CENTENARY REVIEW

THE BIOCHEMISTRY OF MATURATION

By Charles A. Masschelein

(C.E.R.I.A.—1070 Brussels)

Received 30 January 1986

Negative and positive aspects of maturation are respectively related to aroma and taste modifications. Vicinal diketones, hydrogen sulphide, acetyldehyde being primarily responsible for 'green' beer flavours an important feature of maturation is the adjustment of their concentration during the lagering period. The role of secondary fermentation in the removal of these undesirable by-products and the importance of sulphury compounds in determining the typical character of lager beer are reviewed. Particular emphasis is placed upon new enzymatic and genetic approaches to overcome vicinal diketone problems in accelerated fermentation systems using free and immobilized cells.

The presence in beer of amino acids, peptides, nucleotides and organic as well as inorganic phosphates is, in part, due to the secretion of these materials by the yeast during lagering. Most of these compounds are contained in internal pools and their actual participation in flavour maturation depends upon intracellular breakdown and accumulation, changes in cell permeability and subsequent exchange possibilities between the yeast cell and the surrounding beer. Participation and practical implications of medium chain length fatty acids in the development of autolytic and yeasty flavours are discussed.

Key words: *Attenuation, accelerated maturation, α-acetohydroxyacids, α-acetohydroxyacid decarboxylase, amino acid pool, accelerated maturation, autolysed flavour, beer aroma, diacetyl, fatty acid, dimethylsulphide, genetics, lager flavour, secondary fermentation, sulphury flavour.*

Introduction

Biochemical, chemical and physical mechanisms, involved in flavour changes occuring during maturation are of an intricate and complex nature. Most of them are not yet completely elucidated.

As a matter of fact, maturation includes all transformations between the end of primary fermentation and the final filtration of beer. In their classical concept, fermentation and maturation are considered as separate steps in making beer. In traditional lager brewing, modifications of taste and aroma are implicitly associated with a long cold and active secondary fermentation. Thus, carbonation by fermentation of residual sugars, removal of excess yeast by sedimentation, adsorption on the surface of the yeast of various non volatile materials, reduction of the haze-forming potential by precipitation of protein/polyphenol complexes and progressive changes in flavour and aroma are dependent upon the whole of transformations associated with beer clarification and slow stripping of unwanted volatiles by the generation and release of excess carbon dioxide.

It is generally recognized that beer flavour improves during storage. Many studies have shown that the adjustment of the concentration of undesirable compounds such as acetaldehyde, vicinal diketones and sulphur compounds, play an important part from the point of view of the time required to produce a fully matured beer. If this concept of the effect of maturation is true, it should, however, be recognised that many other changes in the composition of the non-volatile fraction occur during that period of time and that these modifications are also known to have a significant effect on the quality of the final product. The observed modifications are generally expressed as a better flavour association with a concomitant increase in palate fullness and mouthfeel. The fact, that such modifications only occur in the presence of yeast, suggests that some correlation must exist between taste improvements and changes in yeast metabolism during lagering. However, release of amino-acids, peptides, nucleotides and organic and inorganic phosphates are not only dependent on the yeast itself, but also on its physiological state and its physical behaviour and on many other variables such as temperature, time, turbulence created by the secondary fermentation, and the shape, geometry and capacity of the storage vessels.

The extent to which excretion and autolysis are important in flavour maturation and its dependency on yeast strain, fermentation conditions and environmental factors are currently ill defined. Hence, to evaluate the contribution of changes in the composition of both the volatile and non-volatile fractions to the quality of the finished beer, a comprehensive understanding of the biochemistry of maturation is useful to prevent the development of off-flavours due to yeast autolysis and to produce beer with the desired flavour characteristics employing a consistent process time. This understanding becomes more critical when accelerated, large scale, single vessel processing is considered.

Future savings in cellar operation may come from the application of immobilized cell technology in the brewing process. However, for such strategies to be successful there is an increasing need to find a solution which satisfies both productivity and the quality of the final product. In this regard, an understanding of biochemical and organoleptic aspects of beer maturation is a prerequisite for the commercial feasibility of using immobilized yeast cells for the production of beer of acceptable quality.

In this paper an endeavour is made to reassess the biochemical background and the contribution of maturation to beer flavour with a view to providing basic information for the optimisation of maturation efficiency in traditional and accelerated systems.

Biochemical and Organoleptic Implications of Carbohydrate Metabolism in Traditional Maturation Systems

The main difference between traditional maturation systems in ale and lager brewing is that ales are conditioned by warm storage, holding the beer at temperatures between 12–20°C whilst lagers are conditioned at much lower temperatures. Under warm maturation conditions residual and priming sugars are rapidly metabolised and removal of 'green' flavours is normally completed in 1–2 weeks depending on the type of beer, yeast strain, wort composition and

TABLE I. Inactivation effect of glucose and maltose on the enzymes involved in the utilisation of maltotriose

% sugar added during growth with 0·5% maltotriose		Maltotriose uptake $\mu/CO^2\ h^{-2}\ mg^{-1}$ dry yeast				Inactivation (%) after 3 hours incubation
glucose %	maltose %	de-adaptation time (hours) 0	1	2	3	with glucose and (or) maltose
2·5	0	374	130	45	24	93
0·5	0	396	238	97	41	90
0·2	0	390	228	147	146	64
0	2·5	346	310	274	270	23
0	5·0	376	262	180	200	45
0·1	5·0	370	190	135	90	76

primary fermentation conditions. In contrast, maturation of lager beers being a low temperature process, the contribution of the lagering period to beer flavour will be significantly dependent upon the length of the lagering period, the amount of yeast in suspension and the quantity of fermentable sugar in the young beer or added in the form of actively fermenting wort.

In a classical two vessel process, lager beers are transferred after primary fermentation and cooling to approx 4°C. to a separate vessel, where residual sugars are fermented so that the beer becomes carbonated. The application of cooling and the resulting decrease in metabolic activity of the yeast as well as the continued contact of yeast cells in an environment that cannot support growth but where fermentable sugars are still present are continual stress conditions which will inevitably tend to poor attenuations, losses in cell viability and altered beer flavour notes. Factors such as yeast concentration, and low fermentation capacity figures for maltotriose are frequently responsible for excess levels of residual wort sugars in the finished beer. In terms of utilization of mono, di-and trisaccharides in commercial brewing yeast strains catabolite inhibition has been found complementary to catabolite repression in explaining the sequential uptake of maltose with respect to maltotriose.[15,30] Catabolite repression explains a mechanism where synthesis rather than activity of the enzyme are concerned. Inactivation of the pre-formed enzyme is known as catabolite inhibition. The distinction between catabolite repression and inhibition is important because it is only catabolite inhibition which can reduce the fermentation speed of maltose and particularly maltotriose to a critical value thus causing hanging or tailing fermentations and concomitant flavour anomalies in the final product.

Interestingly, in contrast to ale yeasts, almost all lager strains are characterized by a variable but rapid decline in their ability to ferment maltotriose.[15] Moreover, very low concentrations of glucose (0·1%) interact synergistically with maltose leading to a rapid inactivation of maltotriose permease. (Table I) Obviously catabolite inhibition and wort composition play an important role in determining fermentation efficiency and the amount of residual fermentable matter in the final product. A similar situation exists for fructose. Indeed, in brewery fermentations, the preferential uptake of glucose over fructose assumes major importance in fermentations using high percentages of sucrose as adjunct, particularly in high gravity brewing. Such selectivity may not be manifest by all yeast strains and those that show selectivity may exhibit varying degrees depending upon the metabolic state of the yeast cell. Such strains may ultimately show a 'fructose block' where high levels of residual fructose remain in the finished beer. Sucrose is hydrolyzed by invertase into α-D-glucopyranose and β-D-fructofuranose. Gotsschalks investigations[3] showed that fructofuranose is converted reversibly to fructopyranose creating selectivity for glucose. Panchal and Stewart[4], however, have recently suggested that the pyranose form of fructose is preferably utilized. The anomeric form of the hexose is clearly significant and selectivity may be explained at the level of cell membrane carriers. Equally competition for the target sites of hexokinase isoenzymes, or even subsequently as an anomeric phosphorylated derivative for other glycolytic enzymes, may play a role in understanding this phenomenon. Considering the industrial impact in terms of beer quality, further information is needed on the 'fructose block' associated regulatory mechanisms to secure more efficient and controlled fermentations.

Scope and Limitations of Secondary Fermentation in Flavour Maturation

In considering the strong flavour potential of volatile sulphur compounds and vicinal diketones an important feature of maturation is the adjustment of their concentration during the lagering period. However, actual participation of secondary fermentation in the removal of these volatiles is doubtful taking into account the inefficiency of beer washing by carbon dioxide.

In fact, instead of the expected reduction, an increase in the levels of hydrogen sulphide and vicinal diketones is frequently observed during the first week of lagering.[13] Enhanced overflow of these undesirable by-products is probably due to the growth promoting conditions created by agitation and aeration during transfer and the resulting derepression of biosynthetic reaction sequences to balance the demand of valine, isoleucine and methionine at the low nitrogen levels encountered at the end of the primary fermentation. It should further be noted, that a longer maturation period is required when kräusen are added in order to activate the secondary fermentation. Indeed, such conditions will lead to an over-production of hydrogen sulphide depending on the relative proportions of threonine and methionine carried forward from primary fermentation.

However, quantities of hydrogen sulphide formed are extremely variable and depend on numerous factors such as wort treatment, heavy metals, fermentation conditions and yeast strain. Therefore, a far better approach is to control the formation of volatile sulphur compounds at a stage of the brewing process prior to maturation.[19] In this respect, hot wort aeration, removal of cold break, supplementing worts with vitamins, minerals and amino acids, and the choice of appropriate fermentation regimes and yeast strain are effective means to prevent excess levels of hydrogen sulphide in the finished beer. Nevertheless, some restrictions seem to be imposed with regard to flavour assessment of sulphur compounds and their effect on beer flavour. This will be especially the case when comparing ales and lagers. Indeed, although their negative attribute to beer flavour, it is almost certain that the presence in trace amounts of

mercaptans, hydrogen sulphide and organic sulphides is a prerequisite for giving lagers their typical character.

Of these compounds, dimethyl sulphide (DMS) has been reported to have quantitatively the greatest significance and is well reviewed as denoting a positive attribute to the flavour of lager beers.[2] Three proteins, thioredoxin, thioredoxin reductase and sulphoxide reductase has been shown to be involved in the reduction of dimethyl sulphoxide (DMSO) with NADPH as hydrogen donor.[3,10] However, the main purpose of thioredoxin is not to reduce DMSO but to generate reducing power in the growing yeast cell.

Moreover, sulphoxide reductase has a much higher affinity for methionine sulphoxide which, if present in wort prevents reduction of DMSO.[10] Thus, non-growth conditions as those encountered during maturation would certainly favour DMS production, especially if methionine sulphoxide contents are low. With regard to taste improvements modifications are apparently related to yeast sedimentation and to the elimination of polyphenol-protein complexes either by precipitation or by adsorption on the yeast cell wall. Cell surface adsorption phenomena that occur during lagering may therefore be considered as an important factor contributing to the physicochemical stability of beer. Notwithstanding this beneficial effect, it would be unrealistic to expect complete stabilisation by lengthening storage time. Obviously, other more economic and more efficient means exist to extend shelf life of the finished beer. With this background it might be useful to re-assess the overall contribution of secondary fermentation to beer flavour.

Biochemical and Genetic Approaches to Accelerate Flavour Maturation of Beer

In contrast to higher alcohols vicinal diketones are only produced through anabolic processes from the carbon source. Diacetyl and 2,3 pentanedione are formed by spontaneous oxidative decarboxylation of the corresponding acetohydroxy acids, metabolite intermediates of the common biosynthetic pathway leading to isoleucine and valine.[16]

Conversion of α-acetolactate and α-acetohydroxybutyrate to diacetyl and 2,3-pentanedione is the rate limiting step in the balance between formation and removal of vicinal diketones.[17,21] Actively metabolising yeast are able to reduce diacetyl to acetoin and butanediol and 2,3-pentanedione to 2,3-pentanediol. Alcoholdehydrogenase with NADH as cofactor and diacetyl reductase using NADPH as a coenzyme have been shown to reduce diacetyl.[9,33] However, diacetyl reductase has a three-fold higher affinity for diacetyl compared to acetoin. Although the ability to reduce vicinal diketones is strain dependent and declines after the primary fermentation enzyme activities are still non-limiting and sufficient to secure very low diacetyl levels during lagering.

This is also the reason why the application of diacetyl reducing enzymes to accelerate beer maturation has never been successful. In this regard, greater promise may lie with the use of acetolactate decarboxylase which catalyses the conversion of α-acetohydroxyacids into acetoin and acetyl ethyl carbinol.[11]

α-acetolactate decarboxylases are known to occur in several species of Enterobacter, Lactobacillus and Bacillus. Addition of these enzymes to freshly fermented beer results in a rapid removal of α-acetohydroxy acids to a level below the taste threshold value of the corresponding vicinal diketones.[12] Because of the shortened residence time, most of the fermentation systems geared to achieve higher productivities as compared to the traditional batch system, produce beers with high α-acetolactate and diacetyl levels. Accordingly, application of enzymatic procedures to overcome these difficulties, could play an important role for the commercial success of continuous and immobilized cell technologies. As pointed out earlier, accumulation of key-intermediates for valine and isoleucine biosynthesis in the early stages of fermentation suggests that, in brewery yeasts, the regulatory mechanisms involved in gene expression are inadequate to balance demand and utilisation of these amino acids. Of particular interest are the regulatory systems involved in the control of biosynthetic and catabolic amino acid pools in yeast as the regulation of the formation of higher alcohols and vicinal diketones is achieved through similar systems. If brewing strains could take up valine and isoleucine at a similar rate to threonine, such a strategy would help circumvent overflow of undesirable fermentation by-products.[32]

The alternative approach is genetic modification of the anabolic pathways for valine, isoleucine and leucine. Improved control might be expected by breeding strains with reduced or mutationally impaired catalytic function of acetohydroxy acid synthase. Previous studies have shown that valine-isoleucine-leucine auxotrophs deficient in acetohydroxy acid synthase did not produce acetolactate, acetohydroxybutyrate, diacetyl and 2,3-pentanedione[4,31] while a fifty percent decrease of the corresponding gene concentration was not affecting the formation of vicinal diketones.[24] Regarding the potential application of these findings, it should be emphasised that, although mutagenesis appears as an ideal tool for controlling the formation of vicinal diketones in brewing, many problems remain to be solved. Indeed, such mutants showed poor fermentation characteristics in the utilization of maltose and, particularly maltotriose at temperatures below 20°C. The main reason for this was a deterioration of fermentation capacity per unit yeast due to reduced growth characteristics because of the inability of the yeast strain to produce valine, isoleucine and leucine.

Obviously, more information at the molecular genetic level of the coding and controlling regions of ILV genes is needed to provide new and more efficient means of controlling diacetyl formation in brewery fermentations. Considerable progress has been made in this field as a result of studies on the structure and regulation of ILV genes of laboratory and industrial lager strains carried out at Carlsberg laboratory.[18,29,36] Results obtained suggest that reduced levels of threonine deaminase and acetohydroxy acid synthase might be expected from changes in the upstream regulatory sequences of ILV 1 and ILV 2 genes.

Formation of acetohydroxy acids could also be reduced by disrupting the coding regions of the two ILV2 genes of S. carlsbergensis, thus lowering the activity of acetohydroxy acid synthase. However, the question arises of to what extent enzyme activity should be decreased to attain a rate limiting level. Indeed, if rate limiting, acetohydroxy acid synthase being very sensitive to feedback inhibition a decrease in its level would entail derepression of enzyme synthesis by lowering the pool of valine. These considerations are not favouring an approach based on a control of gene action by gene concentration. A more direct strategy would be to increase the anabolic flux of intermediates leading to isoleucine and valine by inserting additional copies of ILV5 and ILV3 genes. We have evidence to suggest that this approach could be renumerative with respect to overcoming diacetyl flavour problems in brewery fermentations.[8]

Changes in the Physiological Behaviour of Lager Yeast During Maturation

In contrast to the vast amounts of studies undertaken to elucidate the importance of volatile flavour-active compounds in determining the characteristic aroma of young beer and aroma changes over the lagering period, the role of nonvolatile materials released by yeast has only received little attention.

The observed modifications are generally expressed as a better flavour association and, particularly, by a gradual increase in palate fullness and mouthfeel. The fact that these flavour characteristics are lacking in beers from which yeast

TABLE II. Changes in the physiological behaviour of a flocculent lager yeast during a 6 weeks traditional lagering process

Determination	Time of storage in weeks 0	1	2	3	6
$Q^{N_2}_{CO_2}$, glucose	250	220	210	225	80
$Q^{N_2}_{CO_2}$, maltose	260	225	220	220	85
$Q^{N_2}_{CO_2}$, maltotriose	80	75	70	75	25
Viability (%)	95	95	90	90	50
Invertase (nM glucose h^{-1} ml^{-1})	70	120	180	250	100
α-glucosidase (nM nitrophenol h^{-1} ml^{-1})	0	0	0	0	5

TABLE III. Analytical characteristics of beer samples taken from the middle and bottom of a horizontal tank after fermentation and after 40 days lagering

Determination	End fermentation Middle	Bottom	40 days storage Middle	Bottom
pH	4·0	4·1	4·15	5·0
FAN (mg/l)	58·0	60·0	80·0	700
Isohumulones (mg/l)	26·0	27·0	27·0	32·0
Anthocyanogens (mg/l)	49·0	51·0	50·0	60·0
Color EBC	7·5	7·5	7·5	10·0
Invertase (nm glucose h^{-1} ml^{-1})	70·0	75·0	100·0	1000
glucosidase (nm nitrophenol h^{-1} ml^{-1})	0	0	0	5

has been removed immediately after primary fermentation supports evidence that some correlation must exist between taste improvements and changes in yeast behaviour during lagering.[21]

A thorough examination of these two fundamental aspects could therefore provide experimental evidence with a view to completely chronicle the physiological events, including changes in cell permeability, when the yeast is maintained for long periods at a very low level of metabolic activity.[22,23]

If, during fermentation, the contribution of yeast to beer flavour appears to result indirectly from biosynthetic activities, the same activities can hardly be expected to take place at low turnover rates and in an environment where growth cannot be supported. Accordingly, taste modifications that are produced during maturation are more likely associated with endogeneous breakdown of cellular components through the initiation of hydrolytic systems.

Some insight into this problem has been achieved by determining changes in physiological behaviour of a flocculent yeast throughout a 6 weeks traditional storage process.[26] The assessments include measurements of fermentative capacity on glucose, maltose and maltotriose and of viability. All samples were taken from the bottom of a horizontal 400Hl tank. The activities of invertase and α glucosidase were measured in the supernatant beer. These enzymes were choosen because of their different location within the yeast cell. Results are given in Table II.

It can be seen that viability and fermentative power are stable within the first 3 weeks of storage. Subsequently the ability to ferment wort sugars declines very rapidly to attain a critical level after 5 to 6 weeks. Of particular interest is the finding that the activity of α glucosidase, which cannot be measured during the early stages of lagering, becomes readily detectable.

One would therefore expect that important changes in cell permeability occur during storage. The continuous secretion of invertase into the beer argues, on the other hand, for a possible participation of cell wall material such as glycoproteins in the maturation process. Thus, the decrease in cell viability and the release of excretion and autolysis products appear as a logical consequence of these particular aspects of cell metabolism under lagering conditions.

It should be emphasized, however, two distinct phenomena are involved. Excretion only modifies selective permeability of the plasma membrane, whereas autolysis entails rupture of the latter with a non selective release of protoplasmic material. Such a distinction is important because one could easily conceive that it is exclusively the evolution of the physiological state of the yeast during storage which will determine the profile of the released compounds.

Composition and Contribution of the Non Volatile Fraction to Flavour Maturation of Beer

The presence in beer of amino acids, peptides, nucleotides and organic as well as inorganic phosphates will in part be due to the secretion of these materials by the yeast during lagering. As with amino acids most of this material is contained in internal pools and its actual participation will depend upon cell permeability and subsequent exchange possibilities between the yeast cell and the surrounding beer. Thus, the overall contribution of the released intracellular material will depend upon many variables such as time and temperature; the type of yeast and its physiological state; the amount, rate of sedimentation and activity of the yeast present in the beer; the motion produced by continued fermentation, and the capacity, shape and surface of the storage vessels.

To evaluate the contribution of excretion and autolysis products to flavour maturation of beer, comparative analyses were carried out on beer samples taken from the bottom and the middle of a horizontal 400 Hl. tank respectively at the end of fermentation and after 40 days storage. Table III shows the principal characteristics of these beers after centrifuging out the yeast.

It can be seen that the composition of the beer taken from the bottom of the fermentation vessel is not altered by the high yeast concentration. In contrast, the analytical data of the samples taken after 6 weeks storage from the yeast layer beer deviate widely from the generally accepted norms. Of particular interest is the increase of pH. invertase activity and free amino nitrogen content of the beer taken from the middle of the tank.

Indeed, these findings support evidence that the nonvolatile material liberated from the yeast contributes to the composition of the finished beer.

In order to obtain a more complete picture of the biochemical processes occurring during maturation the release of amino acids, peptides, inorganic and organic phosphates, adenosine-5'-mono-phosphate as well as changes in pH. and viability have been followed in samples containing 50% yeast suspension in beer. Analyses were carried out immediately after fermentation and every two weeks during storage 0°C. in the laboratory. An industrial sample taken from the bottom layer after 35 days lagering and containing the same amount of yeast has been used for comparison. The aim was to simulate changes occurring in a classical process and to ascertain whether similar results are obtained under both conditions. Results are summarized in Table IV.

Results obtained for free amino nitrogen confirm those obtained previously. While variations in amino acids and peptides are extremely important those corresponding to components of a molecular weight more than 1000 are only small.

TABLE IV. Changes in the composition of beer containing 50% yeast during six weeks storage at 0°C

Determination	Time of storage in weeks				
	Laboratory				Industry
	0	2	4	6	5
α-NH_2 Nitrogen (mg/l)	40·0	286	680	1010	570
α-NH_2 Nitrogen (mg/l) MW < 1000	21·0	230	618	925	527
α-NH_2 Nitrogen (mg/l) MW > 1000	19·0	56	72	75	43
Inorganic Phosphates (mg/l)	170	414	670	870	602
Organic Phosphates (mg/l)	13	106	130	200	41
5'-AMP	0	1·3	8·0	38·4	10
pH	4·00	6·56	6·82	6·67	5·61
Viability (%)	95	80	70	40	60

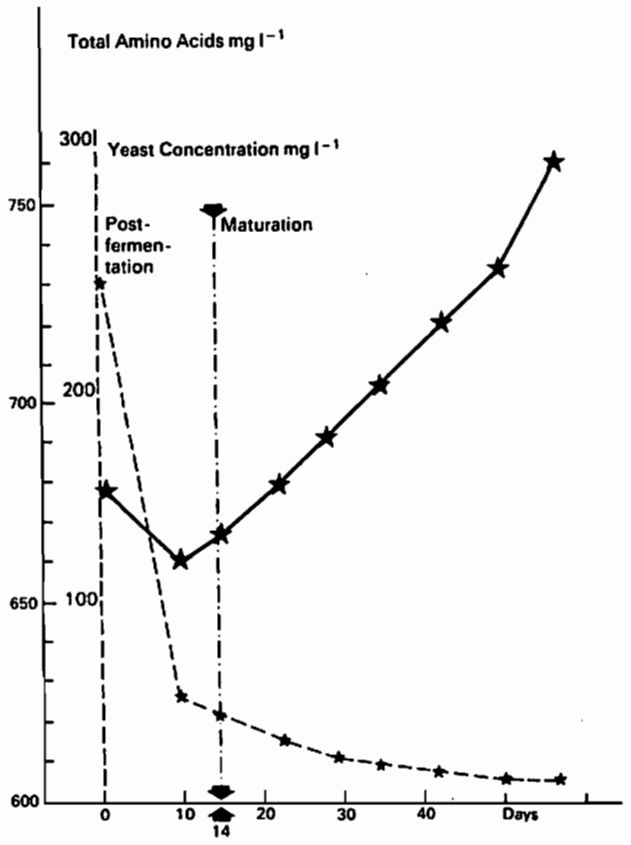

Fig. 1. Changes in total amino acid content and yeast concentration during 8 weeks secondary fermentation with a flocculent lager yeast.

Noteworthy is the fact that concentrations of this material are significantly higher in beers stored in the laboratory (1010 mg/l) compared to those obtained after industrial lagering. The same applies to adenosine-5'-monophosphate and phosphates. These findings are of particular interest as they support evidence that there is a significant contribution of the compounds accumulated in the sedimented yeast to the overall composition of the finished beer.

The question raised by the demonstration that non-volatile compounds are released by yeast during maturation is to what extent this material is contributing to the composition of the final product.

In order to define in more quantitative terms the exchanges in the amino acid composition of a 13·4% Pilsner beer have been followed during 8 weeks traditional lagering.

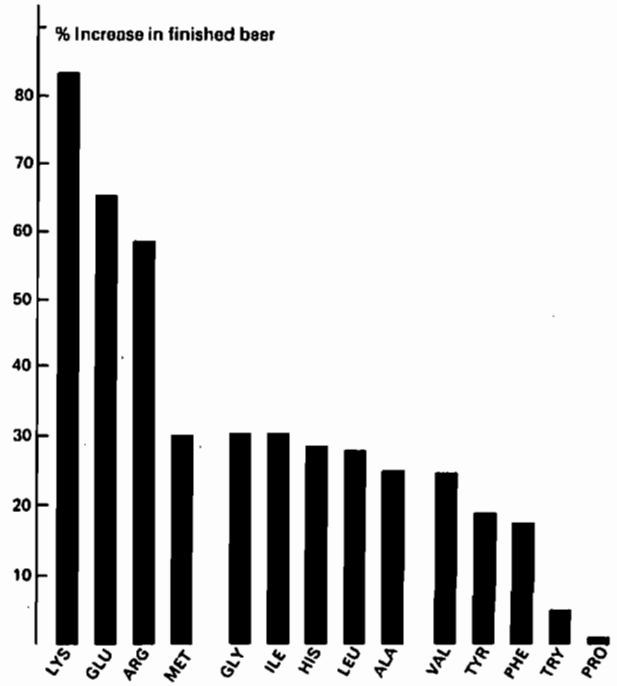

Fig. 2. Contribution of amino acids released by flocculent lager yeast to the composition of the finished beer after 8 weeks storage.

Samples were taken every week in the middle of a 400 Hl horizontal tank. A sample of the sedimented yeast collected at the end of lagering has been used for comparison.

Noteworthy is the fact that changes in the amino acid contents during secondary fermentation occur in two distinct stages—a first stage of approximately 14 days during which yeast completes transformations discontinued by transferring the beer from the fermentation vessel and a second stage corresponding to the actual release of nitrogen material into the surrounding beer.[25] This is illustrated in Fig. 1.

Considering the rapid fall in yeast concentration, it can be assumed that from the third week of storage most of the amino acids which appear in the beer must be derived from the settled yeast. Supposing there is a constant exchange between yeast and beer one can expect a significant correlation between the rate of accumulation of amino acids in barm beer and their proportional increase in the finished beer.

It has been shown that the amino acid composition of barm beer deviates widely from that of beer taken at the end of fermentation. The greatest variations occur for proline, the concentration of which decreases from 47 to 8% in barm beer, and for lysine, glutamic acid and arginine where a 5 to 13 fold increase is observed.

As can be seen from Fig. 2, the rate of secretion of each amino acid and its contribution to the composition of the final beer are indeed closely related.

Metabolic Sequences Involved in Flavour Maturation of Beer

As outlined above yeast contains metabolic pools of amino acids and nucleotides. During growth the outflow of these metabolites is limited by the selective effect of the plasma membrane. However, accumulation and subsequent release from the internal pool may be expected under storage conditions. If so, the maturation process appears to be primarily controlled by intracellular breakdown and cell permeability.

Following the lines of such an approach the effects of storage on accumulation and outflow of free amino nitrogen and amino acids has been studied.[26,34] Results obtained

indicate considerable changes in the amino acid composition of the yeast pool and barm beer. A marked increase in the intracellular concentrations of individual amino acids is observed throughout the lagering period except in the case of proline, aspartic acid, glycine and alanine.

Noteworthy, however, is the fact that the rate of release of these compounds is almost identical to that found for the other amino acids.

Accordingly, while pool accumulation provides the necessary amount of amino acids inside the yeast cell, the availability of this material outside the yeast cell will depend upon specific changes in cell permeability.

These striking qualitative as well as quantitative similarities suggest that the major metabolic sequences involved in maturation of beer are breakdown of cell constituents and accumulation of the degredation products in endogenous pools gradual loss in selectivity of the plasma membrane and release of amino acids, peptides, nucleotides, phosphates and other materials, and decrease in viability with simultaneous release of autolysis products.

One Tank Operation Accelerated Maturation and Beer Flavour

The combination of unit processes of fermentation and maturation provides the opportunity to reduce total costs of the cellaring operation as a whole. The association with the concepts of larger volume processes and higher gravity brewing is a further necessary step to improving productivity. In applying a one tank large volume batch process it is essential to give the bottom of the vessel an adequate deep slope to enable the yeast to be easily and rapidly removed at the end of primary fermentation to prevent autolysis products from adversely affecting the flavour of the beer.

However, construction costs are considerably reduced by minimizing the ratio of surface to unit volume. Minimal ratio are obtained when the diameter equals the height. From that standpoint the nearly flat bottomed ASAHI vessel and UNI-TANK with height to diameter ratios close to 1/1 are the most economical, the NATHAN type cylindro-conical vessels with steep coned angles being the most expensive. Of the several types of multi-purpose large volume tanks operating in different breweries throughout the world the most suitable fermenter for an accelerated one-tank process is obviously a vessel with a sharp edged conical bottom.

To take maximum advantage of benefits offered by such large tank fermenters, the brewer must choose process parameters consistent with efficiency and beer flavour. Thus, the next question to be considered is how to make use of the knowledge gained by investigating a traditional lagering process when changing existing conditions or designing new fermentation systems to achieve higher productivities.

Among the factors controlling the rate of maturation, temperature appears to be one of the most important. The removal of diacetyl is generally considered as one of the principal reasons for maturation. Therefore, many brewers have introduced a warm period of maturation towards the end of fermentation. Obviously, such periods of warm storage at low levels of fermentable extract must influence yeast metabolism by activating degradative systems similar to those operating in traditional lagering.[21]

If so, a temperature assisted maturation could lead to an enhanced breakdown of cell constituents and a more rapid loss of the selective function of the plasma membrane with simultaneous release of autolytic products.[26,34] Although some of these compounds have a desirable effect on beer flavour, others are adversely affecting taste and aroma of the final product. High yeast concentrations long contact times and higher temperatures, which normally occur at the base of a deep fermenter, should be avoided by appropriate cooling and by programmed removal of yeast immediately

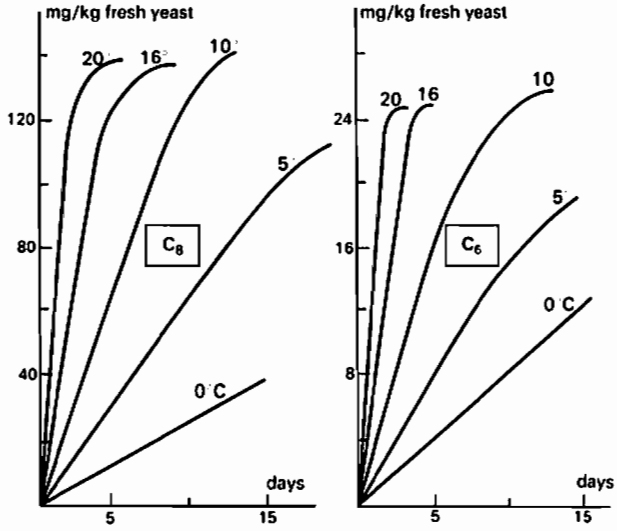

Fig. 3. Effect of temperature on the release of caprylic (C_8) and caproic (C_6) acids during storage of a 50% yeast suspension in beer.

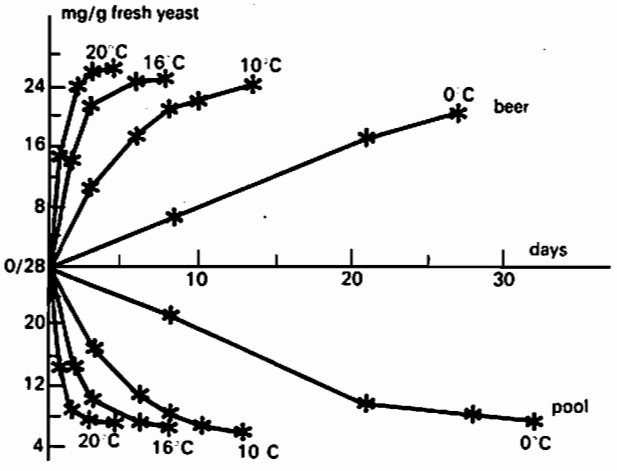

Fig. 4. Effect of temperature on the internal (pool) and external (beer) concentrations of caproic acid during storage of a 50% yeast suspension in beer.

before and immediately after the warm maturation period and then at regular intervals until the end of storage.

It has been shown[35] that incomplete or delayed removal of sedimented yeast may lead to an increased release of short and medium chain length fatty acids.

The significance of caproic, caprylic and capric acids has been studied by Clapperton.[6,7] In considering the threshold concentrations[7] of octanoic acid (4,5 mg/l), decanoic acid (1,5 mg/l) and dodecanoic acid (0,6 mg/l) and their respective concentrations in beer it might be assumed that all are significant contributors to the so called caprylic flavour. The influence of hexanoic acid is less pronounced. Most important to the technical brewer is the increased flavour potential which results from the additive effect of C_6–C_{12} acids on beer flavour. It has been reported by Clapperton[7] that threshold concentrations are reduced by half when a mixture of octanoic and decanoic acids are added to beer.

It must therefore be pointed out that even when threshold values of individual beer fatty acids are not exceeded, small changes in their respective concentrations may have a significant influence on flavour. Some brewers recognize the caprylic characteristics as yeasty.

Direct evidence for the contribution of these acids to autolytic and yeasty flavours has come from the odour assessments at different pH values of beer of tank bottoms taken at the end of a six weeks traditional storage process. Interestingly, acidification from pH 6 to pH 4 shifts the

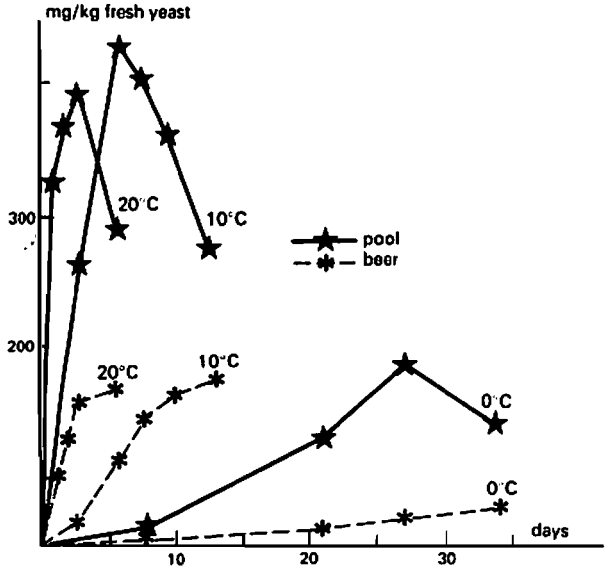

Fig. 5. Effect of temperature on the internal (pool) and external (beer) concentrations of capric acid during storage of a 50% yeast suspension in beer.

flavour spectrum from a distinct sulphury thiamine like odour note towards a typical fatty acid aroma of a butyric-caprylic type. Moreover, the alternation between these two flavour impressions is totally reversible and entirely a function of beer pH.[35] These observations are the expected consequence of the complexity, in terms of analytical interpretation and instrumental measurement of autolytic flavour. In view of these facts the effect of temperature on the excretion of fatty acids by lager yeast has been examined.

According to results obtained[35] release of butyric, isovaleric, caproic, caprylic and capric acids by yeast is increased within the range 0–20°C. Although maximum levels are extremely different from one fatty acid to another, these maxima are not temperature dependent. As can be seen from Fig. 3 the maximum values obtained for caproic and caprylic acids are respectively 25 and 140 mg/kg fresh yeast. They are reached after 3 days at 20°C, 10 days at 10°C and 60 days at 0°C. Excretion rates are highly temperature dependent. A substantial increase is observed above 10°C. The strongest effect is obtained for capric acid, the excretion rate at 16°C. being ten times higher than at 10°C. Only a twofold increase is observed for the other fatty acids.

Synthesis of caproic and caprylic acids seems therefore to be suppressed as early as the beginning of maturation. As a result release is continued until an equilibrium is reached between pool and beer contents. Fig. 4.

In contrast, incubations within the range 0–20°C. did not deplete the pool content of capric acid.[30] As shown in Fig. 5 both internal and external concentrations are increasing. These results suggest that the synthesis of the C_{10} acid is still active in the course of maturation.

The low and additive threshold values of beer fatty acids together with their rapid release, when high yeast concentrations are associated with long contact times, do not leave any doubt with respect to the predominant role played by these acids in the development of yeasty flavours in the course of maturation. The higher the temperature at which maturation is conducted, the greater the risk of off-flavour formation and the more critical will be the effect of yeast concentration and contact time on beer flavour.[27,34]

References

1. Anness B. J. & Bamforth, C. W. *Journal of the Institute of Brewing*, 1982, **88**, 24.
2. Bamforth, C. W. *Federation of European Microbiological Societies, Microbiology Letters*, 1980, **7**, 55.
3. Bamforth, C. W. & Anness, B. J. *Journal of the Institute of Brewing*, 1981, **87**, 30.
4. Cabanc, B., Ramos-Jeunehomme, C., Lapage, N. & Masschelein C. A. *American Society of Brewing Chemists, Proceedings* 1973, 94.
5. Chevallier, P., Chollot, B., Chapon, L. & Urion, E. *Proceedings of the 8th European Brewery Convention, Congress, Vienna*, 1961, 246.
6. Clapperton, J. F. & Brown, D. G. *Journal of the Institute of Brewing*, 1978, **84**, 90.
7. Clapperton, J. F. *Journal of the Institute of Brewing*, 1978, **84**, 107.
8. Dillemans, M., Goossens, E., Goffin, O. & Masschelein, C. A., *Journal of the American Society of Brewing Chemists*, 1986, (in press)
9. Galzy, P., Ratomahenina, R. & Louis-Eugène, S. *Proceedings of the 19th European Brewery Convention Congress, London*, 1983, 505.
10. Gibson, R. M., Large, P. J., Anness, B. J. & Bamforth, C. W. *Journal of the Institute of Brewing*, 1983, **89**, 215.
11. Godtfredsen, S. E. & Ottesen, M. *Carlsberg Research Communications*, 1982, **47**, 93.
12. Godtfredsen, S. E., Ottesen, M., Siqsgaard, P., Erdal, K., Mathiasen, T. & Ahrenst-Larsen B. *Proceedings of the 19th European Brewery Convention, Congress, London*, 1983, 161.
13. Gombert, J. *Brauwelt*, 1975, **118**, 598.
14. Gotschalk, A., *Wallerstein Laboratories Research Communications*, 1947, **10**, 109.
15. Haboucha, J. & Masschelein, C. A., *Cerevisia*, 1976, **1**, 97.
16. Inoue, T., Masuyana, K., Yamamoto, Y., Okada, K. & Kuroiwa, Y., *Proceedings of the American Society of Brewing Chemists*, 1968, 158
17. Inoue, T. & Yamamoto, Y., *Report of the Research Laboratories of Kirin Brewery*, 1970, **13**, 79.
18. Kielland-Brandt, M. C., *Proceedings of the 18th European Brewery Convention, Congress, Copenhagen*, 1981, 263.
19. Masschelein, C. A., Ramos-Jeunehomme, C. & Devreux, A., *Proceedings of the 8th European Brewery Convention, Congress, Vienna*, 1961, 148.
20. Masschelein, C. A. & Haboucha, J., *Society of Chemical Industry, Monograph 19, Surface Activity of the Microbial Cell, London*, 1965, 202.
21. Masschelein, C. A., Ramos-Jeunehomme, C., Jenard, H., & Devreux, A. *Proceedings of the 13th European Brewery Convention, Congress, Estoril*, 1971, 211.
22. Masschelein, C. A., Vande Meerssche, J., Haboucha, J. & Devreux, A. *Proceedings of the American Society of Brewing Chemists*, 1973, 114.
23. Masschelein, C. A., *Proceedings of the 13th Convention of the Australian & New Zealand Section of the Institute of Brewing*, 1974, 59.
24. Masschelein, C. A., *Southern African Yeast and Fermentation Techniques Symposium*, 1977, 6.
25. Masschelein, C. A., *Monatschrift für Brauerei*, 1975, **28**, 189.
26. Masschelein, C. A., & Vande Meerssche, J., *Masters Brewers Association of the Americas Technical Quarterly*, 1976, **13**, 240.
27. Masschelein, C. A., *European Brewery Convention, Flavour Symposium, Copenhagen, Monograph VII*, 1981, 211.
28. Panchal, C. J. & Stewart, G. G., *Journal of the Institute of Brewing*, 1982, **88**, 86.
29. Petersen, J. G. L., *Proceedings of the 20th European Brewery Convention, Congress, Helskinki*, 1985, 275.
30. Ramos-Jeunehomme, C., Haboucha, J., Devreux, A. & Masschelein, C. A. *Proceedings of the 14th European Brewery Convention, Congress, Salzburg*, 1973, 111.
31. Ramos-Jeunehomme, C. & Masschelein, C. A. *Proceedings of the 16th European Brewery Convention, Congress, Amsterdam*, 1977, 267.
32. Ryder, D. S. & Masschelein, C. A., *European Brewery Convention, Symposium on Biotechnology, Nutfield, Monograph* IX, 1983, 2.
33. Van den Berg, R., Harteveld, P. A. & Martens, F. B. *Proceedings of the 19th European Brewery Convention, Congress, London*, 1983, 497.
34. Vande Meerssche, J., Devreux, A. & Masschelein, C. A. *Proceedings of the 16th European Brewery Convention, Congress, Amsterdam*, 1977, 561.
35. Vande Meerssche, J., Devreux, A. & Masschelein, C. A., *Proceedings of the 17th European Brewery Convention, Congress, Berlin*, 1979, 787.
36. Wettstein, D. von, *Proceedings of the 20th European Brewery Convention, Congress, London*, 1983, 97.