CENTENARY REVIEW
TECHNOLOGICAL FACTORS OF FLAVOUR STABILITY

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The flavour stability of a beer primarily depends on the oxygen content of the bottled beer, but the individual steps of wort production are of similar importance viz:-

(a) preservation of reducing substances by avoidance of oxygen pick-up during mashing, lautering and wort boiling.

(b) elimination of substances which are prone to react to flavour active compounds like carbonyls by good mash and wort separation procedures,

(c) avoidance of an excessive exposure of the wort to heat, to limit the formation of Maillard reaction products and related substances.

Existing brewhouses offer some possibilities to improve the process and thus flavour stability. For analytical control Gas Chromatography and High Performance Liquid Chromatography are excellent tools, even simple analyses help to avoid unwelcome changes in the day to day practice.

Key words: *Oxygen uptake, oxidation, lautering, wort turbidity, hops, fatty acids, mash and wort separation, melanoidins, wort boiling, carbonyls, N-heterocycles, colour, 'HMF'-value.*

1. General

Together with a well developed character, foam properties and biological as well as the physical–chemical stability, flavour stability is a significant quality factor for a beer. For that reason, the subject 'Flavour stability' was the subject of a great many scientific and practical publications during the last 1½ decades. Among these, there are some surveys representing the relevant state of knowledge in 1974,[11] 1977[5] and 1981.[7,31] These papers have contributed brick by brick to the building of our knowledge today. Questions from the brewing industry as well as cooperation on an international basis, in particular within the EBC Biochemistry Group initiated four doctors theses which were dedicated either exclusively or partly to this subject.[2,13,15,30] The result of these dissertations has already been or is being published. This publication is an attempt to correlate these results with those obtained by full scale trials to demonstrate the contribution of research in brewing technology, to the improvement of flavour stability in beer.

In the discussions on flavour stability or as Dalgliesh[5] proposed—on 'flavour instability' mainly the change of the aroma component of the flavour was cited. This can be traced and identified by gas chromatography and high performance liquid chromatography. But there are also changes in the taste of beer, which cannot or can hardly be identified by analysis and which nevertheless cause a noticeable alteration of the body, the liveliness and the bitter taste and thus on the balance of a beer.

They are attributable to changes of the state of the colloidal matter such as proteins or protein-tannin complexes, β-glucans, dextrins and bitter substances. By the use of good quality raw materials and good brewing practice along with a suitable stabilisation of the beer it is easier than 10–15 years ago, to keep these alterations within certain limits.[2]

2. The Changes of Beer Aroma

This most remarkable appearance that occurs within a shorter or longer period can be explained mainly by the formation of a great many flavour active substances mainly carbonyls. As the flavour thresholds, particularly of the long chain aldehydes are very low, only minimal quantities are necessary to impair beer flavour. A multitude of mechanisms have been suggested as pathways for carbonyl formation. The most important ones can be depicted as follows.

a) Strekker degradation of amino acids,[22,24,43]
b) oxidadative degradation of isohumulones;[3,6,8,18,19,20,42]
c) oxidation of alcohols to aldehydes;[3,8,16,18,19,20]
d) autoxidation of fatty acids;[9,21,25,26,27,28,45]
e) enzymatic degradation of lipids,[11,10,12,14,47,49,52]
f) aldolcondensation of aldehydes;[17,29,44]
g) secondary autoxidation of long chained unsaturated aldehydes.[16]

All these processes are occurring simultaneously and thus they promote or inhibit one another. An increase of the melanoidins fosters the Strekker degradation of amino acids and the oxidation of alcohols. A high isohumulone content promotes this reaction at the expense of the others; the presence of polyphenols supports the Strekker reaction of amino acids, however inhibits the photodegradation of alcohols. The formation of trans-2-nonenal is diminished by its degradation. Riboflavin catalyses some reactions and inhibits others, the same is true for the effect of light or darkness and temperature.

The *light struck* flavour also contributes to changes in beer aroma during the storage of the bottled beer. It is attributable to the formation of a sulphur compound (3-methyl-2-buten-1-thiol) from hydrogen sulphide, from a light-activated nascent sulphydryl compound which comes mainly from sulphur containing amino acids and iso prendiene from the 4-methyl-3-pentenoyl-side chain of the iso-alpha-acids. Very light coloured beers are particularly susceptible to this effect. Investigation of a great many beer samples indicated that a high polyphenol content and possibly a certain amount of residual amino acids in beer diminished the sensitivity of sunstruck flavours. This photo reaction which is very rapid in green or colourless bottles also exists in brown bottles during a longer storage time and the exposure to natural or artificial light.

Thus, the brewer might be at a loss what measures should be taken to suppress the formation of substances which impart an aged note to the beer. But appraising the importance of the individual reactions it can be deduced:

a) oxygen plays always a crucial role, in particular in the bottled beer, where it accelerates reactions or even makes them possible.
b) oxygen is also of importance during wort production, as oxidation diminishes the amount of reducing substances or the reductive capacity of substances like the polyphenols. Oxidation catalysed by the lipoxygenases causes the the formation of flavour active compounds.
c) long chained fatty acids are prevalent in malt and hops. The turbidity during lautering as well as trub separation determines the amount of fatty acids of the wort which give rise to carbonyls in the finished beer.

TABLE I. Taste panel results of beers with different oxygen content

Fresh 0·27 mg/litre	Smell	clean, hoppy aroma
	Taste	clean, hoppy aroma, full body, lively
	Bitterness	well balanced
Aged 0·27 mg/litre	Smell	slightly oxidised, hoppy aroma
	Taste	slightly cheesy, a bit stronger oxidised than smell, full body, lively
	Bitterness	normal, slightly stringent
Aged 5·4 mg/litre	Smell	strongly oxidised, cardboard flavour
	Taste	strongly oxidised, cardboard flavour, wort like
	Bitterness	harsh and unbalanced

TABLE II. Oxygen content and beer aging carbonyls ppb

	Fresh 0·27 mg O_2/litre	Aged 0·27 mg O_2/litre	Aged 5·4 mg O_2/litre
Acetone	10·3	17·1	31·4
t-2-Butenal	9·4	22·1	34·1
Iso-Butanal	4·7	31·9	35·8
Benzaldehyde	11·4	3·8	8·6
2-Phenylacetaldehyde	5·7	4·3	7·7
2-Methyl-2-Butenal	0	Trace	0
Iso-Valeral	12·2	38·5	56·6
t-2-t-4-Hexadienal	3·6	2·3	2·5
t-2-Hexenal	2·2	2·0	3·2
Hexanal	3·9	3·9	3·8
t-2-Heptenal	0	0·10	3·45
t-2-t-4-Octadienal	7·5	5·0	3·4
t-2-c-6-Nonadienal	0·74	0·16	0·47
t-2-Octenal	Trace	Trace	0·40
Octanal	0·22	0·53	1·18
t-2-5-4-Nonadienal	1·06	0·72	0·26
t-2-Nonenal	<0·02	0·16	0·15
Nonanal	<0·10	<0·10	<0·10
t-2-t-4-Decadienal	0·68	0·68	0·85
t-2-t-4-Undecadienal	0·49	0·42	0·69

d) Maillard products are formed during kilning, mashing, wort boiling and the hot wort stand. They are part of the beer flavour; their uncontrolled formation supports an upgrading development of substances responsible for the aged flavour of beer.

f) amino acids are involved in some reactions; thus the amino acid content of beer should be low. This claim contradicts the requirements of a satisfactory wort quality. Proline, which fosters aldol condensation of aldehydes abounds in wort and beer;

g) metals catalyse oxidations. The replacement of copper or mild steel by stainless steel has minimised this factor, although the effect of cleaning agents on metal surfaces should not be neglected;

h) the influence of light was thought to be overcome by using brown bottles. But even in brown bottles, light exercises an influence during long storage times (for instance on the shelves in supermarkets).

The factors a–d can be influenced by technological means. Referring to this institute's research work, the main attention will be directed to these four items.

3. Oxygen Content of the Bottled Beer, Carbonyls and Beer Aging

To investigate the influence of oxygen uptake during bottling, the same beer was bottled with the lowest possible oxygen content of 0·27 mg O_2/litre and with a high amount of 5·4 mg. Both groups of beers were stored for 7 days at 40°C and compared with the fresh beer of low oxygen content. The result of the taste panel is shown in Table I.

The original beer had an excellent quality, but it deteriorated during storage even under the conditions of an almost air free bottling (pre-evacuation, CO_2-counterpressure etc.). Nevertheless it maintained its character, whilst the beer with the high oxygen content showed an inferior quality: it had totally changed its properties.

This can be traced by analysis of the carbonyls (determined by HPLC) according to Table II.

It is noteworthy that acetone, t-2-butenal, iso-butenal, 2-phenylacetaldehyde, t-2-heptenal, octanal, t-2-nonenal, t-2-t-4-decadienal and t-2-t-4-undecadienal increased markedly whilst t-2-t-4-octadienal and t-2-t-4-nonadienal diminished parallel to aging. t-2-nonenal which is always postulated as an indicator of beer aging did not show a higher level in the bottle of 5·4 mg O_2/l. Obviously, the oxygen caused a degradation of the t-2-nonenal[16] to other compounds which are not yet known, but impart an aged taste.

TABLE III. Oxygen content and beer aging total carbonyls

	Fresh 0·27 mg/l O_2/litre	Aged 0·27 mg/l O_2/litre	Aged 5·4 mg O_2/litre
Total carbonyls ppb	74	134	194
Relative change %	100	180	262
Amount of oleic acid ppm	16	9	10

Table III shows the change of the total carbonyl fraction under the described conditions.

The long chained fatty acids did not show any change during aging, save oleic acid which decreased by 44%. Linoleic and linolenic acids which are described as more reactive remained unaltered.

The results of these trials are interesting, but it should be emphasised, that the oxygen level of 0·27 mg/litre was very low. It implied the air in the bottle neck as well. It requires a lot of measures to attain it in the day-to-day brewery operation: Using only CO_2 to move the beer, de-aeration of the water for filling the filter, for the initial coating mixture and body feed, de-aeration of kieselguhr, Co_2 counterpressure in bright beer tanks and filling machines, pre-evacuation, over-foaming etc. The beer before the filler contained only 0·05 mg O_2/litre, the oxygen pick-up in the bottle was 0·05 mg as well and the air content in the bottle neck amounted to only 0·30 ml/litre resp. 0·17 mg O_2/litre.

Nevertheless, the aging of this beer was noticeable. The flavour stability can be improved by better control of the earlier processes.

4. Oxidation during Wort Production and Effect on Beer Aging

The oxidation during the individual steps of mashing, lautering, wort boiling and hot break separation has different effects, depending on whether there are oxidase systems still active or whether the oxidation occurs without enzymatic catalysis. Furthermore the fineness of the distribution of the absorbed air and the length of the reaction time is important.[39,48]

During mashing at 45°C peroxidases foster the oxidation of polyphenols[2] and lipoxygenases the oxidation of unsaturated fatty acids like linoleic and linolenic acids; at 65°C polyphenoloxidases catalyse the oxidation of polyphenols again. As these latter enzymes are stable below 80°C, they may act even during the later stages of mashing and during lautering. A simulation of the mash procedure with sodium sulphite revealed, that 60–200 mg oxygen are consumed during mashing, depending on the handling of the process, the stirrers, the pumping of mash etc.[23,51]

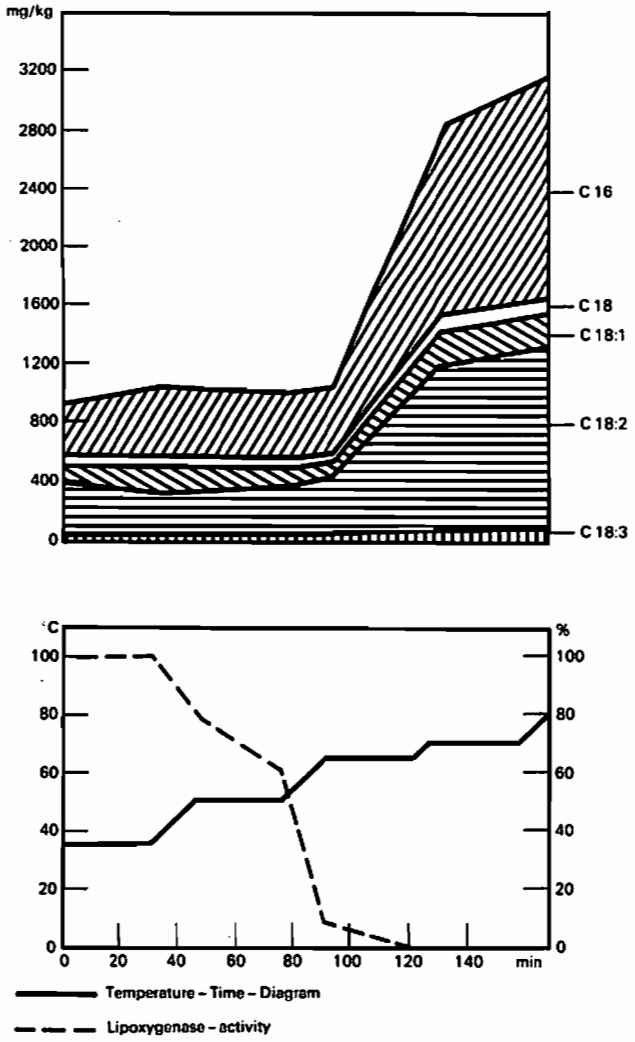

Fig. 1. Higher fatty acids during mashing

In Figure 1, a good impression of the behaviour of fatty acids during mashing is given. In this trial, where the oxygen content of the mash was not observed, there was a marked loss of oleic acid during the stands at 35° and 50°C, but also of linoleic and linolenic acids. During the first rests, lipase activity released the fatty acids which were oxidised by lipoxygenase. But this enzyme was readily inactivated during heating up to 65°C. Thus, at 65–70°C a second optimum of the lipases set free noticeable quantities of C16 and C18:2 acids.

The aeration of the mash exercises its influence mainly on the polyphenols,[2,34,35] but also on proteinaceous material which hinders the effect of the β-glucanases, the amylases and the proteases.[1,46] There is an adverse effect on the extraction of the endosperm material during mashing as well as a slower lautering process due to coarse protein particles blocking the grain bed.[46,50] As Table IV shows, a continuous aeration during mashing in a pilot scale brewhouse caused a very pointed reduction of the tannoids. This group of substances, according to Chapon[4] accounts for the reducing power of mash, wort and beer. In addition aeration during the individual rests at 50°C, 65°C or in particular during the long rest at 70°C brought about a decrease of the tannoids. The deterioration of the beer taste, in particular of the balance of the bitterness was obvious. The flavour stability (i.e. the flavour and bitterness of the aged beers) decreased markedly. Even the aeration at the end of mashing, at 76°C was effective, as the air was mixed into the mash and more widely distributed during pumping to the lauter tun. Thence, there was an unfavourable influence on the speed of run off which has already been mentioned.

Table V gives a survey of the various sources of air uptake during mashing.

Aeration during *lautering* occurs by deficiencies in the equipment or during pumping of wort from the wort hopper to the kettle. At temperatures of 70°C, there are still phenoloxidases active, thus the oxidation of polyphenols is accelerated. As Table VI shows the more oxygen that is mixed into the wort, the more the anthocyanogens and tannoids decrease, causing a deterioration of beer stability,

TABLE IV. Aeration during mashing and its effect of flavour stability

	Control	'Air'	Nitrogen continuous	Nitrogen mashing-in	Aeration 50°C/30′	Nitrogen except during Aeration 65°C/30′	Aeration 70°C/60′	Aeration 76°C/8′
Wort tannoids mg PVP/l 12%	89	38	94	92	75	81	55	80
Beer stability								
0/40/0°C days	2·1	2·5	2·1	2·1	1·6	1·8	1·7	0·9
ACT EBC	17	22	21	20	56	50	54	58
Foam Ross & Clark	129	128	126	128	126	128	128	128
Taste test of the fresh beer								
Odour	clean	astringent	clean	clean	slightly husky	slightly husky	slightly husky	clean
Taste	clean	slightly astringent	clean	clean	slightly husky	slightly husky	slightly husky	clean
Body	full	full	full, smooth	full	full	full	full	full
Liveliness	good	moderate	good	good	moderate	moderate	moderate	moderate
Bitterness	good	harsh	good	good	harsh	harsh	medium harsh	slightly harsh
Taste test of the aged beer (4 weeks 20°C)								
Odour	strongly oxidised	strongly oxidised	clean	slightly oxidised	moderately oxidised	moderately oxidised	oxidised	moderately oxidised
Taste	strongly oxidised	strongly oxidised	clean	slightly oxidised	moderately oxidised	moderately oxidised	oxidised	moderately oxidised
Body	full	moderately full	full	full	full	full	full	moderately oxidised
Liveliness	good	less lively	good	good	moderate	moderate	moderate	moderate
Bitterness	slightly harsh	very harsh, strange	good	good	harsh	harsh	harsh	harsh

TABLE V. Causes for oxidation during mashing

Milling	Wet-milling, 'homogenisation', air-uptake during pumping to the mash tun
Mashing-in	water level too low at the start, too early too high speed of the stirrer(s)
Transfer of mashes	pumping into the mash copper or back to the mash tun from the top
Transfer to the lauter-tun	from the top
Introduction of 'air strings' during pumping, defective bearings of the pumps	
High oxygen content of brewing liquor	

TABLE VI. Analysis of beers. Aeration of Lauter Wort at 70°C

O_2-Content mg/litre	0·2	2·0	3·0	10·0
pH	4·40	4·40	4·41	4·41
Colour EBC	5·5	6·0	7·0	8·0
Polyphenols mg/litre 12%	188	190	187	191
Anthocyanogens mg/litre 12%	66	61	50	41
P.I.	2·8	3·1	3·7	4·6
Tannoids mg/litre 12%	58	56	49	42
Total-N mg/100 ml 12%	78	78	78	80
High mol. N mg/100 ml 12%	14·0	13·8	13·5	14·2
Coag. N mg/100 ml 12%	2·2	2·1	2·3	2·4
Bittersubstances EBC	28·8	28·8	28·8	29·0
Alpha-acids mg/litre	2·0	2·2	2·5	2·3
Iso-alpha-acids mg/litre	28·9	29·1	28·1	27·6
Foam figure Ross & Clark	121	122	121	121
ACT (Chapon)	3·1	4·2	5·8	9·6
Taste test—fresh beer				
Average DLG*	4·4	4·3	4·2	3·7
Bitterness	4·2	3·5	3·2	2·3
Aged beer				
Average DLG*	4·0	3·5	not judged	
Bitterness	4·0	3·0	not judged	

* Test according to 'Deutsche Landwirtschafts-Geseluschaft'.

beer taste and flavour stability. The latter suffers particularly in beers which have originally a pronounced hop aroma. Ultimate investigations of new lautertuns show, that the oxygen content at that stage can be reduced to 0·05 mg/litre.

Wort boiling: The usual 'open' wort boiling brought about a better yield of bitter substances than the wort boiling in 'closed' systems using either internal or external boiling systems. However, the composition of the various polyphenol fractions was less favourable, the colours of worts and beers were darker and the beer taste less attractive and harsher. This was particularly found in the aged beers.

Similar results were observed, when the hot wort was aerated after boiling, either by an 'air-string' during the transfer from the copper to the hot wort tank, in some whirlpool designs or in the hop strainer. A colour pick-up of ca. 1·5 EBC and a harsher bitter taste of the beer accompanied the impaired flavour stability.

5. *The Clarification of Wort during Lautering or during Trub Separation*

It is well known, that the individual lauter systems vary in the brightness of wort according to the design and the mode of handling the process. By improvement of the technique of lautering it is possible to reduce the amount of solids in the unboiled wort by 25% (given the same equipment). But the level itself is determined, whether a lautertun or a mash filter or a strainmaster is used. As for lautertuns the type of the grist plays a part (dry or wet milling).

A survey is given in Table VIII.[32]

Investigations have shown, that the solid content of a lauter wort, respectively its turbidity correlates highly with the amount of palmitic and linoleic acids, whilst oleic and linolenic acids were not affected.[41]

A comparison of beers made of turbid and bright lauter worts was made in the pilot scale. The turbid wort was the first of a mash filter, the bright one the same wort after filtration by means of coarse kieselguhr. After boiling the hot break was removed and eventually one part of the cold break by sedimentation for 16 hours. There was always a separation of hot and cold break, thus the tendencies of the results of

TABLE VII. Comparison of beers. Wort boiling trials with and without acess of air

Mode of boiling	'Open'	'Closed'
Colour EBC	6·5	5·5
Polyphenols mg/litre 12%	172	172
Anthocyanogens mg/litre 12%	52	62
P.I.	3·3	2·8
Tannoids mg/litre 12%	48	53
Total-N mg/100 ml 12%	74	75
High mol. N mg/100 ml 12%	13·2	13·2
Bittersubstances EBC	26·0	24·0
Iso-alpha-acids mg/litre	26·1	22·3
Alpha-acids mg/litre	1·8	1·8
Foam figure Ross & Clark	133	133
ACT EBC	9·0	8·0
Taste *fresh*		
Ø DLG	4·3	4·5
Bitterness	3·5	4·5
aged		
Ø DLG	3·7	4·0
Bitterness	3·0	4·0

TABLE VIII. Solids of Lauter Worts—Different Systems Lautertuns (mg/litre)

Mill	Dry cond	Dry cond	Dry cond	Wet	Wet
Solids	120	155	84	190	680
	Mash filter				
Recirculation	no	no	yes	yes	yes
Solids	810	470	370	400	320
	Strainmaster (mg/litre)				
Trub utilisation	no	yes	yes		
Solids	200	280	340		

TABLE IX. Fatty acids in turbid and bright lauter worts (mg/litre)

	First worts		Boiled worts		Pitching worts	
	turbid	clear	turbid	clear	turbid	clear
C 14	0·50	0·05	0·26	0·06	0·03	0·02
C 16	29·10	5·20	9·50	1·20	0·72	0·27
C 16:1	0·20	0·09	0·12	0·04	0·02	0·02
C 18	1·60	0·30	0·65	0·07	0·05	0·03
C 18:1	2·40	0·16	1·25	0·12	0·11	0·03
C 18:2	27·70	3·00	12·55	0·90	0·82	0·15
C 18:3	2·50	0·30	1·25	0·47	0·10	0·09
C 20	0·47	0·03	0·08	0·03	0·01	0·01

the 'turbid' and 'bright' worts were weakened. But this corresponds to the brewing practice.

Nevertheless, a turbid wort gives rise to a less efficient adsorption of fatty acids on the trub, furthermore the trub removal, respectively the residual level of fatty acids was unfavourable in the originally turbid worts. This is shown in Table IX.

It is evident, that the final worts, i.e. the worts ready for pitching showed fatty acid contents which were determined by the turbidity of lauter wort. Even by careful trub separation it was not possible to avoid the disadvantage of a deficient lauter system.

The beers made from these worts were tested in the fresh and in the aged state. The results are demonstrated in Table X.

TABLE X. Taste test of beers made of clear and turbid worts

Beers made of clear wort		
fresh	odour	clean, sl. estery
	taste	clean, sl. estery
	body	full, smooth
	liveliness	good
	bitterness	good
aged	odour	clean, estery
	taste	sl. oxidised, estery
	body	full
	liveliness	good
	bitterness	good, sl. aftertaste
Beers made of turbid wort		
fresh	odour	yeasty-estery, slightly strange
	taste	yeasty estery
	body	full
	liveliness	good
	bitterness	slightly harsh
aged	odour	oxidised, strange
	taste	oxidised, sl. strange
	body	full
	liveliness	good
	bitterness	harsh, astringent

The HPLC analysis shows the differences of the beers in Table XI.

The aged beer from the turbid wort contained remarkably higher levels of some of the aldehydes than the beer from the bright wort, like t-2-butenal, iso-butanal, 2-phenylacetaldehyde, iso valeral, hexanal, t-2-nonenal, nonanal, t-2-t-4-decadienal and t-2-t-4-undecadienal. But it must be admitted that already the aging of the bright beer caused already a marked increase of the carbonyls. Surprisingly some diminished during aging of that beer, for instance, iso-butanal, benzaldehyde, 2-phenylacetaldehyde and the two decadienals. They react obviously with other substances, whereas during ageing of the 'turbid' beer the formation of these carbonyls superimposes and exceeds the destruction. It is also noteworthy that the fresh beer from the turbid wort showed already a high carbonyl content as Table XII indicates.

In this trial the reduction of linoleic and linolenic acids is more pronounced than in the first test with the different oxygen uptake during bottling.

Some researchers tried to reproduce the effect of the turbid worts by adding the individual fatty acids to fermentation.[40,41] Within this work, a mixture of the four C18 acids (the 15 fold quantity) were added, was less informative and significant, but it is well possible, that the fatty acids promoted a much stronger degradation of carbonyls, thus showing too low a carbonyl content. The decrease of oleic, linoleic and linolenic acids during ageing was significant.

It has already been mentioned and shown in Table IX that turbid worts aggravates trub separation. But it is also an unsatisfactory hot trub separation per se that impairs the taste of the beer and in particular the flavour stability. As Table XIII shows, in the breweries 2 and 6 of the hot break separation was inferior, in brewery 3 it was not satisfactory. This resulted in beers which aged already during the short storage in the warehouse! It was pointed out at some occasions, that a defective hot break removal disturbs flotation, leading to problems during fermentation, and according to the Table, in the final product.

Another question is how far the hops contribute to the fatty acid contents of worts. The individual hops contain different amounts of long chained fatty acids, calculated on basis of the alpha-acid content, due to variety, area and year. Hop products like powders usually show minor deviations, whereas hop extracts vary to some extent, depending

TABLE XI. Carbonyls in beers made of clear and turbid worts ppb

	Clear wort		Turbid wort	
	fresh	aged	fresh	aged
Acetone	3·7	7·2	10·8	11·2
t-2-Butenal	0	9·4	2·0	13·4
Iso-Butanal	4·6	1·9	3·2	8·0
Benzaldehyde	12·1	8·7	7·3	8·1
2-Phenylacetaldehyde	8·1	3·7	4·8	6·1
2-Methyl-2-Butenal	0	1·7	0	1·2
Iso-Valeral	3·7	5·2	14·0	12·3
t-2-t-4-Hexadienal	2·7	4·6	4·0	4·6
t-2-Hexenal	0	4·6	4·3	2·9
Hexanal	0	0·4	3·0	3·4
t-2-Heptenal	0	6·9	3·8	4·7
t-2-t-4-Octadienal	0	3·8	4·2	3·6
t-2-c-6-Nonadienal	0·02	0·08	0·06	0·10
t-2-Octenal	0·02	0·08	0·11	0·26
Octanal	7·0	15·2	12·9	8·6
t-2-t-4-Nonadienal	0·02	0·02	0·02	0·02
t-2-Nonenal	0·05	0·12	0·08	0·15
Nonanal	0·20	0·10	0·12	0·17
t-2-t-4-Decadienal	2·50	0·63	1·15	1·42
t-2-t-4-Undecadienal	2·8	0·76	1·3	1·51

TABLE XII. Total carbonyls and fatty acid contents of the beers

Wort / Beer	Clear		Turbid	
	fresh	aged	fresh	aged
Total carbonyls ppb	47·5	75	77	92
Relative change %	100	158	162	193
Fatty acids ppm				
oleic acid	12	11	14	12
linoleic acid	19	9	29	14
linolenic acid	12	7	12	6

TABLE XIII. 'Cold break'-contents (mg/litre) of the hot worts after boiling, after trub separation after cold break removal and flavour stability

Brewery	1	2	3	4	5	6
After boiling	170	242	214	245	205	234
After hot break separation	WP 185	WP 360	C 310	C 300	WP 225	WP 450
After cold break removal	F 90	F 300	S 250	F 130	F 140	F 420
Flavour stability days at 20°C	70	3	30	60	60	3

WP = Whirlpool.
C = Centrifuge.
F = Flotation.
S = Sedimentation.

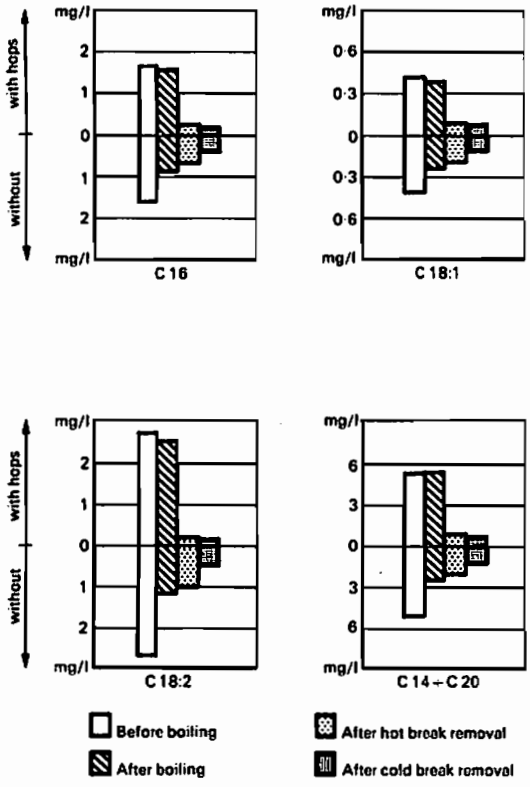

Fig. 2. Fatty acids in worts without and with hops.

on the solvent. Trials have shown, that a normal hopping rate of 65 mg alpha-acids per litre increases the fatty acid content of a wort by 10%. However the linoleic acid content of the sweet wort is almost doubled.[30] Nevertheless the fatty acid content of the hopped worts is markedly lower as Figure 2 shows. Hops support the precipitation of protein during and after wort boiling. By the formation of break, fatty acids are removed from the wort. Thus, hops do not impair flavour stability via this group of substances.

6. *The Effect of Heat*

During the conventional boiling time of, say, 100 minutes and an ensuing time of wort transfer and wort cooling of 120 minutes, the individual wort particles are exposed to temperatures of 95–100°C for 160–220 minutes. The

TABLE XIV. Carbonyls in aged beers from worts of different holding times at 95°C ppb

Time min	85	300
Acetone	5·1	9·7
t-2-Butenal	8·4	10·7
Iso-Butanal	5·2	8·7
Benzaldehyde	7·1	9·1
2-Phenylacetaldehyde	5·2	5·1
2-Methyl-2-Butenal	6·8	1·3
Iso-Valeral	10·2	13·1
t-2-t-4-Hexadienal	2·8	2·3
t-2-Hexenal	0·6	1·3
Hexanal	0·70	3·3
t-2-Heptenal	0	2·4
t-2-t-4-Octadienal	4·9	3·5
t-2-t-6-Nonadienal	0·27	0·74
t-2-Octenal	0·22	0·20
Octanal	5·48	7·06
t-2-t-4-Nonadienal	Trace	Trace
t-2-Nonenal	0·18	0·22
Nonanal	0·15	0·10
t-2-t-4-Decadienal	0·82	1·58
t-2-t-4-Undecadienal	0·46	1·16

TABLE XV. Taste test of aged beers from worts of different holding times at 95°C

Holding time 85 minutes	odour	oxidised
	taste	oxidised
	body	full
	liveliness	good
	bitterness	good, sl. harsh
300 minutes	odour	strongly oxidised
	taste	strongly oxidised, wort like
	body	moderate
	liveliness	moderate
	bitterness	harsh, not to be judged

TABLE XVI. Formation of colour during wort production and flavour stability (colour calculated on 12% P)

Brewery	R	S	T	U	V
First wort	5·0	4·5	6·0	5·0	7·0
Before boiling	7·5	6·0	8·2	7·3	9·0
After boiling	9·5	8·0	11·0	9·0	11·0
After 60 min	10·8	8·8	12·0	9·5	12·0
After 120 min	12·0		14·0		
End of wort cooling total time	13·0	10·0	14·5	10·5	13·5
End of boiling wort cooling min	(150')	(100')	(130')	(110')	(110')
Beer	9·0	6·2	10·8	7·0	9·5
'HMF'-value mg/litre after boiling	16·5	13·8	19·5	14·0	18·5
after wort cooling	21·9	16·4	25·3	16·2	22·0
Flavour stability days at 20°C	<30	>90	<20	>90	~40

TABLE XVII. Comparison low pressure boiling (brewery F). High temperature wort boiling (brewery D)

	Pils control	Pils L.P.	Export control	Export L.P.	Pils HTW
Original wort % P	11·54	11·61	12·95	12·79	12·2
Colour EBC	8·3	8·1	9·7	9·7	8·3
'HMF'-value	19·2	18·7	22·2	21·7	19·4
EBC-BU	30·3	31·5	25·9	26·3	29·4
Taste fresh					
Ø DLG	3·9	4·0	4·3	4·3	4·4
bitterness	3·8	3·8	4·2	4·1	4·2
Taste aged					
Ø DLG	3·7	3·7	3·7	3·8	4·0
bitterness	3·5	3·7	4·0	4·0	4·0

Maillard reactions during wort boiling are continued and it is a question what amount of these 'melanoidins' is acceptable or at which level an impairment in flavour stability occurs.

The influence of different holding times after the end of wort boiling is presented in Table XIV.

There was a well defined increase of most of the carbonyls, like t-2-butenal, iso-butanal, benzaldehyde, iso-valeral, t-2-hexenal, hexanal, t-2-heptenal, t-2-t-6-nonadienal, octanal, t-2-nonenal, t-2-t-4-decadienal and t-2-t-4-undecadienal. Some of these compounds, for instance the two decadienals were obviously more influenced by this heat treatment than by a higher oxygen content in the bottle. Even t-2-nonenal exceeded the 0·20 ppb margin. Iso-valeral was more affected by oxygen, likewise t-2-butenal. It must be admitted that the original level of the beers was different, as the trials took their time, mostly caused by the difficult analysis.

The results of the taste testing are shown in Table XV.

These extreme holding times are not usual in the brewing practice, but, as Table XVII shows, even deviations of 30

TABLE XVIII. GC-analysis of N-heterocyclics in beers (ppb). Comparison: low pressure wort boiling—normal boiling and high temperature—wort boiling.

	Pils control	Pils L.P.	Export control	Export L.P.	Pils HTW
Thiazole	5·6	5·4	*	5·0	3·3
2,6-Dimethylpyridine	—	—	—	—	0·2
2-Methylpyrazine	3·3	2·7	3·1	4·3	3·9
4-Methylthiazole	0·4	0·3	0·3	0·4	0·5
3-Methylpyridine	—	+	+	+	+
2,5-Dimethylpyrazine	0·9	1·0	0·8	1·0	2·5
2,6-Dimethylpyrazine	0·5	0·5	0·5	0·6	0·9
2-Ethylpyrazine	0·1	0·1	0·1	0·2	0·2
2-Methoxypyrazine	0·4	0·1	0·1	0·4	0·1
2,3-Dimethylpyrazine	0·4	0·3	0·3	0·4	0·4
Trimethylpyrazine	0·3	0·4	0·3	0·4	0·7
2-Vinylpyrazine	0·4	0·3	1·5	0·7	0·4
2-Ethyl-3,5-Dimethylpyrazine	0·3	0·2	0·3	0·3	0·3
2,3-Diethylpyrazine	0·2	0·5	—	—	1·2
2-E-3·6-Dimepyrazine	—	—	+	+	+
Tetramepyrazine	+	+	0·2	0·3	0·5
Pyrrole	0·6	0·7	0·7	0·8	0·9
2-Acetylpyridine	0·6	0·6	0·7	0·7	1·0
6,7-DIH-5H-5-ME-CPP	0·2	0·6	0·2	0·3	0·3
2-Acetylthiazole	0·3	0·2	0·2	0·3	0·3
2-Methylbenzoxazole	—	—	—	—	0·1
Pyrazole	0·4	2·3	1·2	2·1	3·5
NIC-SRE-E-Ester	3·5	2·6	6·5	3·5	4·0
3-Acetylpyridine	22·0	16·3	22·0	14·1	23·9
Acetylpyrrole	81·8	59·9	62·2	62·1	80·5
5-A-2,3DIH-1H-Pyrrolizine	2·1	2·5	2·9	4·0	1·5
5-F-6-M-2,3-DIH-1H-P	—	+	0·3	0·4	0·2
5-A-6-ME-2,3-DIH-1H-P	1·8	—	0·4	0·3	2·8
Indole	2·1	2·0	3·4	1·8	3·1
Malzoxazine	6·0	4·8	7·2	4·6	6·9
Total	159	129	128	171	185

* Peaks not separated.
— Not detectable.
+ Traces.

minutes have an influence on flavour stability. As for quality control reasons, it is sufficient to check the colour of wort at the different stages of wort production; in some cases it might be helpful to determine the 'HMF'-value.[33] Furthermore the colour of the green beer respectively the colour loss during fermentation gives a good information.

The data in Table XVI shows that the increase of wort colour between first wort and the end of wort cooling varies between 5·5 and 8·5 EBC units. It is of course dependent on the composition of malt, its modification and the formation of colour active substances during mashing. Darker malts in the grist give rise to darker wort colours and a stronger colour pick-up, but the worts resp. beers in Table XVI were produced of malts of EBC colours between 2·8 and 3·3. A closer look into the behaviour of colour between the end of wort boiling and the end of wort cooling shows also very interesting differences which match with the increase of the 'HMF-value' and the colour of the beer. Melanoidins are reduced to a lesser extent that polyphenols, thus worts with long holding times give rise to correspondingly higher beer colours. The flavour stability is affected similarly. But as always happens in full scale tests, there are certainly more factors involved and thus contributing to a poor flavour stability: insufficient removal of hot break, turbid worts and oxidations at various stages.

In connection with the effects of heat during wort boiling, it is of interest, whether and how far boiling at higher temperatures than 100°C affects flavour stability. As earlier results have shown[33] the boiling times at 135°C were drastically reduced and the total time above 100°C was limited to ca 500 sec. Using 'low pressure boiling' the holding time at 106°C is only 15–20 minutes and the total time at or over 100°C amounts to 60–65 minutes. It is of particular importance however, that the wort is always in turbulence, thus avoiding partial overheating and providing for an evaporation at every stage of the boiling process. This is realisable with low pressure boiling. Using high temperature wort boiling, evaporation occurs only during the expansion steps from 135 to 117°C and further to 100°C. In these continuous systems it is vital to provide large surfaces for evaporation, either by a low wort level in the expansion vessels or by spraying the wort.

It is very understandable, that the introduction of these processes requires a very subtile analytical control of the carbonyls. The GC analysis is more adaptable for this purpose than HPLC. Thus wort aroma components can be determined ranging from higher alcohols, to acids, carbonyls and hop aroma substances. Very important are in this context the N-heterocyclic substances, which in their total amount, correlate significantly with the 'HMF-value' and the colour pick-up.

Table XVII shows some data of beers which had been produced with the different boiling methods. There is a good comparison between the 'normal' and the 'low pressure' boiling methods, whilst the high temperature wort boiling was without a control brew.

There was virtually no difference between the beers of brewery F. The results of brewery D matched with those obtained from 'normal' brews of other breweries. There was no deterioration of flavour stability.

Table XVIII gives a survey of the N-heterocyclics in beers whose data were presented in Table XVII. The result is, that a careful operation of wort boiling at well defined times and temperatures does avoid striking differences of carbonyls and even such a sensitive group of substances remains within the desirable limits. The total amount of N-heterocyclics varies to some extent, but it is not prefixed by the boiling system. As another investigation has shown, even with conventional coppers the differences from brew to brew can exceed those caused by well controlled modern systems. It is noteworthy that malt oxazine, a derivative of proline has remained within the normal standards.

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