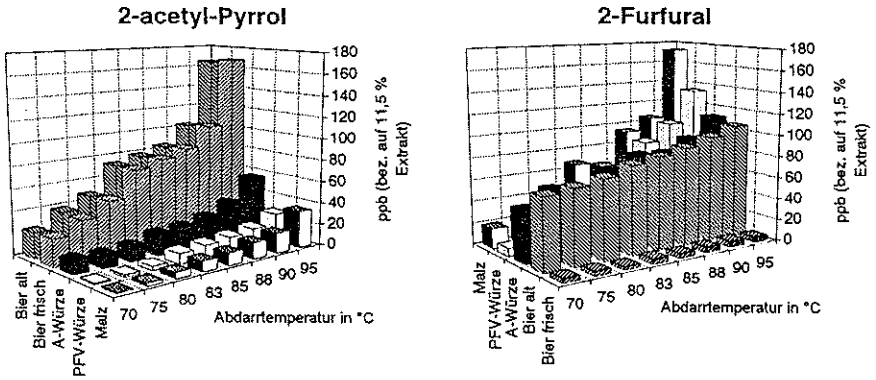


Abb. 7. Indikatoren für thermische Belastung bei unterschiedlichen Abdarrtemperaturen in Malz, Würze und Bier

## SCHLUSSBETRACHTUNG

Mit den dargestellten Methoden wurden die Auswirkungen unterschiedlicher Schwelk- und Darrverfahren auf die Aromastoffzusammensetzung im Malz verfolgt.

- Beim Schwelken geht das Ausdampfen der flüchtigen Fettabbauprodukte mit der Abnahme des Wassergehalts im Malz einher.
- Beim Abdarren erfolgt eine exponentielle Zunahme der Maillard-Reaktionsprodukte mit steigenden Abdarrtemperaturen.

Der Einfluß der durchgeführten Darrverfahren auf den Geschmack und die Geschmacksstabilität der Biere stellt sich folgendermaßen dar:

- Schwelkverfahren: Das Bier aus dem Schwelkversuch 7 mit kontinuierlich ansteigender Schwelktemperatur wurde sowohl im frischen als auch forciert gealterten Zustand am besten bewertet. Darauf folgten die Biere aus dem Schwelkversuch 4 (12 h 55 °C) und Schwelkversuch 8 (Keimdarrkasten).
- Abdarrverfahren: Biere aus Malzen mit Abdarrtemperaturen zwischen 80 °C und 88 °C wurden sensorisch am besten beurteilt. Abdarrtemperaturen über 90 °C ergaben bei gleicher Abdarrzeit Biere schlechterer Geschmacksstabilität.

Für die Praxis bietet sich das Schwelken mit kontinuierlich ansteigender Schwelktemperatur an. Mit mehreren Temperaturmeßstellen in verschiedenen Schichthöhen im Malz, welche als Weiterschaltbedingungen für den Temperaturverlauf unter der Horde dienen, könnte dieses Schwelkverfahren noch optimiert werden.

Das Abdarren kann gezielt durch Kombination von entsprechenden Abdarrtemperaturen und Abdarrzeiten erfolgen, um gewünschte Werte des DMS-Precursors und von Maillard-Reaktionsprodukten zu erhalten. Eine zu hohe thermische Belastung ist in Hinblick auf die Geschmacksstabilität der Biere jedoch zu vermeiden.

Die Analytik der Malzaromastoffe bietet somit die Möglichkeit die Darrtechnologie anhand von Indikatorsubstanzen in Malz, Würze und Bier zu bewerten. Damit kann bereits im Malz das Aromaprofil des fertigen Bieres in gewissen Grenzen vorgegeben werden.



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# Trans-2-nonenal degradation products during mashing

Sophie Noël & Sonia Collin

Université Catholique de Louvain, Unité de Brasserie et des Industries Alimentaires, Place Croix du Sud 2/bte7, B-1348 Louvain-la-Neuve, Belgium

*Descriptors*
*Beer analysis result, 2-nonenal, precursor, staling*

SUMMARY

The formation pathways of trans-2-nonenal in aged beer are still widely controversial.
The stability of trans-2-nonenal from raw materials, studied at the pH of wort, reveal the complexity of the mechanisms involved. Among the major degradation products of trans-2-nonenal, new compounds, unknown in the brewing literature, have been identified and quantified by mass spectrometry in large amounts.
Some of them, releasing trans-2-nonenal during ageing, are suspected to be possible precursors of papery cardboard flavour in beer storing conditions.

COMPOSES DE DEGRADATION DU TRANS-2-NONENAL AU BRASSAGE

*Descripteurs*
*Formation du goût d'éventé, nonénal-2, précurseur, résultat d'analyse de bière*

RESUME

Les voies de formation du trans-2-nonénal des bières vieillies sont encore largement contestées.
La stabilité du trans-2-nonénal, issu des matières premières, étudiée dans les conditions de pH d'un moût, révèle la nature complexe des mécanismes incriminés. Parmi les principaux produits de dégradation, de nouveaux composés, non mentionnés précédemment dans la littérature brassicole, ont été identifiés et quantifiés par spectrométrie de masse, en quantités importantes.
Certains sont apparus comme étant des précurseurs plausibles de l'arôme de carton des bières vieillies vu leur aptitude à se retransformer en trans-2-nonénal lors du vieillissement.

ZUSAMMENSETZUNG DER ABBAUPRODUKTE DES TRANS-2-NONENAL BEIM BRAUEN

*Deskriptoren*
*Altgeschmacksbildung, Ergebnis von Bieranalysen, Nonenal-2, Precursor*

ZUSAMMENFASSUNG

Die Bildungswege für trans-2-Nonenal im gealterten Bier sind immer noch nicht klar bekannt.
Die Stabilität von trans-2-Nonenal (das aus den Rohmaterialien stammt) zeigte bei der Untersuchung in Würzen mit verschiedenen pH Werten, wie komplex die dabei ablaufenden Mechanismen sind. Unter den Hauptabbauprodukten von trans-2-Nonenal wurden neue Verbindungen mittels Massenspektrometrie quantifiziert, die in der Brauereiliteratur noch unbekannt sind.
Einige dieser Verbindungen setzen während der Alterung wieder trans-2-Nonenal frei und sind vermutlich Vorstufen von papierartigen - Cardboard - Flavour Noten bei der Bieralterung.



## INTRODUCTION

Improving the flavour stability of packaged beer is still a goal of many brewers (1). Trans-2-nonenal is considered to be the major aldehyde involved in causing stale flavour.

Currie & al (2) have previously shown that 90% of the initial trans-2-nonenal disappear during mashing, no matter the wort oxygen ratio. This paper aims at evaluating the relative influence of various alcenal degradation pathways (at wort pH and temperature), e.g. : oxidation, hydration and reaction with wort amino acids, pathways possibly involved in the production of card-board aroma in aged beer.

## METHODS

Kinetic curves :

20 ml of a 4 ppm trans-2-nonenal solution at pH 5.2 was flushed with argon, for 5 minutes, prior to the 76°C oxygen free heat treatment in an hermetic bottle. After dichloromethane liquid/liquid extraction and concentration (Kuderna vessel), the extract was injected on a GC-FID or GC-MS system.

18L of an amino acid solution was prepared in a 30L fermentor according to a usual wort composition (see Table I). After flushing with nitrogen, the pH solution was fixed at 5.2. When temperature reached 36°C, 2L of a 100 ppb trans-2-nonenal solution were added. For this experience, the temperature diagram given in figure 4 was applied. After extraction with a C18-Bond Elut cartridge and concentration in a Kuderna vessel, the analysis was performed on a GC-FID or GC-MS system.

Table I : Amino acid composition in a usual wort.

| Amino acid | Concentration (ppm) | Amino acid | Concentration (ppm) |
|---|---|---|---|
| Proline | 350 | Isoleucine | 85 |
| Leucine | 160 | Serine | 70 |
| Arginine | 150 | Glutamic acid | 60 |
| Phenylalanine | 150 | Histidine | 60 |
| Threonine | 150 | Aspartic acid | 55 |
| Valine | 130 | Tryptophane | 45 |
| Alanine | 120 | Glycine | 40 |
| Tyrosine | 100 | Methionine | 35 |
| Lysine | 95 | Cysteine | 0 |

Gas chromatographic analysis :

For gas chromatography, we used a Hewlett Packard Model 7673 automatic sampler, a cold on-column injector, a FID detector, and a Shimadzu CR4A integrator. Analysis was carried out on a 50mX0.32mm, wall-coated,open tubular (WCOT) CP-SIL5 CB capillary column (film thickness, 1.2 µm). The oven temperature was programmed to rise from 30 to 80°C at 20°C/min, then to 200°C at 2°C/min. The carrier gas was helium at a flow rate of 1.5 ml/min. The injector temperature was maintained at 3°C above the oven temperature. The detector temperature was 275°C. The minimum peak area for data acquisition was set at 500 µV.sec.

For GC-MS analysis, the same column was directly connected to an HP 5988 quadrupole mass spectrometer. Electron impact mass spectra were recorded at 70 eV. Spectral recording throughout elution was automatically performed with the HP59970C software.

Synthesis of suspected nonenal-derived compounds :

3-hydroxynonanal synthesis. *First step:* In a 50 ml agitated three-necked flask equipped with an ice-water condenser, 2ml of heptanal were dropped for 30 minutes into 20 ml of a 1 molar allyl magnesium bromide solution in anhydrous ether. After one hour, 15 ml of ice-water were dropped in the solution. The resulting solid was dissolved by adding 4.5M sulfuric acid. After anhydrous magnesium sulfate treatment, the combined organic phases were filtrated and finally dried to obtain 4-hydroxy-1-decene.

*Second step:* In a 30 ml ether / water (50:50) mixture, 0,8 g of 4-hydroxy-1-decene and 6 ml of osmium tetraoxide (0.08M) were stirred for 5 minutes during which the mixture became dark brown (osmate ester formation). Subsequently, 2,3 g of sodium periodate were added over a period of 30 minutes, at 25°C. The mixture was stirred for an additional 1,5 hours. The organic layer was removed and the aqueous layer thoroughly extracted three times with 10 ml of ether. The combined organic layers were filtered through anhydrous sodium sulfate.

2-nonenoïc acid synthesis. In a 250 ml flask equipped with a reflux condenser and a calcium chloride drying tube, 0,5 mole of malonic acid were dissolved in 92 ml of dry pyridine. 0,5 mole of freshly distilled heptanal were added to the solution stirred and maintained at 0°C. After 60 hours, the mixture was heated until completion of the release of carbon dioxide. The solution was washed with the same volume of water. 150 ml of HCl (25%) were added into the organic phase. The resulting precipitate was dissolved in toluene, washed three times with water and, finally, vacuum distilled (nonenoïc acid collected at 162°C).

Putrescine-nonenal Shiff base synthesis. Putrescine has been chosen as an ideal wort amine (around 4-6 ppm) (3,4) to synthesize a nonenal-derived imine. The addition of 0,5 mole of nonenal to 0,3 mole of putrescine was operated at 0°C in dichloromethane, in presence of $K_2CO_3$. After filtration, the imine was dried in a rotavapor system.

## RESULTS AND DISCUSSION

Trans-2-nonenal stability during mashing :

From our experiments under argon, we can first conclude that trans-2-nonenal is relatively stable in a deoxygenated aqueous solution. Around 20-30% of trans-2-nonenal can be destroyed by hydration after 60 minutes at 76°C (see figure 1). Mainly one degradation product has been detected in the final mixture (see figures 2(a) & (b); mass spectrum presented in figure 3(a)).

On the other hand, transformation of trans-2-nonenal into oxidized derivatives (peak nr 5 in figures 2(c) and 3(b)) can be more rapid in presence of oxygen, even at low temperature.

Eventually, the third model system has clearly shown that trans-2-nonenal is also very instable in an amino acid mixture (figure 4). 95% of nonenal had disappeared after 100 minutes (88% during the 15 first minutes at 36°C).

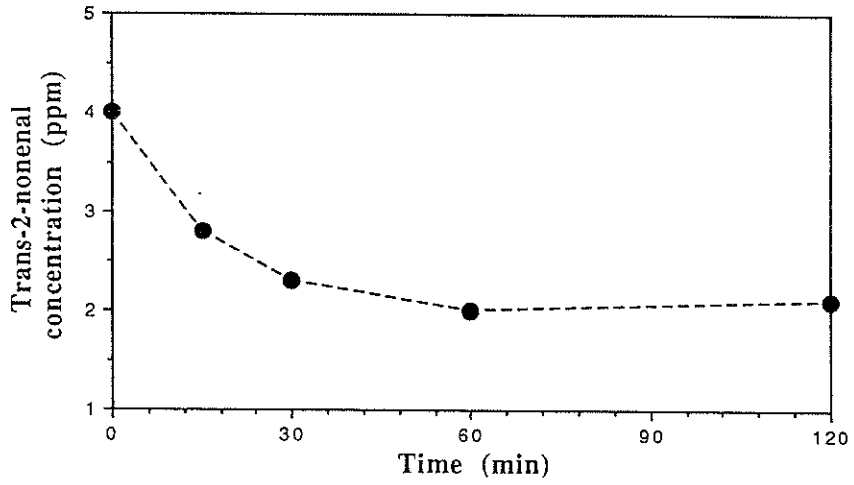



Fig 1. Evolution at 76°C of trans-2-nonenal concentration in a pH 5,2 aqueous solution.

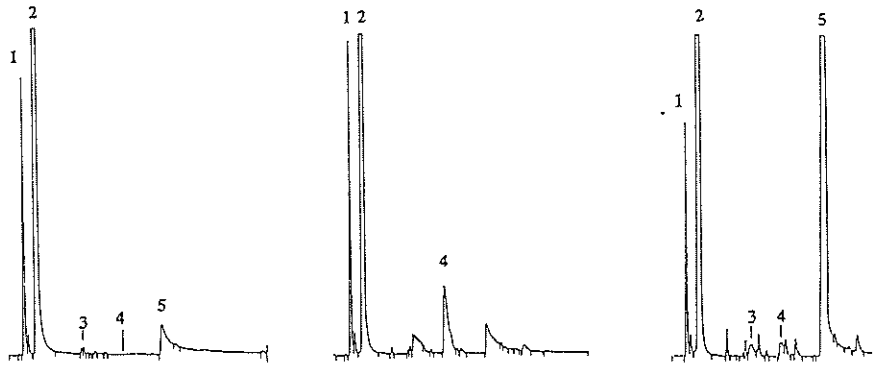



Fig 2. Nonenal degradation products at 76°C in a pH 5,2 aqueous solution. (a) initial time, (b) 60 minutes in an inert atmosphere, (c) 60 minutes in presence of oxygen.
Peaks nr 1 = cis-2-nonenal, nr 2 = trans-2-nonenal.

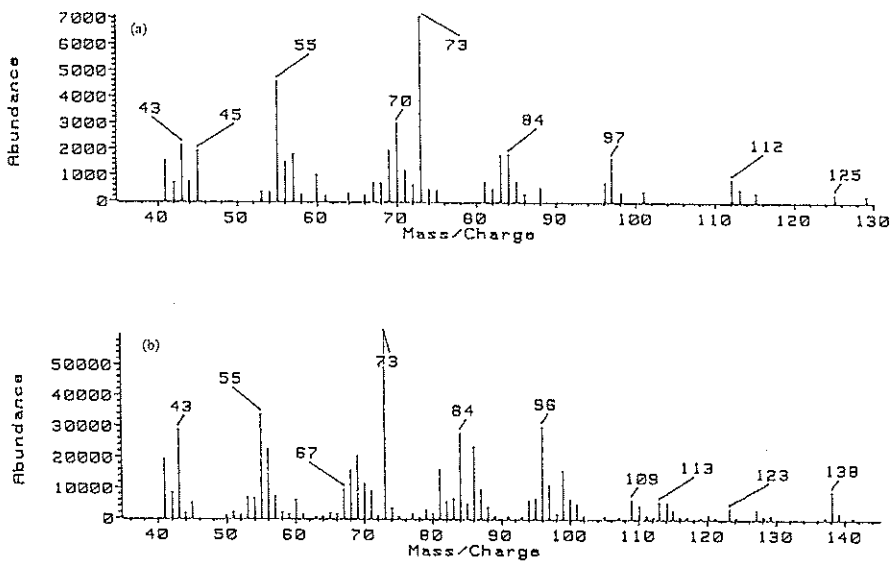



Fig 3. Mass spectra of trans-2-nonenal degradation compounds : (a) nr 4, (b) nr 5 (see figure 2 for peak numbering).

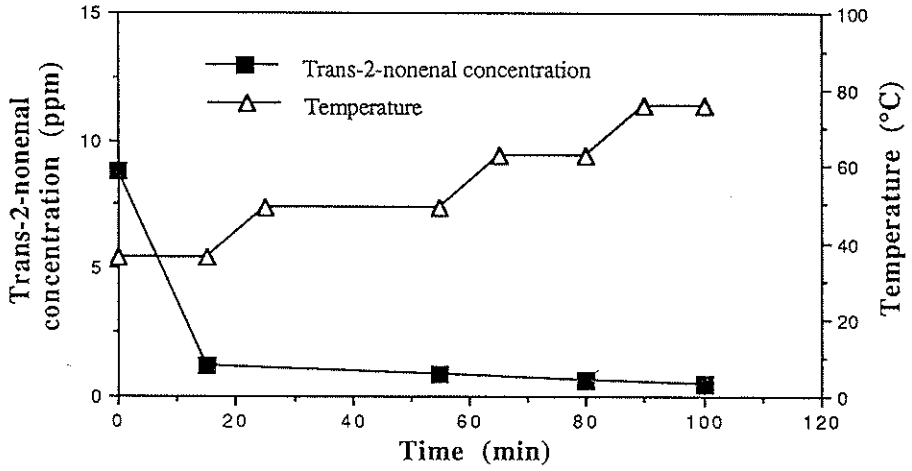
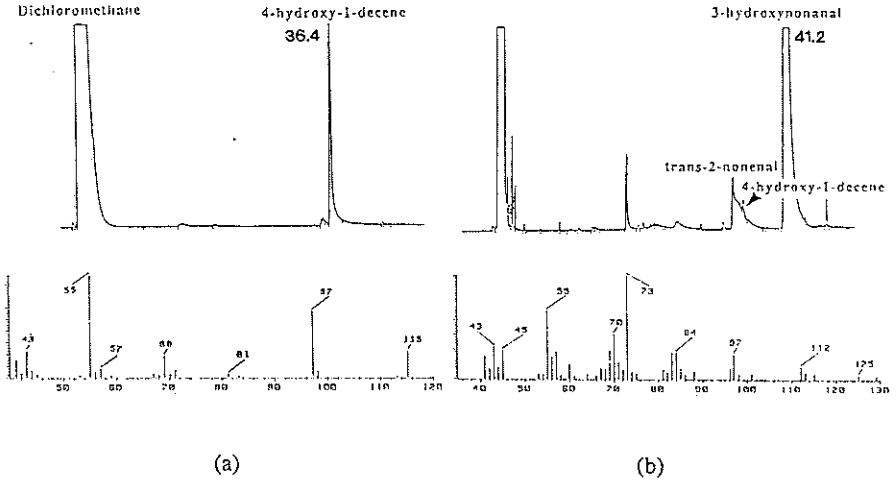



Fig 4. Evolution of trans-2-nonenal concentration during mashing in an amino acid model solution.

Identification of nonenal degradation products :

By the synthesis of 3-hydroxynonanal, we have demonstrated that this aldol can be originated from nonenal hydration (figures 3 (a) & 5 (b) to be compared). This compound proved to be relatively instable in aqueous solution and easily retransformed to nonenal in case of low pH.




(a) (b)

Fig 5. Chromatograms and mass spectra of (a) 4-hydroxy-1-decene and (b) 3-hydroxynonanal.

Retention time and mass spectrum of pure nonenoïc acid (figure 6) confirmed that this alcenoïc acid is the main nonenal oxidation product (nr 5).

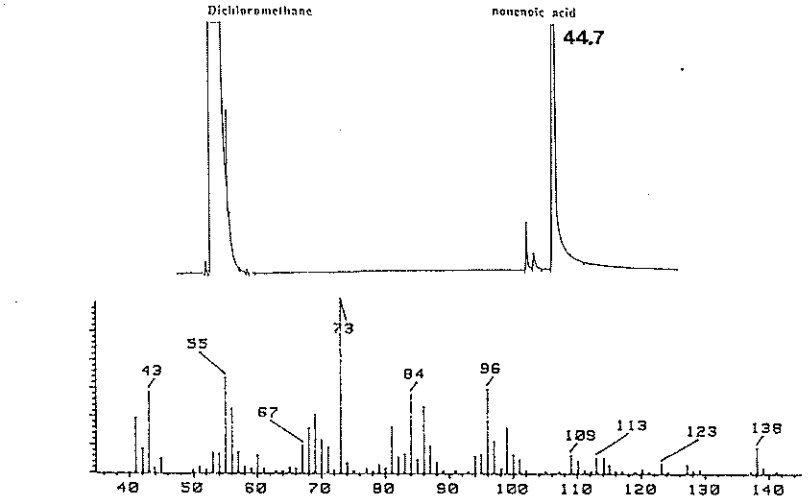
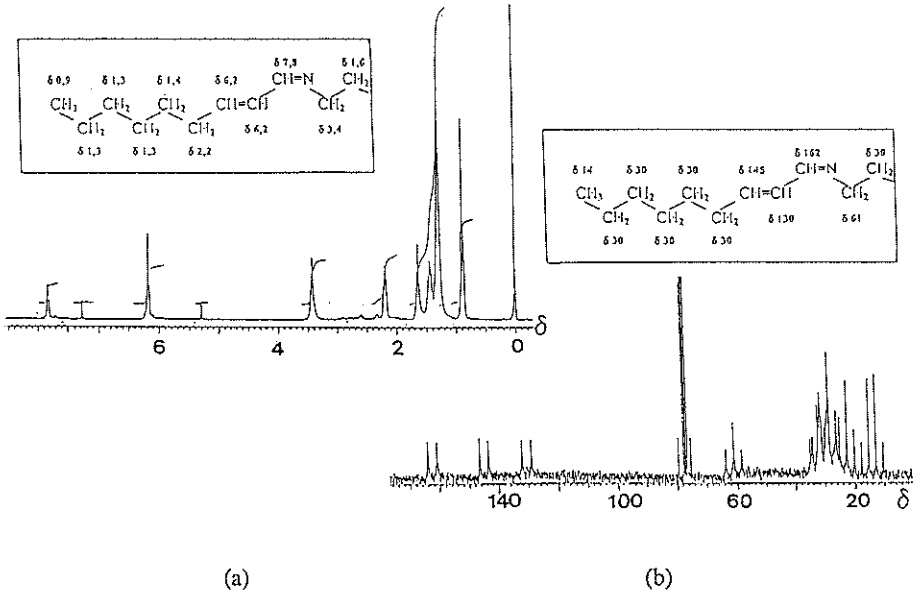



Fig 6. Chromatogram and mass spectrum of nonenoïc acid.

In order to assess the reactivity of nonenal with amino acids or amines, we have tried to synthetize a trans-2-nonenal derived imine with putrescine. As suspected, this Shiff base can be easily obtained in dichloromethane when water is removed (see figure 7). Distillation of aqueous imine solution has shown the relative stability of this compound in water. Less then 0,1% of putrescine-nonenal Shiff base was converted to the free aldehyde after 1,5 hour at 35°C.




(a) (b)

Fig 7. $^{1}H$ (a) and $^{13}C$ (b) NMR spectra of putrescine-nonenal Shiff base ($CDCl_3$ - 200 and 50 MHz, respectively).

## CONCLUSION

Three major nonenal derived compounds suspected of being nonenal precursors during aging, have been clearly identified in this work :

1) 3-hydroxynonanal from nonenal hydration;
2) nonenoïc acid from nonenal oxidation;
3) Shiff base from amine/aldehyde condensation.

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# Bildung von "Alterungscarbonylen" – mögliche Mechanismen und Bedeutung bei der Bierlagerung

B. Thum, H. Miedaner, L. Narziß & W. Back

TU München, Lehrstuhl für Technologie der Brauerei I, D-85350 Freising-Weihenstephan, Germany

*Deskriptoren*
*Aldehyd, Altgeschmacksbildung, Bierlagerung, Precursor*

ZUSAMMENFASSUNG

Da die Konzentration bestimmter Aldehyde bei der Bieralterung zunimmt, sollten deren Bildungswege und ihr Beitrag zum Aroma untersucht werden. Deshalb wurden Modellversuche direkt im Medium Bier durchgeführt.
Verschiedene Vorläuferverbindungen wurden Bier zugemischt, welches nach Lagerung analysiert wurde. Es stellte sich heraus, daß der "Strecker-Abbau" nur bei einem stark erhöhten Gehalt an Aminosäuren eine Rolle spielt. Ansonsten dürfte die Oxidation höherer Alkohole überwiegen. Der Redoxstatus von Bieren mit geringer Sauerstoffbelastung bleibt weitgehend stabil, eine Carbonylbildung kann aber auch in reduzierendem Milieu stattfinden.
Durch sensorische Experimente konnte gezeigt werden, daß bestimmte Aldehyde für eine Verstärkung der süßlich-malzartigen Note verantwortlich sind.

FORMATION OF "AGING CARBONYLS" - POSSIBLE MECHANISMS AND THEIR IMPORTANCE DURING BEER STORAGE

*Descriptors*
*Aldehyde, beer storage, precursor, staling*

SUMMARY

Since the concentration of certain aldehydes increases during storage of beer, the pathways of their formation and their contribution to flavour require investigation. Hence model reactions have been carried out directly in beer itself.
Different precursors were mixed with beer which was analysed after storage. It has been found that "Strecker degradation" only takes place with a strongly increased content of amino acids. Otherwise oxidation of higher alcohols predominates. The redox status of beer with low contents of oxygen remains largely stable although carbonyl formation can take place under reducing conditions.