slops and intercept.

Kinetics Analysis Using Kissinger Method

The absolute temperature at maximum reaction rate was obtained by using the Kissinger method. According to Eq. (14), a plot of $\ln(\beta/T_p^2)$ versus $1/T_p$ express a fitted straight lines as shown in Fig. 5. The activation energy and pre-exponential can be calculated from the slops and intercept.

Model Fitting

The results of the three methods are presented in Table 4. For the Coats–Redfern method, the mean values from five different heating rates were used. The activation energies for decomposition of SMM calculated from Coats–Redfern, Ozawa–Flynn–Wall and Kissinger equations are 81.55, 81.32 and 82.29 kJ/mol, respectively, which are much lower than those of using isothermal methods obtained by other authors (Table 1). The difference can be explained by the following. The initial concentrations of SMM cannot be easily defined under isothermal condition because a part of SMM has been already degraded into DMS during the heating phase. This reaction is very sensitive to temperature. If a study is performed with an external boiler or internal boiler, the wort temperature is not homogeneous in the vessel. In this study, using small amounts of wort (20 mL), the heat transfer problems can be minimized. It is possible to explain the difference between the results obtained with different methods and heating conditions. Lower activation energy is more consistent with degradation of SMM, which proceeds fast and easily. However, as the activation energy (also the energy barrier) can provide the information of critical energy needed to start a reaction, the decomposition activation energy range obtained in this study can help to understand the thermal stability of SMM.

The results from the three methods (Coats–Redfern, Ozawa–Flynn–Wall and Kissinger) are very similar to each other. In the case of a first-order single-step reaction, all the three methods give very accurate results. Practically, different kinetic analysis methods should be complementary rather than competitive.

This modeling focuses on the experiments at constant heating rates. In order to approach the reality, by model-fitting, non-isothermal data were used with various heating rates. The model can fit the main trends in the non-isothermal data, especially at the high temperature side. Two models according to Coats–Redfern and Kissinger method give the better fitting with the experimental results than the one according to the Ozawa–Flynn–Wall method. The

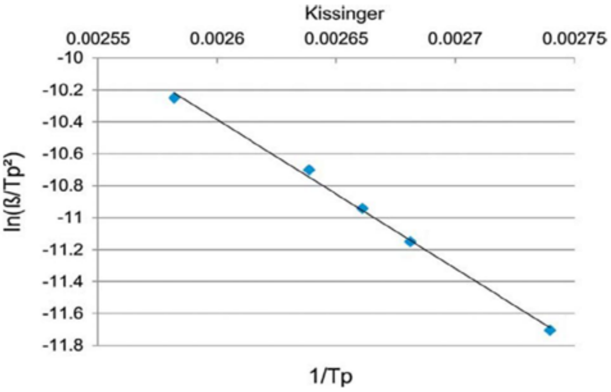

FIG. 5. PLOTS OF LNS VERSUS 1/T AT CONVERSION VALUES (*A*) OF 62 % ACCORDING TO KISSINGER METHOD ($R^2 = 0.989$)

TABLE 4. KINETIC PARAMETERS OF THE THREE METHODS

	Coats–Redfern	Ozawa–Flynn–Wall	Kissinger
E_a (kJ/mol)	81.30	81.51	82.29
ln$A$	21.75	20.61	21.60
A (min^{-1})	2.79E+9	9.29E+8	2.40 E+9
R^2	≥0.9500	0.9795	0.9494
Arrhenius equation	$k = 2.79 \cdot 10^9 \cdot e^{-9778,7/T}$	$k = 9.29 \cdot 10^8 \cdot e^{-9781.2/T}$	$k = 2.40 \cdot 10^9 \cdot e^{-9897.7/T}$

reason can be related to the Dolye approximation, which was used in the Ozawa–Flynn–Wall method. The Dolye approximation has the limitation of an α range of 5–20%, and, generally, the inflection point involved in the Kissinger method far exceeds this range (Chen and Wang 2007).

The concentrations of SMM with different heating rates (0.5–1.5 K/min) during heating, boiling and whirlpool are showed in Fig. 6. The initial temperature of kettle wort is 72C after mashing. After 60 min of wort boiling, the temperature was decreased to 98C within 50 min during cast-out and in whirlpool. Normally, the concentration of DMS reaches less than 100 μg/L by the end of wort boiling because of reduction through the evaporation of DMS; the degradation of SMM during this 50 min leads to an increase in the concentration of DMS. The concentration of DMS can increase to more than 100 μg/L (sensory threshold) again. In order to model this process, the integral method (Coats–Redfern) is used to calculate $G(\alpha)$ during the heating phase. When initial concentrations of SMM and heating rate are set at 400 μg/L and 1 K/min, respectively, the concentration of SMM decreases to 338.3 μg/L during the heating phase. Correspondingly, 14% SMM has already decomposed before wort boiling. The concentration of SMM decreases continuously during 60 min of wort boiling. Using the isothermal method and the kinetic parameters, obtained from this study (Table 4), 148.8 μg/L SMM (38% of initial concentration of SMM) should be theoretically detected in wort by the end of wort boiling. Because of the highly volatile nature of DMS, most amounts of DMS can be evaporated after wort boiling. Therefore, the DMS increase during the cast-out and in whirlpool plays an important role in determining the final taste of beer. According to the kinetic model, 66 μg/L DMS (16.5% of initial concentration of DMS) can be formed during the cast-out and in whirlpool.

If the heating rate is reduced to 0.5 K/min, the change in the concentration of SMM is very similar to the results with 1.5 K/min. But if the heating rate is increased to 1.5 K/min, the concentration of SMM can be significantly higher than 100 μg/L because of short heating time. In order to avoid DMS off-flavor in beer, enough heating and boiling time, boiling with pressure, wort precooling or stripping are necessary process steps to reduce DMS in wort and beer.

This model can predict the concentration change of DMS and SMM with summary of data from practical experiments and brewery.

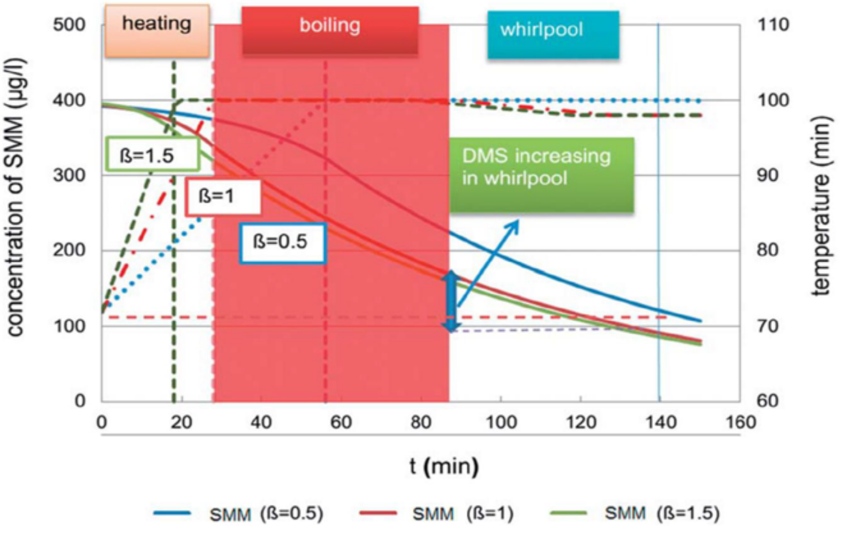

FIG. 6. CONCENTRATION OF SMM WITH KINETIC PARAMETERS ACCORDING TO COATS–REDFERN METHOD DURING HEATING, BOILING AND IN WHIRLPOOL

CONCLUSIONS

The kinetic parameters of degradation of SMM were studied under non-isothermal conditions and analyzed using Coats–Redfern, Flynn–Wall–Ozawa and Kissinger methods. The results from the three methods are very similar to each other. Degradation of SMM was found to have an activation energy of about 82 kJ/mol under non-isothermal condition, which is much lower than the values obtained from isothermal experiments by other authors. The models according to Coats–Redfern and Kissinger methods fit the non-isothermal data with various heating rates better than the model according to the Flynn–Wall–Ozawa methods. Probably, this was the first time that degradation of SMM was closely monitored and from a kinetic view studied during the heating process. Valuable information can be obtained for operational practice, which can be used to better control the heating parameters during heating wort and wort boiling.

ACKNOWLEDGMENTS

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2.5 Kinetic Modeling of Hop Acids during Wort Boiling

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Kinetic Modeling of Hop Acids during Wort Boiling

Yarong Huang, Johannes Tippmann, and Thomas Becker

***Abstract*—The thermal degradation of iso-alpha-acids was investigated at four pH levels between 4.5 and 6.5 at different boiling temperatures (90-130 ℃) during the wort boiling time (0-360 min). The free energy of activation and the reaction rate constant (k) of the degradation of iso-alpha-acids were calculated. Raising pH value from 4.5 to 5.5 and from 5.5 to 6.5 decreased the reaction energy about 20 kJ/mol. The relative proportion of isocohumulone stays almost constant. We developed a kinetic model for the irreversible consecutive reation; iso-alpha-acids are isomerized form of alpha-acids by the application of heat in solution and at the same time iso-alpha-acids are degraded to humulinic acids and other compounds. This kinetic model is useful to calculate time to added hops into boiling kettle.**

***Index Terms*—Activation energy, alpha-acids, degradation, iso-alpha-acids, kinetics, pH value.**

I. INTRODUCTION

Hops (humulus lupulus) are an important ingredient in beer brewing and have been used for centuries for the purpose of contributing a clean bitter taste to beer. The value of hops for beer brewing is determined by the alpha-acids, essential hop oils and polyphenols. Alpha-acids are the quantitatively predominating class of polyketides accounting for up to 10% of hops dry matter and comprising of at least six congeners differing in the alkanoyl side chain. Whereas cohumulone, n-humulone, and adhumulone account for more than 98% of the total amount of alpha-acids, the minor congeners, namely, posthumulone, prehumulone, and adprehumulone, reach a relative quantity of less than 2% [1]. Iso-alpha-acids are isomerized forms of the alpha-acids (humulones) from the hops. The main transformation products account for up to 70% of the amount of alpha-acids initially added during wort boiling [2]. The iso-alpha-acids are mostly responsible for the characteristic, fine bitter taste and the foam properties of beer. The degree of bitterness imparted by hops depends on the degree of isomerizes of the insoluble alpha-acids during the wort boiling.

A thorough study of any chemical reaction must therefore include structure and kinetic investigations. It is known that the iso-alpha-acids are hydrolyzed or converted to humulinic acids and other undesirable compounds (Fig. 1). Some researchers have already investigated the kinetic of alpha-acids isomerization and found that the appearance of iso-alpha-acids did not equal the loss of alpha-acids, especially at the beginning of the wort boiling. For extended heating times, losses of iso-alpha-acids becomes increasingly significant [3]. Up to now the reaction kinetic of the degradation of iso-alpha-acids has been just studied basing on the assumptions. The reaction rates were calculated using mathematic modeling without experimental data.

This research seeks to discover the kinetics of degradation on iso-alpha-acids as a function of temperature and pH value. A purified iso-alpha-acids extract in different pH buffered aqueous solutions was used. The influence of some reaction parameters, such as temperature and pH value on the first-order rate constants involved in the kinetic model, has been investigated.

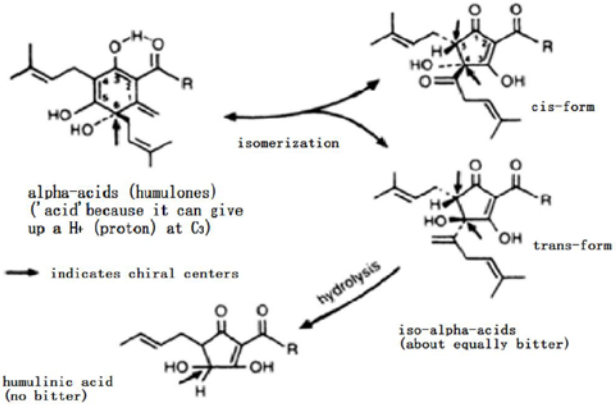

Fig. 1. Hops acid structures and chemical significance [4].

II. PERTINENT LITERATURE

Quantitative studies and model experiments have revealed a huge influence of the stereochemistry on the reactivity of the iso-alpha-acids and demonstrated a rapid degradation of trans-iso-alpha-acids, whereas the corresponding cis-isomers have been found to be comparatively stable. Recent molecular studies led to the discovery of harsh and lingering bitter tasting tricyclohumols, tricyclohumenes, isotricyclohumenes, tetracyclohumols, and epitetracyclohumols as the major transformation products of trans-iso-alpha-acids in model experiments as well as in authentic beer samples [5].

Some researchers have published data regarding the isomerization of alpha-acids during wort boiling with a first-order reaction. *Mostek* et al. [6] stated that during 120 min boiling time the rate of the isomerization of alpha-acids follows the first-order reaction except for the first 10 min. Knowledge of degradation of iso-alpha-acids during the wort boiling is relatively limited.

Schellhammer *et al.* [3] conducted the wort boiling experiments in a model wort boiling system and heated the

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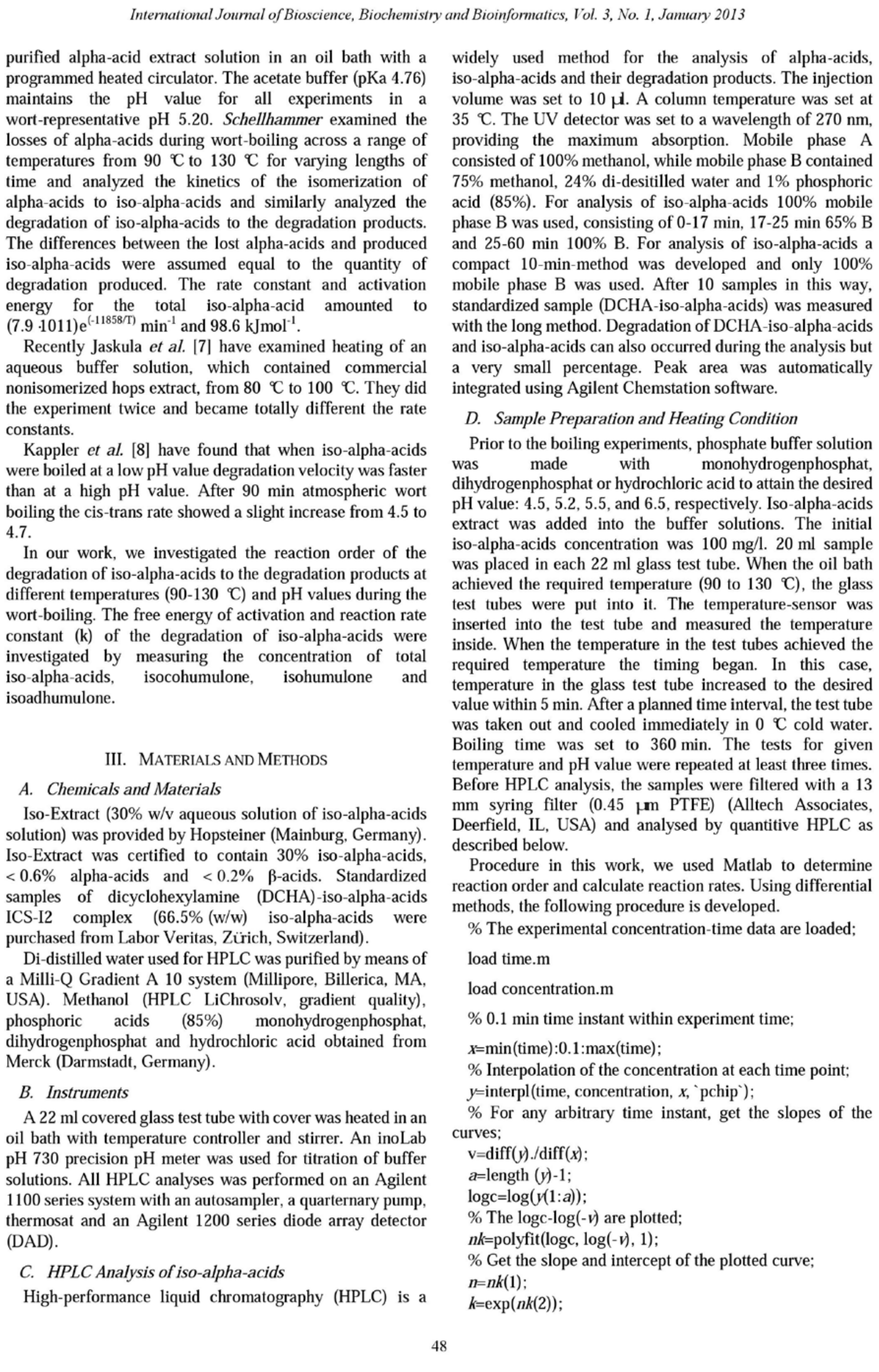

purified alpha-acid extract solution in an oil bath with a programmed heated circulator. The acetate buffer (pKa 4.76) maintains the pH value for all experiments in a wort-representative pH 5.20. *Schellhammer* examined the losses of alpha-acids during wort-boiling across a range of temperatures from 90 ℃ to 130 ℃ for varying lengths of time and analyzed the kinetics of the isomerization of alpha-acids to iso-alpha-acids and similarly analyzed the degradation of iso-alpha-acids to the degradation products. The differences between the lost alpha-acids and produced iso-alpha-acids were assumed equal to the quantity of degradation produced. The rate constant and activation energy for the total iso-alpha-acid amounted to $(7.9 \cdot 1011)e^{(-11858/T)}$ min^{-1} and 98.6 kJmol^{-1}.

Recently Jaskula *et al.* [7] have examined heating of an aqueous buffer solution, which contained commercial nonisomerized hops extract, from 80 ℃ to 100 ℃. They did the experiment twice and became totally different the rate constants.

Kappler *et al.* [8] have found that when iso-alpha-acids were boiled at a low pH value degradation velocity was faster than at a high pH value. After 90 min atmospheric wort boiling the cis-trans rate showed a slight increase from 4.5 to 4.7.

In our work, we investigated the reaction order of the degradation of iso-alpha-acids to the degradation products at different temperatures (90-130 ℃) and pH values during the wort-boiling. The free energy of activation and reaction rate constant (k) of the degradation of iso-alpha-acids were investigated by measuring the concentration of total iso-alpha-acids, isocohumulone, isohumulone and isoadhumulone.

III. MATERIALS AND METHODS

A. *Chemicals and Materials*

Iso-Extract (30% w/v aqueous solution of iso-alpha-acids solution) was provided by Hopsteiner (Mainburg, Germany). Iso-Extract was certified to contain 30% iso-alpha-acids, < 0.6% alpha-acids and < 0.2% β-acids. Standardized samples of dicyclohexylamine (DCHA)-iso-alpha-acids ICS-I2 complex (66.5% (w/w) iso-alpha-acids were purchased from Labor Veritas, Zürich, Switzerland).

Di-distilled water used for HPLC was purified by means of a Milli-Q Gradient A 10 system (Millipore, Billerica, MA, USA). Methanol (HPLC LiChrosolv, gradient quality), phosphoric acids (85%) monohydrogenphosphat, dihydrogenphosphat and hydrochloric acid obtained from Merck (Darmstadt, Germany).

B. *Instruments*

A 22 ml covered glass test tube with cover was heated in an oil bath with temperature controller and stirrer. An inoLab pH 730 precision pH meter was used for titration of buffer solutions. All HPLC analyses was performed on an Agilent 1100 series system with an autosampler, a quarternary pump, thermosat and an Agilent 1200 series diode array detector (DAD).

C. *HPLC Analysis of iso-alpha-acids*

High-performance liquid chromatography (HPLC) is a widely used method for the analysis of alpha-acids, iso-alpha-acids and their degradation products. The injection volume was set to 10 µl. A column temperature was set at 35 ℃. The UV detector was set to a wavelength of 270 nm, providing the maximum absorption. Mobile phase A consisted of 100% methanol, while mobile phase B contained 75% methanol, 24% di-desitilled water and 1% phosphoric acid (85%). For analysis of iso-alpha-acids 100% mobile phase B was used, consisting of 0-17 min, 17-25 min 65% B and 25-60 min 100% B. For analysis of iso-alpha-acids a compact 10-min-method was developed and only 100% mobile phase B was used. After 10 samples in this way, standardized sample (DCHA-iso-alpha-acids) was measured with the long method. Degradation of DCHA-iso-alpha-acids and iso-alpha-acids can also occurred during the analysis but a very small percentage. Peak area was automatically integrated using Agilent Chemstation software.

D. *Sample Preparation and Heating Condition*

Prior to the boiling experiments, phosphate buffer solution was made with monohydrogenphosphat, dihydrogenphosphat or hydrochloric acid to attain the desired pH value: 4.5, 5.2, 5.5, and 6.5, respectively. Iso-alpha-acids extract was added into the buffer solutions. The initial iso-alpha-acids concentration was 100 mg/l. 20 ml sample was placed in each 22 ml glass test tube. When the oil bath achieved the required temperature (90 to 130 ℃), the glass test tubes were put into it. The temperature-sensor was inserted into the test tube and measured the temperature inside. When the temperature in the test tubes achieved the required temperature the timing began. In this case, temperature in the glass test tube increased to the desired value within 5 min. After a planned time interval, the test tube was taken out and cooled immediately in 0 ℃ cold water. Boiling time was set to 360 min. The tests for given temperature and pH value were repeated at least three times. Before HPLC analysis, the samples were filtered with a 13 mm syring filter (0.45 µm PTFE) (Alltech Associates, Deerfield, IL, USA) and analysed by quantitive HPLC as described below.

Procedure in this work, we used Matlab to determine reaction order and calculate reaction rates. Using differential methods, the following procedure is developed.

```
% The experimental concentration-time data are loaded;
load time.m
load concentration.m
% 0.1 min time instant within experiment time;
x=min(time):0.1:max(time);
% Interpolation of the concentration at each time point;
y=interpl(time, concentration, x, `pchip`);
% For any arbitrary time instant, get the slopes of the curves;
v=diff(y)./diff(x);
a=length (y)-1;
logc=log(y(1:a));
% The logc-log(-v) are plotted;
nk=polyfit(logc, log(-v), 1);
% Get the slope and intercept of the plotted curve;
n=nk(1);
k=exp(nk(2));
```

IV. RESULTS AND DISCUSSION

A. *Effect of Temperatures and pH Value on Degradation of iso-alpha-acids*

To determine the relationship between pH value, temperature, and degradation of iso-alpha-acids, experiments were carried out at five temperatures (90, 100, 110, 120, and 130 ℃) and at pH values in the range of 4.5-6.5.The degradation of iso-alpha-acids was strongly dependent on temperature. It is a well-known fact that raising the temperature increases the reaction rate. Typical plots of iso-alpha-acids concentration in % vs. time for pH values 4.5, 5.5, and 6.5 with temperature (100 and 120, 110, and 130 ℃) are shown in Fig. 2 and Fig. 3. As is seen, at 100 ℃ and pH value 4.5 64% of iso-alpha-acids were recovered after a 360 min of boiling, but at 130 ℃ only 2.3%. pH value also has a strong influence on the degradation of iso-alpha-acids, especially at lower temperatures (90, 100, and 110 ℃). At 100 ℃ and pH 4.5 the recovery rate of iso-alpha-acids was double that at 100 ℃ pH 6.5 after 360 min boiling. But for higher temperatures (120 and 130 ℃) the influence of pH decreased significantly. At 130 ℃ almost no changes were observed. At 130 ℃ and pH 6.5 the recovery rate of iso-alpha-acids sank to 1.6% nearly to 0%. Finally, it can be seen from Fig. 2 that raising the temperatures has more influence on degradation of iso-alpha-acids than rising pH value.

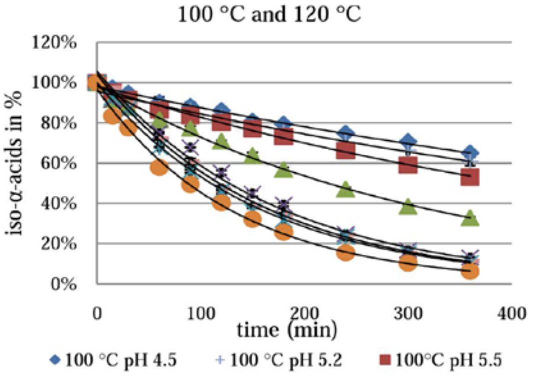

Fig. 2. Recovery rate of iso-alpha-acids after boiling with different pH value at 100 ℃ and 120 ℃.

B. *Determination of Reaction Order*

To explore the reaction order, that the detailed sequential fluctuation of concentration of iso-alpha-acids is analyzed at the time intervals. (1) is a general representation of the reaction rate, k represents the rate constant (min^{-1}), c is the reactant concentration (mg/l), n is the reaction order (no units) and v is the reaction velocity (mg/l·min), negative for reactants (iso-alpha-acids) and positive for degradation products.

$$v = -\frac{d(C_{iso})}{dt} = kC_{iso}^{\;n} \qquad (1)$$

A zero-order reaction yields a linear plot of concentration vs. time. A first-order reaction yields a linear plot of -ln(recovery rate of iso- alpha-acids) or log(recovery rate of iso- alpha-acids) vs. time. The two plots have been done in Fig. 4 and Fig. 5. Typical plots of -ln(recovery rate of iso-alpha-acids) vs. time are shown in Figs. 5-7. The rate constant k is independent of the concentrations of iso- alpha-acids but depends upon environmental factors, such as the temperature and pH value. Comparison between zero-order and first-order Plots has been made. It is clear that the correlation coefficients of -ln(recovery of iso- alpha-acids) vs. time are a marginal better than of concentration vs. time, especially at low temperature (90, 100 and 110 ℃).

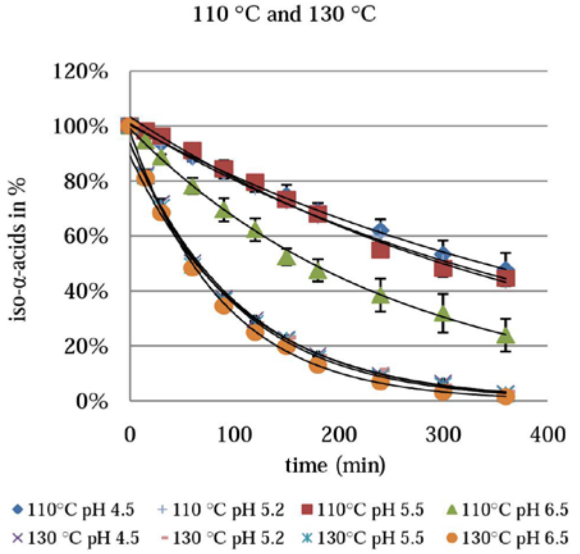

Fig. 3. Recovery rate of iso-alpha-acids after boiling with different pH value at 110 ℃ and 130 ℃

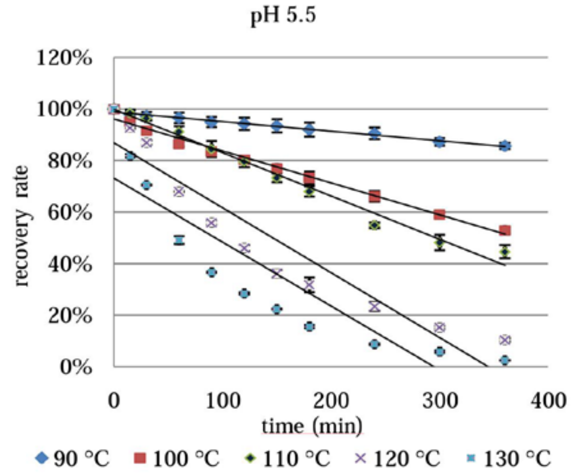

Fig. 4. (iso-alpha-acids) in % vs. time with different temperatures for pH 5.5.

To determine which reaction order (zero-order and first-order) should be used, we used the software Matlab to define the reaction order (2) can be seen by taking the logarithm on the both sides of the (1). lnv and lnCiso express a linear function with intercept lnk and slope n.

$$\ln v = \ln(-\frac{d(C_{iso})}{dt}) = \ln k + n \ln C_{iso} \qquad (2)$$

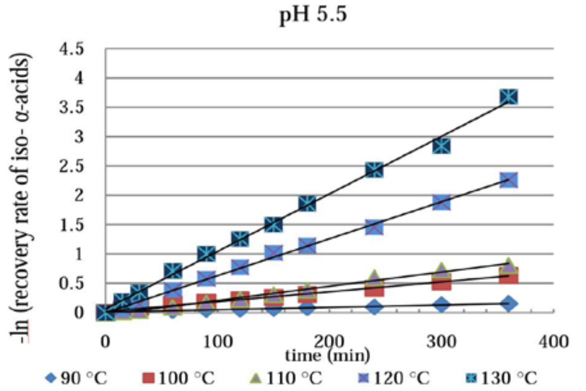

Fig. 5. ln(iso-α-acids)in % vs. time with different temperatures for pH 5.5

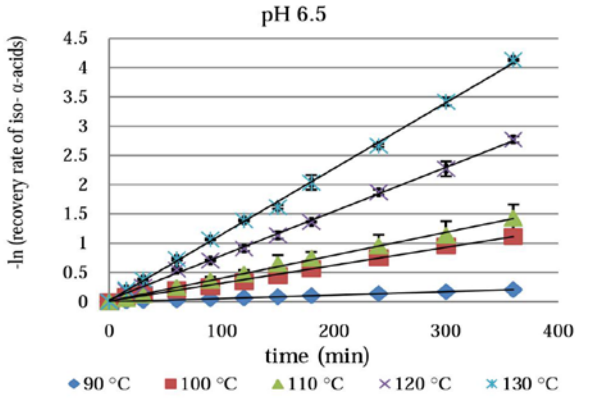

Fig. 6. ln(iso-alpha-acids) in % vs. time with different temperatures for pH 6.5

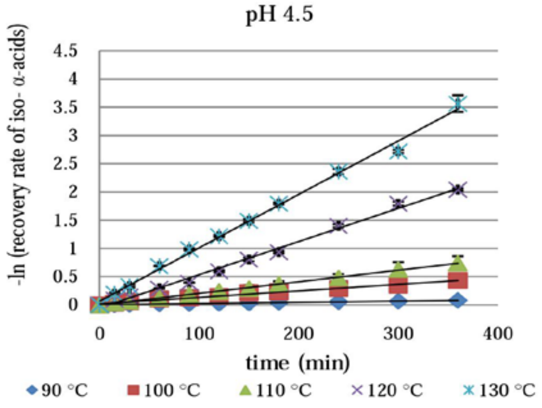

Fig. 7. ln(iso-alpha-acids) in % vs. time with different temperatures for pH 4.5.

The reaction rate constants and order of reactions are presented in Table I. The orders of reaction were calculated as approximately 1 (between 0.87 and 1.30) in all of the experiments. Therefore, it is possible to consider that the degradation of iso-alpha-acids were first-order reactions. The reaction rate constants k can be calculated direct with this program or using the slope of the linear regression line. Both values are very similar. Other authors, in the previously published work, used the slope of the linear regression. To compare the results of other authors in this paper, the reaction rate constants k were from the slope of the linear regression line in Fig 5-7.

It is a well-known fact that raising the temperature increases the reaction rate. Arrhenius equation is an equation that represents the dependence of the rate constant k of a reaction on the absolute temperature T. (Laidler 1996)

A plot of ln k vs. 1/T is shown in Fig. 8, which illustrates the degradation of iso-alpha-acids follows the Arrhenius equation.

$$\frac{d \ln k}{dT} = \frac{E_a}{RT^2} \quad ; \quad k = Ae^{-E_a/(RT)} \qquad (3)$$

Take the natural log of both sides

$$\ln k = -E_a/(RT) + \ln A \qquad (4)$$

$\ln k$ and $1/T$ express a linear function with intercept $\ln A$ and slope $-Ea/R$.

k is a first-order kinetic constant that changes with temperature, as given by the Arrhenius equation.Ea is the free energy of activation (kJmol^{-1}) in alkaline solution.

R is the universal gas constant (8.314 J K^{-1}mol^{-1})

T is the absolute temperature (K)

A is the pre-exponential factor (min^{-1})

The Slop of line ($-E_a/R$) allows calculation of the activation energy (E_a). And the pre-exponential factor A can be calculated with the intercept of the line.

The experimentally determined Arrhenius equations and activation energies derived therefrom for total iso-alpha-acids with different pH values, are summarized in Table I. The results obtained indicated that raising pH value from 4.5 to 5.5 and from 5.5 to 6.5 decreased the reaction energy fast 20 kJ/mol. *Clarke* noted that the iso-alpha-acids can be hydrolyzed or converted to humulinic acid with aqueous alkali. In iso-alpha-acids, splitting of the isohexenoyl side chain at C_4 prefers to at a high pH value.

TABLE I: FREE ENERGIES OF ACTIVATION FOR DEGRADATION OF TOTAL ISO-ALPHA-ACIDS, ISOCOHUMULONE ISOHUMULONE AND ISOADHUMULONE WITH DIFFERENT PH VALUES

Reaction energies (Ea) (kJ/mol)	4.5	5.2	5.5	6.5
total iso-alpha-acids	116.2±2.8	104.4±0.14	100.9±5.2	71.8±1.6
isocohumulone	117.1±2.9	103.7±0.15	101.6±4.8	73.8±2.0
isohumulone and isoadhumulone	115.8.±2.8	102.9±0.12	98.1±4.5	70.5±1.6

Fig. 8 shows that the regression straight lines of isocohumulone, isohumulone and isoadhumulone are almost the same. Arrhenius equation and reaction energies of degradation of total iso-alpha-acids, isocohumulone, isohumulone and isoadhumulone are nearly identical. Degradation of isocohumulone and the sum of isohumulone and isoadhumulone proceeded very similarly to the degradation of total iso-alpha-acids. The acyl side chain at C2 of iso-alpha-acids has no effect on splitting the isohexanoyl side chain at C4. The relative proportion of isocohumulone is around 30%.

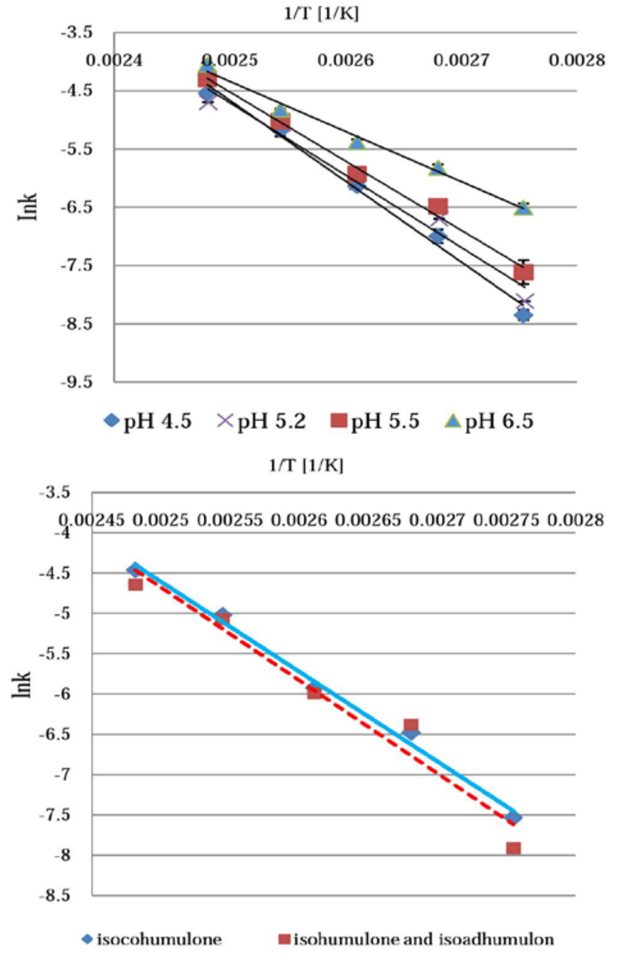

Fig. 8. Arrhenius behavior of degradation of total iso- α-acids (up) and of isocohumulone, isohumulone, isoadhumulone with pH 5.5(down).

The concentration of iso-alpha-acids during the wort boiling depended on the isomerization rate constants of alpha-acids and degradation rate constants of iso-alpha-acids. The concentration of alpha-acids decreased exponentially as a function of time (5). The initial concentration of iso-alpha-acids is zero. For this consecutive reaction, the concentration of iso-alpha-acids can be determined at time by using (8), that came from (5-7) [9].

Iso-alpha-acids are an isomerized form gained iso-alpha-acids by the application of heat in solution. At the same time, iso-alpha-acids are degraded into humulinic acids and other compounds. This reaction is an irreversible consecutive reaction. Each reaction step is first order. When the rate constants of each step was found, the integration of the kinetic Eqs. could also be determined.

Scheme 1: irreversible consecutive reaction of alpha-acids

$$alpha-acids \xrightarrow{k_1} iso-alpha-acids \xrightarrow{k_2} humulinic-acids$$

For the particular case of a two-step reaction (Scheme 1) starting with C_0 (alpha-acids), the solution can be expressed in the following form.

$$\frac{d(C_{alpha\text{-}acids})}{dt} = -k_1 (C_{alpha\text{-}acids})_0 \tag{5}$$

$$\frac{d(C_{iso-alpha-acids})}{dt} = k_1 (C_{alpha-acids})_0 - k_2 (C_{iso-alpha-acids}) \tag{6}$$

$$(C_{alpha\text{-}acids}) = (C_{alpha\text{-}acids})_0 \, e^{-k_1 t} \tag{7}$$

$$(C_{iso-alpha\text{-}acids}) = (C_{alpha\text{-}acids})_0 \left(\frac{k_1}{k_2 - k_1}\right)\left(e^{-k_1 t} - e^{-k_2 t}\right) \tag{8}$$

By using the Software Matlab the concentration of alpha-acids and iso-alpha-acids can be predicted in time series (Fig. 9). If the initial concentration of alpha-acids is 50 mg/l, after a normal boiling time of 60 min, at 100 ℃, the concentration of iso-alpha-acids was calculated to be 23.79 mg/l in the solution. After 90 min, i. e. normal boiling time and half dwell time in a whirlpool, the concentration was calculated to be 30.01 mg/l. The maximum concentration was reached after 200 min. Raising the boiling temperature decreased the time to reach the maximum concentration of iso-alpha-acids significantly. At 110 ℃ 90 min, at 120 ℃ 70 min und at 130 ℃ only 20 min.

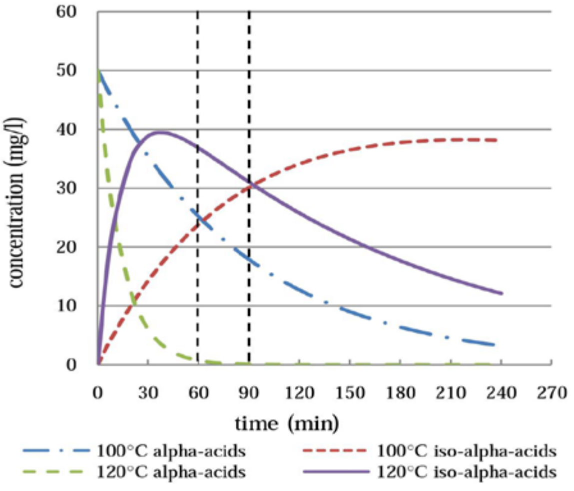

Fig. 9. Concentration of alpha-acids and iso- alpha-acids duing the wort at 100 ℃ and 120 ℃.

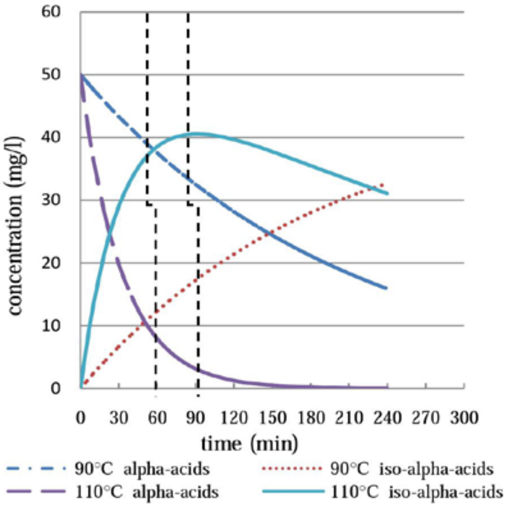

Fig. 10. Concentration of alpha-acids and iso- alpha-acids duing the wort at 90 ℃ and 110 ℃.

V. CONCLUSION

In this paper, the kinetics of degradation of iso-alpha-acids has been studied through boiling experiments in an aqueous buffer model system as a function of time, temperature and pH value. The free energy of activation and the reaction rate constant (k) of the degradation of total and individual iso-alpha-acids were calculated at four pH levels. The determination of the reactions order was run by a program compiled in Matlab R2007a language, which is a powerful software for numerical calculation. By raising the pH value of the reaction mixture increased the degradation of iso-alpha-acids. By raising the boiling temperature decreased the influence of pH value on the degradation of iso-alpha-acids significantly. Together with the kinetic rate of isomerization of alpha-acids, we developed a kinetic modeling for the irreversible consecutive reaction. This knowledge can be used for pre-isomerization of hops in the brewhouse, to save the energy and improved the hops utilization.

ACKNOWLEDGMENT

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Yarong Huang received a B.S.-Degree in human medicine from Shanghai Medical University in the year 1999. She was employed in July 1999 as a physician in the Tongren hospital in Shanghai. She chose to leave this position in 2002, in order to continue her studies in Germany. She studied Brewing Science and Beverage Technology and graduated with a Dipl.-Ing. Degree in brewing and beverage technology from the Technical University Munich (TUM) in the year 2009. Since December 2009 she has been employed as a PhD student in the field of brewing research to investigate the chemical reactions of important process markers during wort boiling processes at the TUM in Freising-Weihenstephan, chair of Brewing and Beverage Technology (Prof. Dr. Thomas Becker).

Errata

According to publications

page	written in publication	it should be
32	atmosphere boiler	atmosphere boiling system
33	P 1013 bar	P 1.013 bar
36	the radio of leucine and isoleucine	the ratio of leucine and isoleucine
36	the “sunlight” defect in milk [12]	the “sunlight” defect in milk [91]
52	85.5 to 101C; 6C; 100C	85.5 to 101 °C; 6 °C; 100 °C
52	Freidman	Friedman
53	take log of both sides	take ln of both sides
55	Tabel 2: TP	Tabel 2: T_P
56	Fig. 4.and Fig 5 Plots of lns versus 1/T	Fig. 4. Plots of lnß versus 1/T
56	slops	slope
58	References: CHEN, D.G.X. 1993	CHEN,D., Gao, X, Dollimore, D. 1993
59	Schellhammer et al. [3]	Malomicki et al. [3]
61	Fig. 2. In addition to ✚120 °C pH 5.2	▲100 °C pH 6.5 ✕ 120 °C pH 4.5 ✕120 °C pH 5.5 ● 120 °C pH 6.2

2.6 Coagulable nitrogen

Lit.: Brauwelt vol. 155 (2015), no. 10, p. 274-277

2.6.1 Importance of coag.-N

The practical brewer has long attached great importance to the concentration of coagulable nitrogen (coag.-N). In recent years, and also in connection with the introduction of modern boiling systems in breweries, the issue of optimum protein coagulation has become very topical in terms of foam stability, full mouth and colloidal stability of the beer.

In addition to the TBA and the DMS concentration, the remaining coag.-N of the hot wort is one of the decisive key parameters of wort boiling. The ratios between these three wort ingredients determine not only the optimum boiling time, but also the later beer quality in terms of mouth feel, taste, foam consistency, colour, beer stability and chemical-physical shelf life. While modern boiling systems with ever shorter boiling times and lower evaporation degrees attempt to achieve the desired wort quality, the TBA values will generally remain below the standard values without any problems. Further process engineering measures, such as wort stripping or post-evaporation, can lower the DMS concentration after the whirlpool rest. This makes it clear how important the decrease in coag.-N is for measuring the boiling time and temperature. The chemical kinetics of the decrease of the coag.-N has not yet been sufficiently investigated.

Depending on the malt quality, the lautered wort contains a wide variety of protein compounds as well as protein degradation products which have dissolved during the mashing process. By adding hops to the lautered wort, the protein concentration is slightly increased by the proteins contained in the hops. In the kettle full wort, the concentration of coag.-N is 3-7 mg/100 ml. The boiling of the wort with the protein denaturation and protein coagulation taking place in the process causes the elimination of a proportion of the total dissolved coag.-N. The wort boiling process is intended to lower the concentration of the coag.-N to 20-30 mg/l. A light-coloured wort contains 950-1150 mg/l total N, thereof 22 % high molecular N (2 % coag.-N), 18 % medium molecular N, 60 % low molecular N (Narziß and Back 2005, 2009).

The coag.-N depends on the amount of total nitrogen. With the Kjeldahl nitrogen measurement method, the concentration of total nitrogen for specific wort is proportional to the extract (Hagen and Schwarz 2000). The role of polyphenolic substances in their reaction with proteins for the decrease of coag.-N during wort boiling is controversially discussed in previous research. At present, the predominant view is that the significance of these polyphenolic substances can only be derived from their reducing properties, but not from their tanning power. The reason is that the hydrogen bond between polyphenols and protein molecules is not stable at temperatures above 80 °C (Miedaner 1986; Hui 2007; Siebert 2006; Biermann 1984). For example, the addition of the tanning agent extract did not cause any additional protein precipitation apart from 9 times the standard value (Biermann 1984, Schuster 1958). However, mixtures of hydrolysable polyphenols, such as tannins, or low molecular weight polyphenols, such as flavonols, catechins and anthocyanogens, are present in hops or malt. Flavonols are monomeric polyphenols with weak affinity for proteins and can be present in clear solutions in the presence of proteins. Catechins (flavan-3-ol) form the monomeric components of proanthocyanidins. The proanthocyanidins known as "anthocyanogens" are dimers, trimers of catechin, epicatechin and gallocatechin. They are important for the formation of the non-biological beer haze (Chapon 1993). Tannoids are dimers and trimers of up to 10 flavanol molecules and have an intermediate affinity for proteins. They produce insoluble compounds under certain conditions (Kühbeck 2006, 2007). Tannins are higher molecular weight reaction products. They precipitate proteins even at low concentrations, but never completely. (Leupold et al. 1981, Chapon 1993). Furthermore, the binding between the ε-amino groups and the iso-α-acids is influenced by the decrease of the coag.-N. From the point of view of process engineering, boiling temperature, boiling time, boiling method, flow conditions and pH value are also of great importance for the formation of coag.-N.

At the isoelectric point, the water solubility is minimal, as the net charge of the protein is zero, which makes it difficult for hydrate envelopes to form. Structure and solubility of barley and wheat proteins are related and influenced by temperature. However, the relationship between these properties varies with the type of protein and the composition of the protein solution.

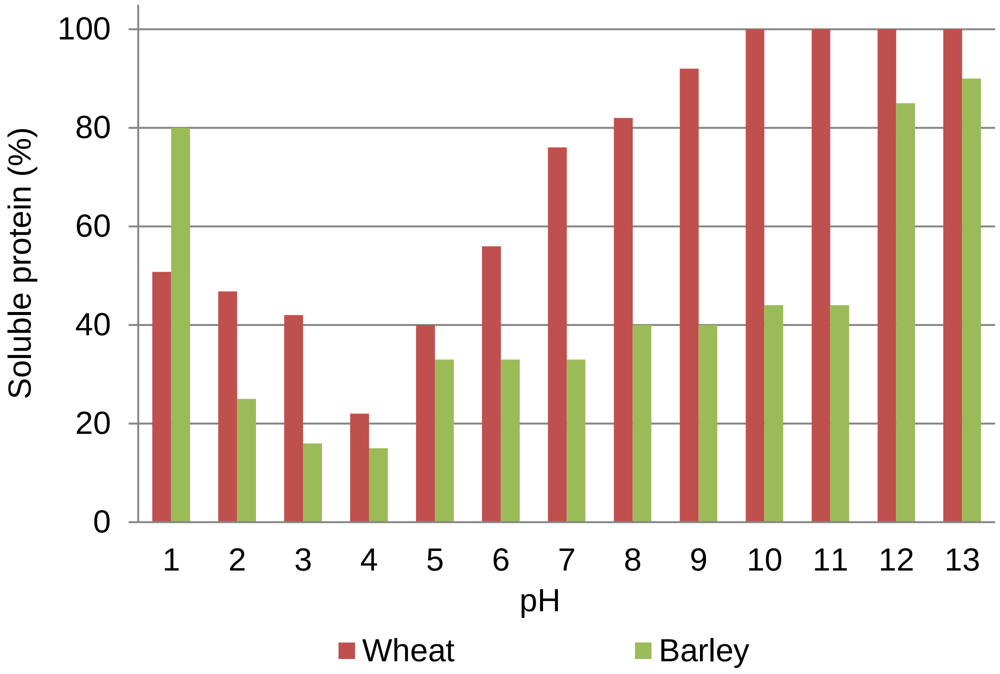

Fig. 11: Protein solubility of wheat and barley at various pH values (El-Hawary 1988)

During wort boiling, the amino acids and proteins are denatured. De Wit et al. investigated the solubility of the individual amino acids at different pH values with and without heat treatment. The solubility values are quite close to each other. There are no significant differences (Tab. 4). Above pH 5 the amount of protein coagulated is constant, but at lower pH values it is substantially reduced. In the brewhouse, the pH of the wort after technological acidification is 5.2, so the pH value was not considered as a variable parameter here.

Tab. 4 : Effect of heat treatments up to 155 °C on the amino acid composition of a 1 % ß-lactoglobulin solution in water at different pH values (De Wit 1984)

amino acids	Without heat treatment	Heated at pH 3	Heated at pH 6	Heated at pH 9
Trp	1.6	1.6	1.6	1.6
Ile	8.4	8.3	8.4	8.3

Tyr	3.8	3.8	3.7	3.7
Phe	3.7	3.8	3.8	3.8
Pro	8.2	8.4	8.1	8.2
Leu	22	22	22	22
Val	8.8	8.6	8.7	8.5
Lys	15	15	14.8	15.0
Met	3.9	4.0	3.9	3.9
Cys	4.8	4.7	4.4	4.2
Ala	13.9	13.8	13.8	13.9
Arg	3.1	3.0	3.0	3.0
His	1.8	2.1	2.0	1.9
Thr	8.0	7.9	7.8	7.9
Ser	7.0	6.9	6.8	6.9
Gly	3.4	3.4	3.5	3.4
Asx	15.0	15.0	14.9	14.9
Glu	25.0	25.1	25	24.9
Lal	0	0	0.031	0.050

2.6.2 Decrease in coagulable nitrogen

With the help of the statistical software Design Expert, version 6.0.11, a test program was created. Boiling temperatures (100, 115 and 130 °C), Reynolds number of stirring flow (950, 1900 and 2850), total nitrogen (1100, 1350 and 1600 mg/l), polyphenols (500, 700 and 900 mg/l) and α-acids (50, 100 and 150 mg/l) were varied as boiling parameters.

$$Re = n \cdot \rho \cdot d_2^2/\eta s \qquad (33)$$

The corresponding rotation frequency n is calculated from the Reynolds number.

$$n = Re \cdot \eta s/ \rho \cdot d_2^2 \qquad (34)$$

The dimensionless performance index (Newton number Ne) is calculated from the stirring power (P).

$$Ne = P/\rho \cdot n^3 \cdot d_2^5 \qquad (35)$$

In order to capture the influences and the significance of the various factors mathematically, the model equations for the decrease of the coag.-N are listed in Tab. 5. The concentrations of total nitrogen and the boiling temperatures are the dominant influences in protein coagulation. The polyphenol concentration and the stirring motion have no significant effect. In comparison, the α-acid concentration is of secondary importance.

Tab. 5 : Model equations for the removal of the coag.-N in the wort within 60 min boiling time

decrease of the coag.-N (mg/l)	model equations
coded	+117.14 + 25.26 · temperature + 6.60 Re + 25.66 · total nitrogen + 8.77 · polyphenols + 1.35 · α-acids
uncoded	-251.42 +1.68 · temperature + 0.01 · Re + 0.95 · total nitrogen + 0.04 · polyphenols + 0.027 · α-acids

To create kinetic models for removing the coag.-N, 11 % wort was heated in 20 ml headspace vials to different temperatures (90, 100, 110, 120 and 130 °C) in the laboratory heating block while being continuously stirred. After certain time intervals (0, 10, 20, 30, 40, 60, 90 and 120 min) the samples were taken. These were filtered immediately to avoid any further reaction. For each point in time, sampling was carried out in three ways. The concentrations of the coag.-N were calculated according to the following equation:

coag.-N in wort = coag.-N after 5 hours of boiling (MEBAK II 3.2.3)
- coag.-N after planned boiling time

During the heating-up phases, acceptance tests of the coag.-N have already taken place. This led to different initial concentrations of the coag.-N. The heating rates were 10 K/min.

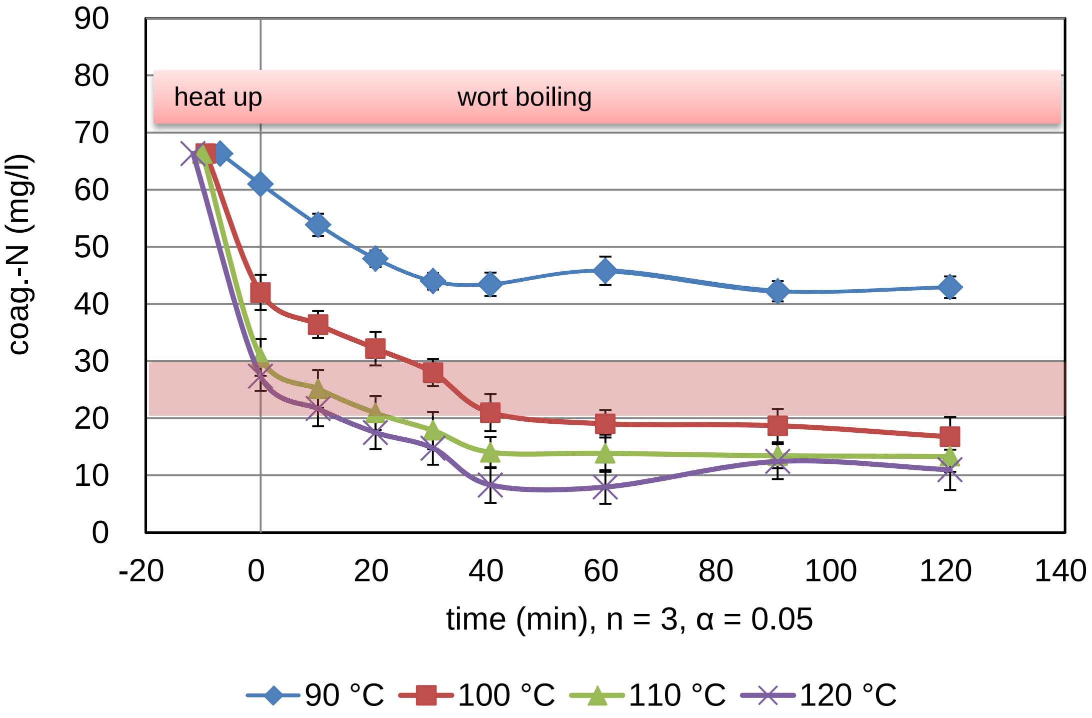

Fig. 12: Decrease of the coag.-N during heating and wort boiling

Optimum concentrations of coag.-N in the hot wort are 20-30 mg/l (marked red in Fig. 12). It can be seen from Fig. 12 that a temperature of 90 °C is not sufficient to achieve the desired concentration for the coag.-N. At 100 °C and a boiling time of 1 h a satisfactory protein excretion is given. Already during the heating process the concentration of coag.-N decreases very strongly. At 110 and 120 °C, the upper limit of 30 mg/l is already reached during heating. More nitrogen is precipitated at 130 °C than during a 5 h boiling period according to MEBAK II 3.2.3. The calculated values of the coag.-N are therefore mathematically negative.

It can clearly be seen that the most nitrogen was excreted within the first 30 min of boiling. After about 40 min boiling time, the concentration of the coag.-N changes hardly more. Therefore only one kinetic model for the first 30 min boiling time was created in the context of this work. In the literature, protein precipitation is often described as a 1st order reaction. In order to check whether a 1st order reaction satisfies the speed law of protein coagulation, the natural logarithm of the recovery rate is plotted against time. A linear regression was performed (Fig. 13).

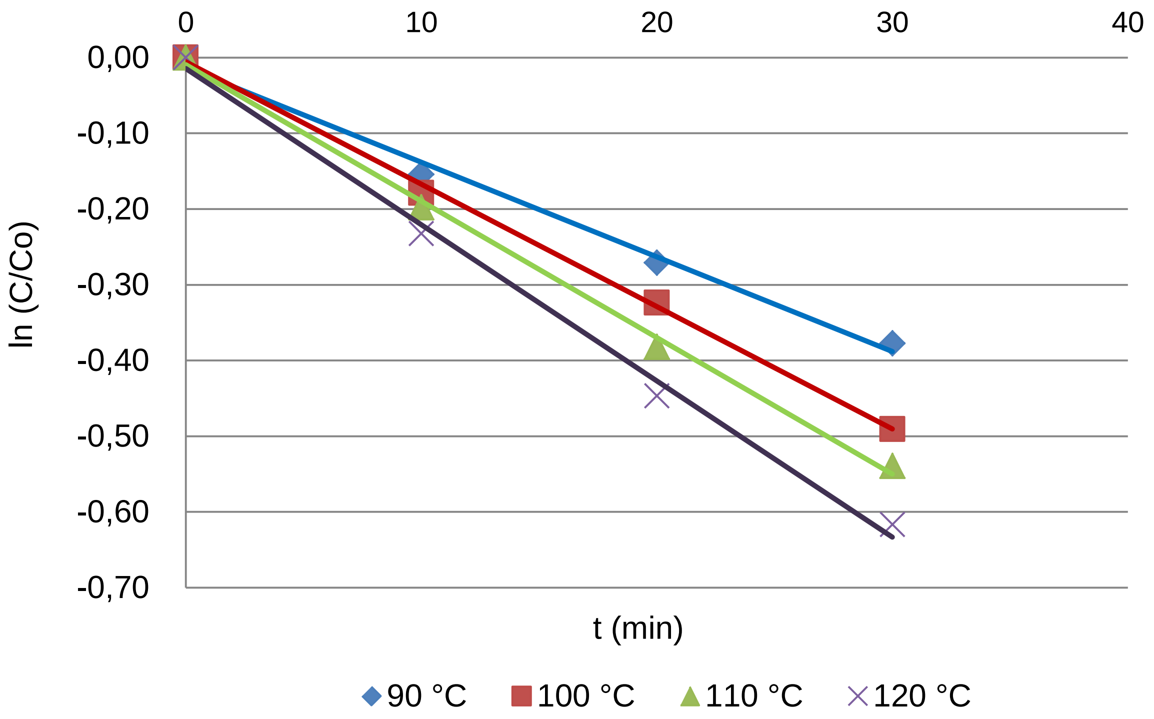

Fig. 13 : Linearization of the coag.-N for the first 30 min boiling time

The logarithmic form of the Arrhenius equation can be used to deduce the required activation energy from experimentally determined reaction rate constants of a reaction:

$$\text{lnk} = -E_a/R\cdot T + \ln A \tag{36}$$

where k is the reaction rate constant, E_a the activation energy, R the universal gas constant (8.314 J $mol^{-1}K^{-1}$), A the pre-exponential factor, and T the absolute reaction temperature in K. The values are summarized in Tab. 6.

Tab. 6 : Kinetic parameters of the decrease of the coag.-N

time (min)	R^2	E_a (kJ/mol)	A (min^{-1})	Arrhenius equation
0-30	0.9683	19.238+/-7.973	7.5625	$k = 7.5625\ e^{(-2313.9156/T)}$

A diagram is created from the kinetic parameters (Fig. 13), which shows the relationships between the boiling time, the boiling temperatures and the decrease of the coag.-N. The diagram shows the relationship between the boiling time, the boiling temperatures and the decrease of the coag.-N. The kinetic parameters are then used as a basis for the calculation.

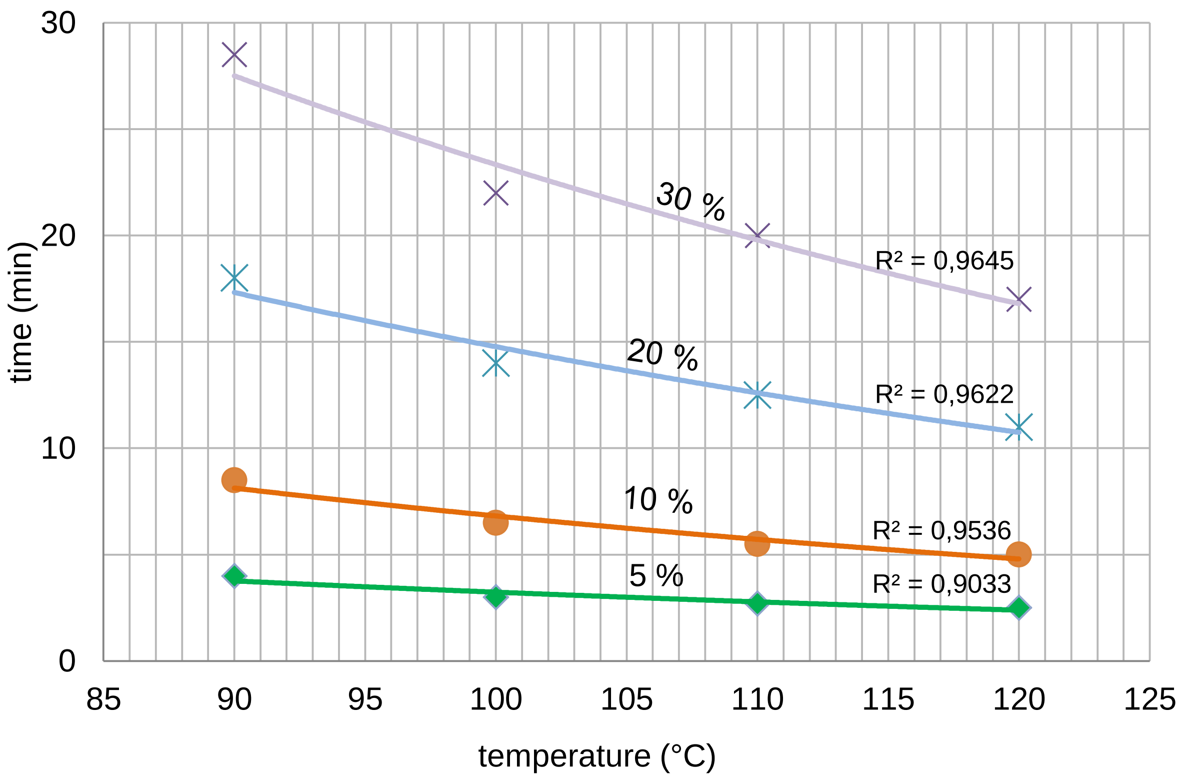

Fig. 14: Calculated decrease of coag.-N in % as a function of boiling time and boiling temperature

Conventional boiling systems tend to have a strong tendency towards protein coagulation, while modern boiling systems are gentler in the sense of lower coag.-N precipitation. In addition to the boiling temperatures, the heating rates on the decreases of the coag.-N also play an important role. To simulate the heating process, from kettle full wort (72 °C) to boiling temperature (90 to 120 °C), the decreases of the coag.-N were theoretically calculated with kinetic parameters and shown in Fig. 14. In general, the kinetic equation of a decomposition reaction can be represented as eq. (37).

$$\frac{\mathrm{d}\alpha}{\mathrm{d}t} = k(T)\,f(\alpha) \tag{37}$$

The dependence of the reaction rate constant k on the temperature k(T) can be represented by the approach of Arrhenius:

$$k(T) = A\exp(-E_a / RT) \tag{38}$$

The heating rate β is a function of temperature (T) and time (t).

$$dT / dt = \beta \tag{39}$$

Eq. (38) and eq. (39) are inserted in eq. (37). After integrating eq. (37), eq. (40) results, where α represents the reaction conversion; T the reaction temperature in K; ß the heating rate and G(α) the integral function.

$$G(\alpha) = \frac{A}{\beta} \int_{T0}^{T} \exp(-E_a / RT) \mathrm{d}T \qquad (40)$$

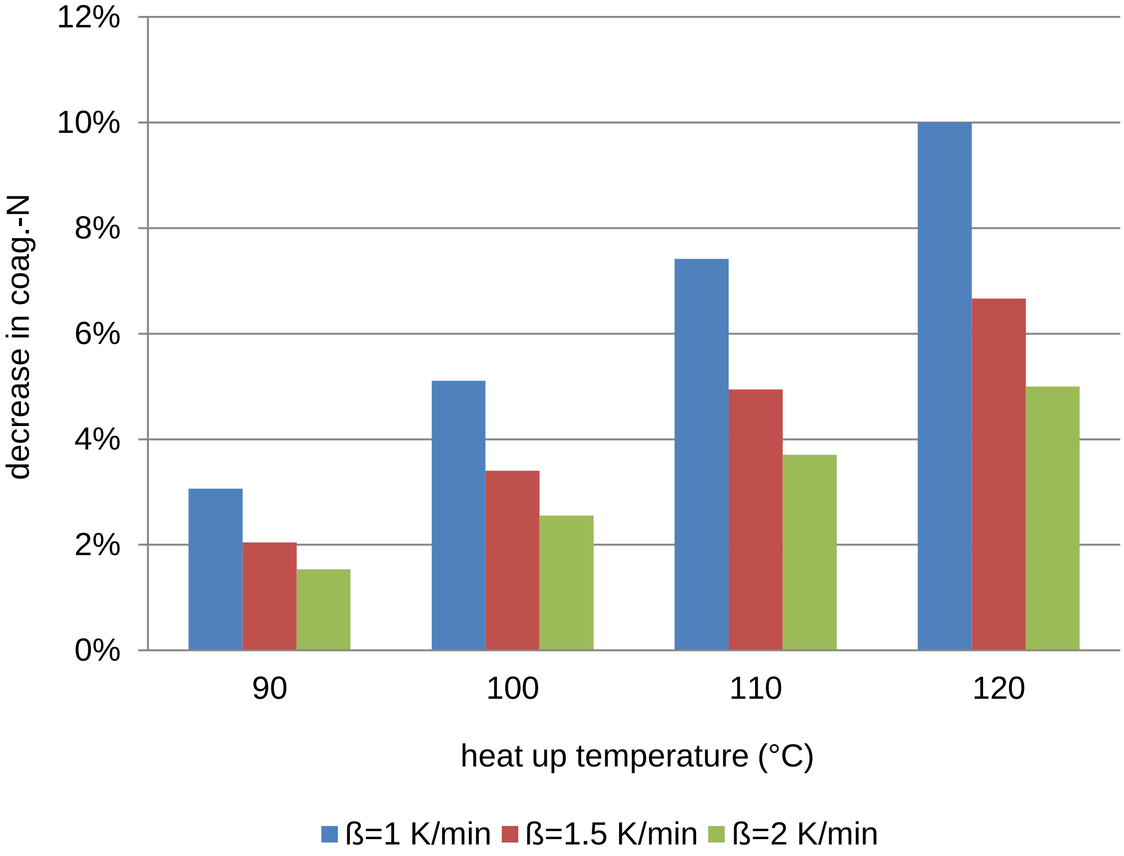

Fig. 15: Calculated decrease of the coag.-N during the heating phase

If the wort is heated at 72 °C with a heating rate of 1 K/min, it takes 28 min to reach the boiling temperature of 100 °C and during this time the coag.-N concentration already drops by around 5 %. If the heating rate is increased to 2 K/min, the heating time is halved and at the same times the decrease of the coag.-N decreases.

3 Discussion

How much time does it actually take to boil the wort? What is the key task? The minimum times required to fulfil the key wort boiling tasks are shown in Table 7. The time required to achieve the desired original wort concentration depends on the initial concentration of the kettle full wort and the heat output of the boiling system. The reaction speed is determined by the boiling temperature and/or pressure. When boiling under atmospheric conditions with a standard hourly evaporation of 8 %, the boiling time is approximately 60 min. The thermal energy consumption is approximately 5 kWh/hl without taking thermal energy losses into account. To vary the boiling temperatures, the temperature or pressure of the heating medium (steam or hot water) can be adjusted if the heat transfer surface is sufficiently dimensioned.

Conventional overpressure boiling systems are LPWB, with temperatures between 102-105 °C, and HTWB (rarely used in breweries) employing temperatures between 120-140 °C. Boiling times are greatly reduced with both methods. With LPWB, there is a constant change between a 3.5 min pressure increase and a 2.5 min pressure release. The total evaporation can be reduced to 4.5 % in relation to the volume of wort. The total boiling time is thereby reduced by 10-20 min. When employing HTWB, wort is normally heated to temperatures ranging from 128-135 °C in three stages through three heat exchangers connected in series. The wort is maintained at this temperature for 2.5-3.0 min in the last stage. The wort temperature can then be gradually reduced in two expansion stages. Evaporation rates in this process are 6-8 % (Hackensellner 2001, Bühler et al. 2003, Scheller 2008). Advantages of the HTWB are on one hand shortening the actual boiling time, not taking into account heating duration, and on the other hand, the low thermal energy demand required resulting from the heat recovery between the heating stages. However, savings from reduced boiling time are somewhat reduced by increased cleaning requirements on the boiling system. A disadvantage of the HTWB is the so-called cooking taste (Schneider 1989). Due to the many formed Maillard products, a cooking taste can be unpleasant (Narziß et al. 1991, 2005). A significant issue with HTWB is fouling on the heat exchanger surfaces. If cleaning is not carried out, a fluctuating heat transfer will occur, which leads to an uneven wort thermal load. A practical solution would be heat transfer stages arranged in parallel, so during boiling in one heat exchanger row the other could be cleaned. The cleaning effect can also be improved by adding hydrogen peroxide to the caustic solution.

Tab. 7 : Reaction constants and activation energies in relation to the different tasks of wort boiling and the time to reach different conditions

		condition	Q (kWh/hl)	k or function		E_a (kJ/mol)	t (min)			
							100 °C	110 °C	120 °C	130 °C
set original wort		8 % evaporation	5.02				60	40-50 min 102-105°C	2 min 120-140 °C	
α-acids isomerization		hopping rate of 5.5 g/hl	0.02	$k_{iso} = 7.90 \cdot 10^{11} e^{-11858/T}$		98.6	time to reach maximum concentration of iso-α-acids			
							140	90	70	20
protein coagulation		2-3 mg/100 ml		$k_{coag.-N} = 7.5625 \cdot e^{-2313.9/T}$ first 30 min		19.23	time to reach 2-3 mg/ml			
							20-40	0-20	0-12	
		decrease of the coag.-N = -251.42 +1.68 · temperature + 0.01 · Re_R + 0.95 · total nitrogen + 0.04 · Polyphenols + 0.027 · α-acids								
Flavours development	2-MB		0.0015	$k_1 = 1.62 \cdot 10^{3} \cdot e^{-5732.6/T}$	$k_2 = 2.65 \cdot 10^{12} \cdot e^{-13259/T}$	157.97				
	3-MB		0.005	$k_1 = 2.04 \cdot 10^{3} \cdot e^{-5448.8/T}$	$k_2 = 6.39 \cdot 10^{12} \cdot e^{-13283/T}$	155.73				
	DMS	<100 µg/l	0.001	$k_{DMS} = 3.51 \cdot 10^{10} \cdot e^{-9757/T}$		81.12	If SMM < 100 µg/l			
							110	47	14	0
Evaporation of unwanted aromatics (DMS)				evaporation heat 437 J/g		0.00036				
sterilizing		bacillus subtilis		Z-value =14.9 K	D-value =10.7 min (90°C)	88	time to kill the bacillus subtilis			
							= 2.6	< 2.6	< 2.6	< 2.6

The isomerization of α-acids to iso-α-acids could be achieved within a wort boiling time of only 20 min at 130 °C. At 100 °C and pH 5.2 the maximum concentration of iso-α-acids is only reached after a theoretical boiling time of 140 min. However, achieving maximum isomerization of α-acids for wort boiling is not mandatory. The costs for hops or hop products are comparatively low. If the hop price increases in the future and the energy price decreases, it is recommended to aim for maximum isomerization. There are other ways to achieve a higher degree of isomerization. On the one hand already isomerized hop products could be added directly and on the other hand the first hop dosage, if an appropriate dosing device is available, could be added already in the flow vessel at a higher temperature (up to 130 °C). In this way a higher hop yield could be achieved with a considerably reduced boiling time. The dissolving of the aroma-intensive hop oils could partly also be carried out outside the brewhouse by adding hops in downstream fermentation or storage vessels.

The hot wort residual coag.-N value was used to assess protein precipitation. The concentration of coag.-N is another key parameter. Protein coagulation during wort boiling is a first-order reaction during the initial 30 min. While heating at a rate of 10 K/min, the concentration of coag.-N decreases rapidly. After a boiling time of approximately 40 min, the concentration exhibited minimal change. A kinetic model for a boiling time during the first 30 min boiling was developed. The corresponding decrease of coag.-N in % coinciding with the boiling time can be read from the temperature/boiling time diagram (Fig. 14). With the developed formula (Tab. 5), it was found that temperatures play the most important role for the decrease in coag.-N. With a value of 5.2, the pH is not considered a variable in this context, but as a constant. The initial concentration for total nitrogen is the second most important influencing variable. The α-acids concentrations as well as stirring speeds have essentially no influence. The homogenization of the wort samples is described by the Reynolds number (Re). The Reynolds number depends on one hand on the properties density ρ and dynamic viscosity η of the wort and on the other on the characteristic variables of the stirrer rotation, i.e. its speed n and the diameter d. Interestingly, in the so-called "SchoKo" gentle boiling process, the wort must be stirred during the heat retention time. Primarily, convection makes protein coagulation possible.

Employing atmospheric boiling, the residual concentration of coag.-N is already at 2-3 mg/100 ml after boiling 20-40 min. After boiling more than 40 min, the residual coag.-N concentration decreases no further at all boiling temperatures (90-120 °C) (Fig. 12). Boiling at 110 °C, approximately 20 min is required to achieve 2 mg/100 ml, boiling at 120 °C approximately 10 min. It is stated in the literature that boiling at 135 °C 3 min, the residual concentration of coag.-N decreases to 1.4 mg/100 ml (Narziß et al. 2009). However in laboratory tests it was discovered that the residual coag.-N concentration falls below 1 mg/100 ml even during heating. However, evaporation is not required for sufficient protein elimination. It is sufficient if the wort is kept at boiling temperature for a certain period of time. The temperature must be just below boiling temperature. Fig. 12 shows at 90 °C after a boiling time of 120 min the residual coag.-N remains essentially unchanged at 4.4 mg/100 ml. Higher concentrations of the residual coag.-N lead to a smearing of the yeast and to a reduced colloidal stability. Too low concentrations can lead to poor foam head retention in the finished beer.

The 3-MB taste threshold in beer is 0.6 mg/l, which is significantly lower than for 2-MB at 1.2 mg/l (Meilgaard et al. 1970). Both flavouring components are described as indicators for wort boiling and beer ageing (Hertel 2007, Feilner 2013). Due to their high distribution factor, the entire portions of the 2 and 3-MB formed during wort boiling are evaporated (Feilner 2013, Ditrych et al. 2019). After boiling, the rate of evaporation decreases, but 2-MB and 3-MB are still reproduced and their concentrations in wort increase again in during the whirlpool rest. During beer ageing, the concentrations will continue to rise even at low temperatures. It is understandable that the intermediates of the Maillard reaction will continue to form 2- and 3-MB at lower temperatures. An increased formation of the two Strecker aldehydes during beer aging causes a deterioration of flavour stability. To date, a maximum allowed concentration of the intermediates in the wort has not been defined. The intermediate products are already formed during malting and mashing. The possibility of optimizing flavour stability would be to boil the wort for a shorter period of time and to lower the kilning temperature during malting.

DMS is formed from SMM at temperatures >85 °C. Due to the slight volatility of DMS and the strong movement of the wort in the brew kettle, DMS is quickly evaporated after formation. The reproduction of DMS in the whirlpool is an important parameter for the DMS concentration in the later beer. The later DMS concentration in the beer depends on the SMM concentration in the whirlpool. According to the kinetic model shown, 14 % SMM are already eliminated when the wort is raised to boil and 16 % SMM are subsequently eliminated in the whirlpool after wort boiling. Free DMS is essentially completely evaporated within a 30 min boiling period (Narziß and Back 2005). The DMS newly formed during boiling is evaporated immediately. The DMS concentration remains well below 100 µg/l until the end of boiling. If the SMM concentration in the whirlpool remains below 100 µg/l, the DMS concentration will always remain below the limit value of 100 µg/l. The SMM initial concentration depends on the malt quality and the mashing process. Investigations of kettle full wort revealed SMM concentrations of approximately 380 µg/l. In order not to exceed a DMS concentration in cooled wort of 100 µg/l, a boiling time of about 55 min at 100 °C is required. At 110 °C, a boiling time of 30 min is sufficient.

A shortened boiling time can lead to insufficient SMM cleavage and increased DMS replication in the whirlpool. Possible countermeasures are: Pre-cooling of the wort before entering the whirlpool, evaporation under vacuum, thin-film evaporation and wort stripping. Further possibilities are an increase in the boiling temperature and a shortening of the whirlpool rest or the use of other systems and processes in order to simulate less DMS. With long boiling times, good evaporation is achieved, but too low values for the residual coag.-N. With shorter boiling times, excessive DMS values are achieved, but good values for the residual coag.-N.

To only sterilize the wort, a boiling temperature is not required. Apart from a few heat-resistant spores, the microflora of malt, hops and malt substitutes in the wort cannot survive the heating phase. The D- and z-values given in the technical literature allow a calculation of the killing kinetics of spore-forming bacteria (Oliver-Daumen 2010).

An ideal minimum boiling time cannot be determined. With gentle boiling processes, the boiling temperature is not attained. With dynamic boiling processes, the boiling time is reduced to a few min, based on the actual boiling temperature. When using a wort stripping process, the desired DMS concentrations are easily achieved. A complete isomerization of the α-acids need not to be forced employing extended boiling times, as the costs of hop products are not economically significant.

The concentration of the coag.-N and the TBA values are two important parameters for determining the boiling time. The kinetic knowledge of the formation of the flavouring substances on the one hand and their evaporation on the other hand cannot be considered independently of each other. Only if both processes are considered simultaneously, the changes in aroma substance concentrations can be described comprehensively. Decisive for determining the boiling period is ultimately setting the desired original wort concentration goals under economic aspects, i.e. taking into account the current raw material and energy costs.

Vigorous boiling of wort is not essential to complete the defined tasks of wort boiling. But the efficient time- and energy-saving benefits of high-temperature boiling make it potentially interesting for breweries. While the idea of continuous wort boiling is not new, research into the formal kinetic description of chemical reactions of important process markers during boiling processes may prove the process advantageous. Variation of process markers with temperature can be calculated by the formula for chemical kinetics. In the process of continuous wort boiling, the wort in the balance tank is heated through a plate heat exchanger and then heated to 130 °C with steam. After being held at this temperature for a several seconds, the wort is returned to the heat exchanger to preheat the incoming wort. As can be seen from Tab. 7， most of the commonly quoted reasons for wort boiling are completed with the exception of hop isomerization. The use of post-fermentation bittering removes the need for extracting hops in the kettle.

A complete automation of the production process requires a multitude of process data. The kinetic mechanism of the formation of important process markers in the wort plays an important role in an automated brewhouse process. On this basis, the process parameters can be scientifically adjusted or programmed.

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