

Branched chain aldehydes: production and breakdown pathways and relevance for flavour in foods

Bart A. Smit · Wim J.M. Engels · Gerrit Smit

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Abstract Branched aldehydes, such as 2-methyl propanal and 2- and 3-methyl butanal, are important flavour compounds in many food products, both fermented and non-fermented (heat-treated) products. The production and degradation of these aldehydes from amino acids is described and reviewed extensively in literature. This paper reviews aspects influencing the formation of these aldehydes at the level of metabolic conversions, microbial and food composition. Special emphasis was on 3-methyl butanal and its presence in various food products. Knowledge gained about the generation pathways of these flavour compounds is essential for being able to control the formation of desired levels of these aldehydes.

Keywords Fermentative flavour formation · Lactic acid bacteria · Amino acid converting enzymes · Branched chain aldehydes · 3-Methylbutanal

Introduction

Branched-chain aldehydes, such as 3-methyl butanal, 2-methyl butanal and 2-methyl propanal, are potent flavour compounds. In many food products, such aldehydes are key-flavour compounds. Sensorially, they are generally perceived as malty, chocolate-like. An important process leading to the formation of compounds like 3-methyl butanal is the non-enzymic, heat-induced, Strecker degradation of amino groups with reducing sugar moieties (Strecker 1862). Since foods during their production are in many cases subjected to some kind of heat treatment and protein and carbohydrates are generally present, the conditions for the formation of branched-chain aldehydes are favoured. In addition to chemical formation, branched-chain aldehydes are formed during fermentation of many foods. In foods like chocolate/cacao, the combination of fermentation followed by heat treatment gives rise to optimal flavour formation (Counet et al. 2002).

An (branched-chain) aldehyde is an organic compound containing a terminal carbonyl group. This functional group, which consists of a carbon atom bonded to a hydrogen atom and double-bonded to an oxygen atom (chemical formula $O=CH-$), is called the aldehyde group. The slightly positive carbon atom in the aldehyde group, caused by the electronegative oxygen atom, is susceptible to attacks by nucleophiles, and this makes an aldehyde relatively reactive. Aldehydes can therefore relatively easily be reduced to the corresponding alcohols or oxidised to the corresponding acids. Consequently, aldehydes are generally present only in low concentrations, however, the taste thresholds of aldehydes are also rather low; for 2-methyl propanal and 2- and 3-methyl butanal, they were reported as 0.10, 0.13, and 0.06 mg/l, respectively (Sheldon et al. 1971).

B. A. Smit
Campina Innovation,
Nieuwe Kanaal 7C,
6709PA Wageningen, The Netherlands

W. J. Engels
Department of Flavour, NIZO Food Research,
PO Box 20, 6710 BA Ede, The Netherlands

G. Smit
Department Food Chemistry, Wageningen University,
PO Box 8129, Wageningen, The Netherlands

G. Smit (✉)
Department of Flavour Generation and Delivery,
Unilever Food and Health Research Institute,
PO Box 114, 3130 AC Vlaardingen, The Netherlands
e-mail: gerrit.smit@unilever.com

Formation and conversion

Although 3-methyl butanal and the other short-chain, branched aldehydes are important flavour compounds in many foods, biochemical conversion routes have been studied mainly in fermented dairy products and chemical conversions mainly in relation to Strecker (Maillard) reactions. This paragraph will focus on influencing the conversion rates leading from and to 3-methyl butanal in several food systems, leaving the biochemical details in the referred papers.

Leucine catabolism

3-Methyl butanal is an intermediate in the catabolism of leucine. A general summary of this catabolism is shown in Fig. 1. The numbers in this figure will be referred to in the next sections and in paragraph headings in parenthesis.

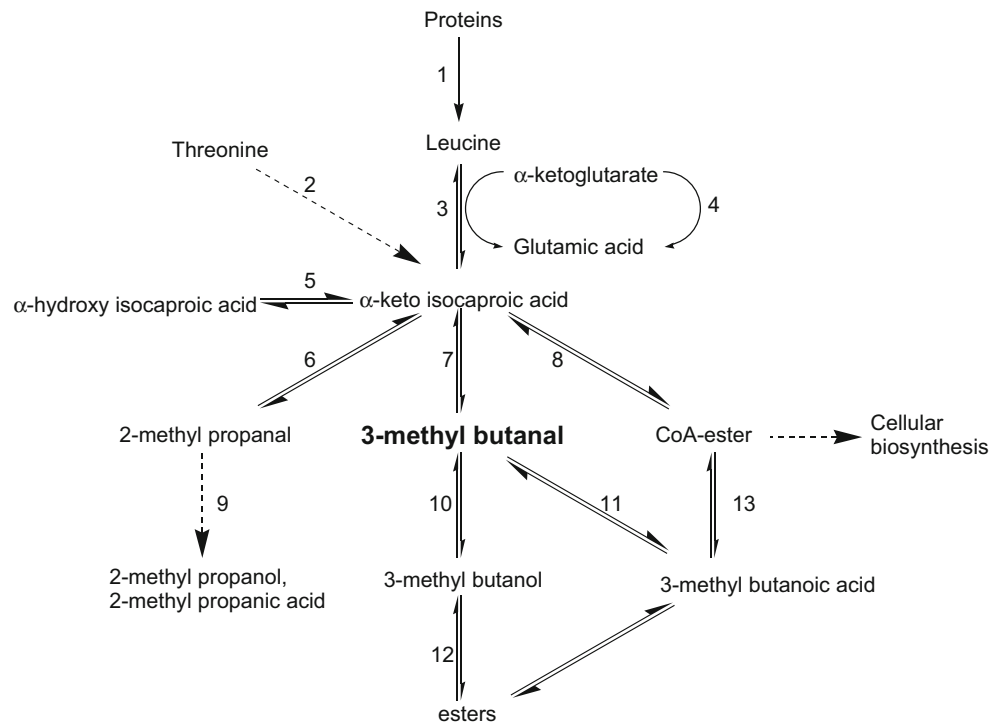
The pathway from leucine via the corresponding α -keto acid and aldehyde to alcohol is referred to as the Ehrlich pathway, which was identified in yeasts as the main route for fusel alcohol formation (Ehrlich 1907). In addition to this main route, leucine catabolism may result in hydroxy-acids, CoA-esters and other high flavour-impact aldehydes, alcohols and esters. This scheme has extensively been reviewed for lactic acid fermentations (Yvon and Rijnen 2001; Smit et al. 2005b) and alcoholic fermentations (Dickinson 2000b). The catabolism of valine, isoleucine, phenylalanine and methionine proceeds very similarly to

the pathway described in this paper for leucine (suggested reviews: Kohlhaw 2003; Fernandez and Zuniga 2006).

Amino acid pool (reactions 1 and 2)

In food systems, leucine is generally liberated from protein by extracellular and intracellular proteolysis (1), although many micro-organisms can also make leucine from threonine (reaction 2). In *Lactococcus* spp., leucine biosynthesis is encoded by the ILV and LEU operon (Godon et al. 1992, 1993). The last step in the leucine anabolism and first step Leucine catabolism are the same: the conversion between α -keto-isocaproic acid and leucine by a transaminase (Godon et al. 1992). CodY is an important regulator of the internal amino acid pool by controlling peptidase, transporter and transaminase genes, based on isoleucine levels (Chambellon and Yvon 2003). The concentration of isoleucine hereby also affects the concentrations of other amino acids. Proteolysis is essential for liberating enough amino acids for full flavour development. The proteolytic system of micro-organisms is extensively studied and reviewed (Ogrydziak 1993; Fox and McSweeney 1996; Kunji et al. 1996; Christensen et al. 1999; Savijoki et al. 2006). The large variation in peptidase activities among strains of many species may yield a good approach for controlling protein degradation in foods (Ayad et al. 1999; Gatti et al. 2004; Di Cagno et al. 2007; van Hylckama Vlieg and Hugenholtz 2007). In addition to the natural diversity, strains over-expressing peptidases were developed (Van De Guchte et al. 1990; Courtin et al. 2002;

Fig. 1 Metabolic network around 3-methyl butanal



Bockelmann et al. 2006). The increase of amino acids in cheese curd, obtained by the application of peptidase over-producing starter cultures, not only leads to increased flavour perception due to the flavour of the amino acids themselves but also to increased levels of amino acid derived flavour compounds, such as 3-methyl butanal (Exterkate and Alting 1995; Courtin et al. 2002). The same effect was observed in dried sausages (Dura et al. 2004; Herranz et al. 2005; Casaburi et al. 2008) and sourdough (Gänzle et al. 2007).

Transaminase activity (reaction 3)

An initial step in leucine conversion of leucine towards 3-methyl butanal is the formation of α -keto isocaproic acid. Reaction 3 in Fig. 1 yields this important intermediate and can be catalysed by transaminases and leucine dehydrogenase. In food systems, the transaminases play a major role (Yvon et al. 1997; Engels et al. 2000; Larroure et al. 2000), while lactic acid bacteria (LAB) related spoilage organisms such as *Bacillus* spp. and *Clostridium* spp. possess a leucine dehydrogenase (Zink and Sanwal 1962; Hummel and Kula 1989). Several transaminases have been described, including leucine, branched-chain amino acid, aromatic amino acid and methionine transaminases. These enzymes have overlapping substrate specificities (Gao and Steele 1998; Rijnen et al. 1999a,b; Engels et al. 2000; Yvon et al. 2000; Hansen et al. 2001). Several studies have shown that transaminase presence and activity varies largely among bacterial species and strains (Smit et al. 2004c; Fernandez De Palencia et al. 2006; Liu et al. 2008; van Hylckama Vlieg and Hugenholtz 2007). Knocking out the branched chain amino transferase in *Lactococcus lactis* resulted in roughly 90% reduction in leucine transamination (Yvon et al. 2000). The other 10% conversion was caused by the aspecificity of other transaminases (Engels et al. 2000; Yvon et al. 2000). Knocking out two transaminases in yeast did not lead to a full repression of the fusel alcohol formation because anabolism provided the corresponding α -keto acids converted in the Ehrlich pathway (Eden et al. 2001; Schoondermark-Stolk et al. 2005). Knocking out the amino acid anabolism on top of the transaminases still led to minor amounts of 3-methyl butanal and isoamyl alcohol, indicating that an additional pathway or source for α -keto isocaproic acid was also present (Eden et al. 2001).

Yvon et al. (1999) were the first to show that adding α -keto glutarate to cheese curd leads to increased flavour levels due to increased transamination capacity. Later, this was also shown for other cheese types and for sausages (Larroure et al. 2000; Banks et al. 2001; Ur-Rehman and Fox 2002; Beck et al. 2004; Herranz et al. 2004; Tjener et al. 2004a; Williams et al. 2004). Instead of adding α -ketoglutarate to cheese, the α -ketoglutarate can be recycled using the enzyme glutamate dehydrogenase (GDH,4). This was shown by introducing a

GDH gene from *Peptostreptococcus* in a *L. lactis* strain (Rijnen et al. 2000). Later, the same group showed the presence of this gene in LAB and the natural transfer of the GDH property to starter lactococci (Tanous et al. 2006).

Although increasing amino acid concentrations and enhancing transamination activities were proven to be effective in increasing flavour formation by many food related organisms, only a minor part of the converted leucine accumulates as 3-methyl butanal in food products (Yvon et al. 1999; Kieroneczyk et al. 2003; Smit et al. 2004c). This indicates that reaction rates of α -keto-isocaproic acid converting enzymes influence the formation of 3-methyl butanal. Several approaches for controlling the competition between various α -keto isocaproic acid consuming reactions are described in the following paragraphs.

Branched chain keto acid decarboxylase activity (reaction 7)

This enzyme catalyses the decarboxylation of α -keto isocaproic acid to 3-methyl butanal. Its activity in lactococci (*Streptococcus lactis*) was identified by Tucker and Morgan (1967). In yeast, the presence of such an enzyme besides the specific pyruvate decarboxylase (PDC) was postulated in relation to the production fusel alcohols (Chen 1977; Oku and Kaneda 1988; Ter Schure et al. 1998). Wild lactococcal strains, *Corynebacterium* (Ayad et al. 1999; Smit et al. 2004c), *Carnobacterium* (Larroure et al. 2000), and *L. delbreuckii* subsp. *lactis* (Helinck et al. 2004), appeared to possess this activity more generally and with higher activities than LAB used as starter cultures in dairy applications. Smit et al. first discovered the gene encoding the branched chain keto acid decarboxylase enzyme (KdcA) in *L. lactis* by screening a mutant library of a decarboxylase-positive strain in a decarboxylase-negative strain (Smit 2004). De la Plaza identified a similar enzyme (KivD) by N-terminal sequencing the partially purified protein (De La Plaza et al. 2004). The gene occurs rarely in the sequenced LAB genomes (Liu et al. 2008). In contrast to PDC, KdcA has a very broad substrate specificity, hereby being able to produce various flavour compounds (Smit et al. 2005a; Vuralhan et al. 2005; Yep et al. 2006; Gocke et al. 2007). This broad specificity makes this enzyme also very interesting for use as biocatalyst (Berthold et al. 2007; Gocke et al. 2007). Over-expression of the enzyme was very successful, but in synthetic medium, the 3-methyl butanal production was (only) similar to the wild strain. This indicates that under these conditions, the decarboxylase capacity of the wild strain was not limiting. A test with an over-expression mutant in cheese with or without α -ketoglutarate has, to our knowledge, not been described. Food-grade non-starter LAB (“wild-LAB”) exhibiting high activity of this enzyme have successfully been applied in cheese (Ayad et al. 2000; Whetstone et al. 2006).

Hydroxy acid and keto acid dehydrogenases (reactions 5 and 8)

In lactococci, a relatively large amount of leucine is converted in the α -hydroxy isocaproic acid (Yvon et al. 1999; Smit et al. 2004c). This is in line with the general desire of converting NADH to NAD⁺ by metabolically active lactic acid bacteria (Schlegel 1997). Over-expression of α -hydroxy acid dehydrogenase in *Lactobacillus casei* followed by applying this strain as adjunct starter in the preparation of cheddar cheese resulted in a decrease of keto-acid derived flavour compounds. This confirms the high impact of this enzyme on the α -keto-acid conversion, even when used as adjunct culture (Broadbent et al. 2004; see also below). This implies that knocking out the gene coding for this dehydrogenase in combination with high decarboxylase activity should most probably yield increased flavour levels.

The overall reaction catalysed by the α -keto acid dehydrogenase complex is a substitution of CO₂ by the cofactor CoA while reducing NAD⁺. The enzyme is present and active in yeasts, bacilli, enterococci, propioni bacterium and *Lactococcus lactis*. The CoA coupled acid can be used in fatty acid biosynthesis or hydrolysed to the branched chain organic acid (reaction 3). This reaction does not lead to the formation of 3-methyl butanal or the corresponding fusel alcohol but produces relatively high amounts of corresponding organic acids, for example by *Lactobacillus helveticus* (Namba et al. 1969; Derrick and Large 1993; Ward et al. 1999; Dickinson 2000a,b; Hester et al. 2000; Zhu et al. 2005).

Chemical keto-acid conversion (reaction 6)

Besides various enzymatic conversions, chemical oxidation of α -keto-isocaproic acid may occur under cheese-like conditions. This reaction is catalysed by manganese and results in 2-methyl propanal (Smit et al. 2004a). This aldehyde is generally associated with (enzymic) valine catabolism. The activity can be modulated by the Mn²⁺, oxygen concentration and redox potential (Smit et al. 2004a; Kieronczyk et al. 2006). The variation in the formation of volatiles in a meat model system was mainly determined by pH and bacterial species and, to a lesser extent, by the manganese concentration (Olesen and Stahnke 2004; Tjener et al. 2004b). This indicates that this chemical conversion is not dominant in this system.

3-Methyl butanal conversion (reactions 10 and 11)

Once formed, 3-methyl butanal can be converted to compounds such as 3-methyl butanol, 3-methyl butanoic acid, via reduction and oxidation respectively, and esters. In many organisms, 3-methyl butanal is therefore only a

transient metabolite (De Vos Petersen et al. 2004). Products from 3-methyl butanal as mentioned clearly add to the (balance in) flavour perception, but their odour threshold is much higher than that of 3-methyl butanal. When maximal flavour is desired, a reduction of these 3-methyl butanal conversions might therefore be beneficial. The natural variation in activity of the enzymes, found in various microbial strains, involved is an appropriate way to control this balance.

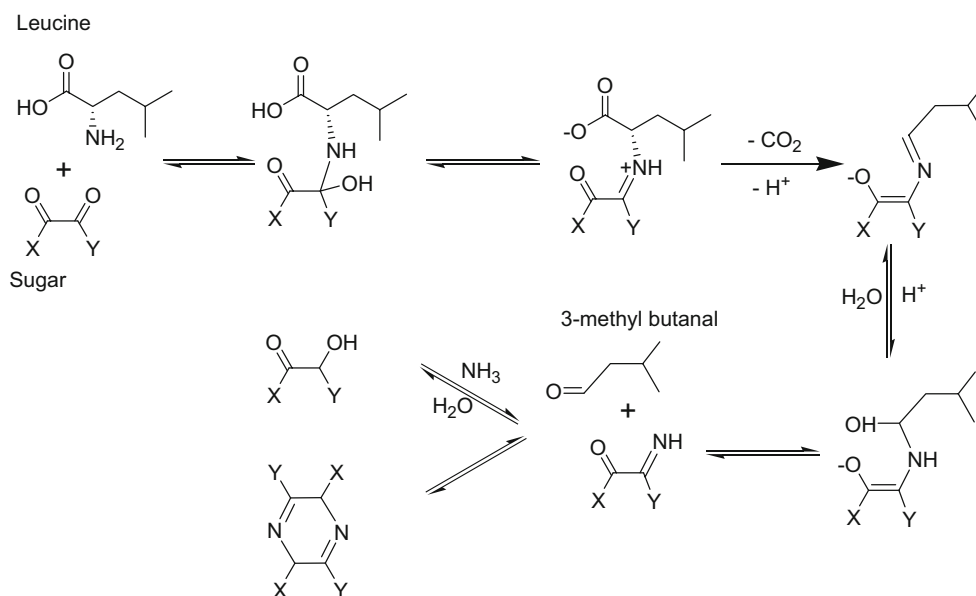
Alcohol dehydrogenase (10) catalyses the reduction of 3-methyl butanal to 3-methyl butanol. This is the last step in the Ehrlich pathway. This reaction is very active in yeasts (Chen 1977). In many lactic acid bacteria, alcohol dehydrogenase is present, but its activity is much lower (Hatanaka et al. 1974; Schneider-Bernlohr et al. 1981; Bradshaw et al. 1992; Arnau et al. 1998; Temino et al. 2005).

Aldehyde dehydrogenase (11) is also active in many organisms. In *Staphylococcus xylosus*, it has been shown that 3-methyl butanoic acid is mainly formed by the consecutive decarboxylation of the keto acid followed by oxidation of the aldehyde by a wide spectrum aldehyde dehydrogenase. It has also been shown that the oxidation activity is higher than the decarboxylation activity, resulting in low 3-methyl butanal concentrations (Beck et al. 2002). In yeast, the organic fusel acids can be transported out of the cell by the PDR12p ABC transporter (Hazelwood et al. 2006).

Chemical formation of 3-methyl butanal by Strecker degradation

The Maillard reaction is very important for the formation of brown colour and flavour in especially heat-treated products such as bread and malt. In short, the Maillard reaction starts with the condensation of an amino group with a reducing sugar leading to a so-called Amadori product. Rearrangement of this Amadori product can lead to dicarbonyls. The reaction of such a dicarbonyl with an amino acid resulting in flavour-active aldehydes is called the Strecker degradation (Strecker 1862; Schonberg and Moubacher 1952). In the case of leucine, deamination followed by decarboxylation results in 3-methyl butanal as shown in Fig. 2 (Pokorny et al. 1973; Baltes 1982; Oberparleiter and Ziegler 1997; Rizzi 1998). The major control parameters are temperature, but substrate concentrations and substrate characteristics also affect their conversion rates. Already in 1957, Keeny and Day (1957) postulated that this reaction might add to the flavour of cheese, since amino acids and reducing sugars are available in a suitable environment and a long (ripening) time enables the reaction to proceed. Later, several kinetic studies were done with other substrates at elevated temperatures, but no kinetic data under food fermentation conditions (relatively

Fig. 2 Reaction scheme of the Strecker degradation of leucine (adapted from Rizzi 1998)



low temperature) have been presented (Hofmann et al. 2000; Martins et al. 2003; Martins and Van Boekel 2005).

Biodiversity, lysis, co-operation

The intracellular processes, as discussed previously, are influenced by the total food matrix. The chemical and physical properties of the surrounding matrix influence the behaviour of the microbial cell. Other organisms potentially add to the total set of possible reactions in the system. For example, lysis of bacteria is a process where the intracellular enzymes suddenly end up in a total different environment, with not only different substrate and cofactor concentrations but also physical parameters such as pH.

Impact of matrix

Apart from being present or not, activity of amino acid converting enzymes can also be affected by the growth and culture conditions of the bacteria. This is highly relevant because it also offers opportunities for practical implication. Stress responses to salt and pH for example stimulate several pathways. In yoghurt, acid pushes the bacterial culture into stationary phase, the phase where most exopolysaccharides and flavours are formed. In cheese, the stationary phase is reached due to a lack of lactose. In this phase, the cofactor pool will change, hereby also affecting the flavour formation. Proteolysis, peptidolysis, transamination and decarboxylation can still proceed, while dehydrogenases will probably be inactive due to a lack of NADH. If this reasoning holds, the main 3-methyl butanal increases should be measurable during carbon-source limited stationary phase.

Extracellular molecules can influence the regulation of several reactions. As stated earlier, isoleucine is able to regulate the amino acid metabolism via CodY (Guedon et al. 2001; Chambellon and Yvon 2003). Adding isoleucine to the cheese matrix should theoretically lead to lower 3-methyl butanal levels by high-3-methyl butanal producing strains.

Besides an impact on regulatory pathways, the previously discussed α -ketoglutarate example shows that also changing (limiting) substrate concentrations in the medium can be very effective (Yvon et al. 1999). The addition of amino acids have been described above, but instead of adding free amino acids or peptides, also the addition of proteolytic enzymes has been successfully researched and applied in cheese and sausage productions (Ansorena et al. 2000; Fernandez et al. 2001; Azarnia et al. 2006).

Natural biodiversity

A large variety in amino acid conversion capacities is found in lactic acid bacteria, particularly among strains isolated from a non-production environment, the so-called wild isolates. Wild strains are often found in environments low in amino acids, which makes them more dependent on their own biosynthesis of amino acids compared to industrial strains, and consequently, they possess more amino acid converting enzymes. In wild lactococci for example, not only a larger enzyme potential is present, but concomitantly also produce rather unusual flavour components and/or flavour profiles (Ayad et al. 1999). This variety is a great source for finding more optimal, more characteristic production organisms. Knowledge on the pathways as described above enables specialized and high-throughput screening of culture collections (Lavery et al. 2001; Smit et al. 2004b; Ingham et al. 2007; van Hylckama Vlieg and

Hugenholtz 2007; Pastink et al. 2008). The large natural biodiversity could potentially offer specific traits for new products; some examples of using wild starter bacteria in products such as cheese have already been reported (Ayad et al. 2003).

Co-operation between strains

It is not often that the optimal combination of enzymes can or will be found within one strain. However, in many fermented products, a combination of microbial strains is used, which could potentially result in co-operation of enzymes in a pathway between strains, for instance, upon lysis of the strains (see below). This interaction can be negative, where desired compounds are broken down by another organism, or positive. A combination of strains should be selected, where the total set of conversions is optimal. Screenings, as used for screening the natural biodiversity, are also relevant for the screening of optimal co-operation. For example, Ayad et al. (2001) showed that the combination of two strains, of which each had only a limited set of enzymes in the pathway leading to 3-methyl butanal, were able to complement each other. Kieronczyk et al. (2003, 2004) found similar results when combining strains lacking and possessing GDH activities, and Broadbent et al. (2004) showed an example where a D-hydroxyisocaproic acid dehydrogenase over-producing strain could reduce the flavour forming, e.g. 3-methyl butanal, ability of other strains in a mixture. The latter was most likely caused by the effective conversion of keto-acid to non-tasting components (see also above). Genomics approaches offer new opportunities for unravelling and applying microbial co-operation in food fermentations (Sieuwerds et al. 2008).

These examples of co-operation between strains offer new possibilities for the construction of tailor-made starter cultures because it means that not all the required enzyme activities in a certain flavour pathway need to be present in one strain. It was shown that such a co-operation between strains can also work in a real cheese, with again the example of production of 3-methyl butanal as a key compound in that cheese (Ayad et al. 2003; Amarita et al. 2006).

Role of lysis in the formation of branched-chain aldehydes

One could question whether the above-mentioned co-operation can be explained by a diffusion of intermediate compounds in a pathway from one bacterial strain to another or whether this is due to a (partial) lysis of bacteria. In the latter case, the total potential of enzymes would become available as if it was one incubation mixture. The role of cell lysis has extensively been studied with regard to

the level of proteolysis and peptidolysis of lactic acid bacteria. Meijer et al. (1998) and Lepeuple et al. (1998) showed that lysis of lactic acid bacteria greatly improved the peptidolytic activity under cheese conditions. Their results indicated that the cell membrane can be a barrier between the enzymes, located intracellularly, and the peptide substrates present in the cheese matrix. Apparently, there is not enough active transport by the starter cultures, for taking up the peptides, once they are present in the cheese matrix, and lysis then is essential for enhancing enzyme–substrate interaction.

In contrast to the activity of peptidases, where lysis generally enhances the activity enzymes, enzymes that require cofactors or cosubstrates (e.g. PLP, NAD, and NADP) could be negatively affected by lysis of the cells. It likely depends on the type of enzyme(system) whether lysis will improve the activity (and formation of flavour) or not. Despite this precaution, it appears from a recent work by various studies that lysis of the bacteria in general seems to increase the formation of flavour components, such as branched-chain aldehydes.

Martinez-Cuesta et al. (2006a,b) reported that cell membrane permeabilisation by lactacins positively influence the formation of aldehydes in lactic acid bacteria. Bourdat-Deschamps et al. (2004) took this further towards cheese models by showing that the conversion of phenylalanine to flavour compounds was enhanced by autolysis. In a study by De Palencia et al. (2004), bacteriocin-sensitive strains of *L. lactis*, with BcAA activity and α -keto acid decarboxylase activity, were used as adjunct together with a bacteriocin-producing (Lacticin 3147) *L. lactis* strain in cheese making. In control cheese making, a non-bacteriocin producing strain was used. The bacteriocin produced enhanced lysis of the adjunct strains, which led to an increase in isoleucine transamination. The concentration of the flavour compound 2-methylbutanal was about doubled again, indicating that increased aldehyde formation can be obtained due to lysis.

Taken together, lysis appears to improve the conversion rate of intermediates towards branched-chain aldehydes, and this finding may also explain why a combination of strains (with different enzyme activities for the pathway to these aldehydes) can be an effective way to generate branch-chain aldehydes in products like cheese.

Presence and impact of branch-chain aldehydes in various food products

After having discussed pathways of formation of branched-chain aldehydes and opportunities to influence and control their formation, in this section, we focus on the impact of 3-methyl butanal as flavour compound and its formation in

various important foods. In a large number of foods, 3-methyl butanal is a (key) flavour compound, and it is mainly produced by the transaminase-initiated enzymic pathway and by (non-enzymic) Strecker degradation. We will only focus on those products that are subject to a processing step (i.e. heating and fermentation) and not on the presence of these aldehydes in products like tomato or grain, where they can also be detected pre-harvest.

Bread/wheat/sourdough

Taste and smell are undoubtedly the most important attributes determining the quality of bread or baked cereals in general. 3-Methyl butanal, phenylacetaldehyde and 3-(methylthio)propanal are amino-acid-derived key flavour compounds in bread (Schieberle 1996). They are formed essentially in two ways: The enzymatic (Ehrlich) reaction is dominant in the crumb, while the chemical Strecker degradation proceeds fast in the crust during the baking process. Aldehyde concentrations in the crumb generally are low, whereas in the crust, a stronger (Strecker) formation of aldehydes 2- and 3-methyl butanal and 2-methyl propanal is observed during baking (Zehentbauer 2001; Ruiz et al. 2003).

The type of starter cultures used in the fermentation process and the fermentation regime (temperature, time, pH, etc.) differs considerably between several bread types resulting in large differences in 3-methyl butanal levels (Zehentbauer 2001; Ruiz et al. 2003; Gänzle et al. 2007). Sourdough breads are, in addition to bakers yeast, *Saccharomyces cerevisiae*, produced with lactic acid bacteria (LAB), such as *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus*. The majority belongs to the genus *Lactobacillus*, e.g. *Lactobacillus sanfrancisco* (Hansen and Schieberle 2005). In sourdough breads, these LAB also significantly influence the amounts of 3-methyl butanal and many other odorants, e.g. acetic acid, butanoic acid, phenylacetic acid, 2- and 3-methylbutanoic acid and pentanoic acid (Czerny and Schieberle 2002; Gänzle et al. 2007; Van Der Meulen et al. 2007). 3-Methyl butanal concentration in the flour is low, and LAB may increase the concentration by a factor of 4. In addition, yeast fermentation yields 3-methyl butanal. The results of research on sourdough breads suggested that, in some cases, no free leucine was left over after fermentation, thereby indicating the need to form sufficient amounts of the free amino acids during fermentation (Czerny and Grosch 2000; Hansen et al. 2001; Kirchhoff and Schieberle 2002; Hansen and Schieberle 2005; Corsetti and Settanni 2007; Corsetti et al. 2007).

Zehentbauer and Grosch (Zehentbauer 2001) observed, in addition to differences in formation of Strecker aldehydes depending on the ingredients and recipe used for bread making, also differences in losses of 2-methyl propanal, 2- and 3-methyl butanal. It can be assumed that the formation

and losses of both desired and undesired compounds (leading to off-flavour) determine overall flavour balance (Zehentbauer 2001; Czerny and Schieberle 2002).

In rice cakes, produced without fermentation, various volatile flavour compounds were formed in the whole product due to the baking process, including aldehydes 2- and 3-methyl butanal (Buttery et al. 1999).

Chocolate/cocoa

The secret of the flavour of chocolate resides mainly in its volatile aromatic fraction. At least 35 key aroma compounds could be identified in chocolate (Counet et al. 2002; Frauendorfer and Schieberle 2006). Three of those compounds had a strong chocolate-like flavor: 2-methylpropanal, 2-methylbutanal and 3-methyl butanal. Many others were characterized by cocoa/praline-flavoured/nutty/coffee notes, e.g. pyrazines (Counet et al. 2002).

The first processing step of cocoa beans involves fermentation with LAB, acetic acid bacteria and yeasts (Nielsen et al. 2007). This cocoa fermentation is crucial not only to the formation of significant volatile fractions (alcohols, esters and fatty acids) but also for the development of cocoa–chocolate flavor precursors (amino acids and reducing sugars). Cocoa is dried to minimize the formation of moulds and to reduce the acid level and astringency of the beans by decreasing the total quantity of polyphenols. Via Maillard reactions, subsequent cocoa roasting converts flavor precursors formed during fermentation to two main classes of odorant compounds already mentioned: pyrazines and aldehydes (Kattenberg and Kemming 1993). Concentrations between 20 and 60 mg/kg have been found for 3-methyl butanal (Ziegleder 1991).

Meat

For storage and preservation purposes, meat is treated in various ways, of which some have a strong impact on flavour. Examples are fermentation and drying. These processes may yield significant levels of 3-methyl butanal. Two types of starter culture, lactic acid bacteria and *Micrococcaceae*, are often used in combination when producing fermented sausages. Lactic acid bacteria, e.g. *Lactobacillus sakei* and *Pediococcus pentosaceus*, cause a lowering of the pH, thereby preventing growth of many pathogenic microorganisms. The *Micrococcaceae*, e.g. *S. xylosus* and *Staphylococcus carnosus*, are added due to their nitrate and nitrite reductase activity, which assists in color formation (Lucke 1998; De Vos Petersen et al. 2004). Furthermore, the *Micrococcaceae* also produce pleasant flavors, such as those associated with the branched-chain aldehydes contributing significantly to odor perception of the final product (Montel et al. 1996).

The microbial catabolism of leucine by LAB and *Staphylococcus* species and by, e.g. *Carnobacterium* species and *Moraxella* has been studied by various groups (Montel et al. 1996; Stahnke 1999a,b; Marco et al. 2007). Masson et al. demonstrated catabolism of leucine by *S. carnosus* yielding 3-methyl butanal, 3-methyl butanol and 3-methyl butanoic acid (Masson et al. 1999). It appeared that the preculture and incubation conditions strongly influenced the level of production of the three metabolites. The reactions yielding 3-methyl butanal were those already described for LAB in the previous section (Fig. 1). Oxidation of 3-methyl butanal then might yield 3-methyl butanoic acid. In addition, direct oxidative decarboxylation yielding 3-methyl butanoic acid from the keto acid was suggested (Masson et al. 1999). *Carnobacterium piscicola* is also able to form 3-methyl butanal and 3-methyl butanol via α -ketoisocaproic acid (Larrouture-Thiveyrat and Montel 2003; Larrouture-Thiveyrat et al. 2003). *Moraxella phenylpuruvica* degraded both leucine and phenylalanine yielding 3-methyl butanal and benzaldehyde/benzacetaldehyde (Møller et al. 1998).

In dry-cured ham, 2- and 3-methyl butanal have been associated with nutty, cheese and salty notes, e.g. in Parma ham (Andres et al. 2002), Iberian ham (Andres et al. 2005) and American country ham (Song et al. 2008). The formation is probably non-enzymic via Strecker reaction, since no fermentation takes place in the product. This is a slow process, at temperatures applied during dry-cured ham ripening, but long ripening times may facilitate this reaction (Andres et al. 2002).

Soy fermentations

Soy sauce is traditionally used as seasoning in eastern Asia, and its popularity in the Western part of the world is growing due to its intense umami taste accompanied by a very characteristic aroma (Steinhaus and Schieberle 2007). Japanese soy sauce (shoyu) is traditionally produced by fermentation of heated soybeans and wheat flour with *Aspergillus oryzae* or *Aspergillus sojae* to koji. In the next step, the koji is fermented with *Pediococcus halophilus* and *Zygosaccharomyces rouxii* to yield moromi. Pressing of moromi then yields the soy sauce, which is finally pasteurized and bottled. For the aroma development of Japanese soy sauce, all these steps are important. For Chinese soy sauce, only soybeans but no cereals are used, whereas Korean soy sauce is produced from soybeans, barley meal, and various spices (Nunomura and Sasaki 1992). 3-Methyl butanal and 2-methyl butanal are amongst the most important odorants in soy sauce and are thought to be essentially produced by microbial action via both the Ehrlich pathway and via amino-acid biosynthetic pathways of branched-chain amino acids leucine, valine and isoleu-

cine. In addition to the aldehydes, the oxidation and reduction products such as 3-methylbutanoic acid and (fusel) alcohols are regarded as key aroma compounds (van der Sluis et al. 2000, 2002; Steinhaus and Schieberle 2007).

Beverages

In beverages such as (fermented) black tea and coffee, 3-methyl butanal is thought to be a key contributor to aroma (Czerny and Grosch 2000; Kumazawa and Masuda 2001; Wright et al. 2007). In wine, branched-chain alcohols, e.g. isoamyl alcohol and isobutanol, are synthesised in the yeast cell through the Ehrlich pathway by degradation of branched-chain amino acids (Ehrlich 1907). Mitochondrial and cytosolic enzymes of *S. cerevisiae* are involved in the initial α -keto acid formation from the amino acids. A decarboxylase converts the resulting α -keto acid to the corresponding branched-chain aldehyde, e.g. 3-methyl butanal, with one carbon-less atom, and the alcohol dehydrogenase catalyses the NADH-dependent reduction of this aldehyde to the corresponding fusel alcohol. Alternatively, the aldehyde might be oxidised to a carboxylic acid (Derrick and Large 1993; Dickinson and Norte 1993; Didion et al. 1996; Swiegers et al. 2005; Swiegers and Pretorius 2005).

Although the branched-chain aldehydes are generally not regarded as key flavour compounds in wine, in some wine or wine products, 3-methyl butanal definitely contributes to flavour, e.g. in Pedro Ximenez, Fino, botrytized Sauternes and Cava wines that contain relatively high concentrations of this aldehyde (Campo et al. 2008). Sherry wines and Port may also have large amounts of branched aldehydes (Cullere et al. 2007). Potential key aroma compounds of freshly distilled Calvados and Cognac were 3-methyl butanal and hexanal (Ledauphin et al. 2006a, b).

Beer is made by fermenting a malt extract with *S. cerevisiae*. Malt is predominantly produced by germinating barley followed by a drying process called kilning (Fickert and Schieberle 1998). 3-Methyl and 2-methyl butanal were determined as the most odor-active compounds in malt (Zhou et al. 2002; Cramer et al. 2005). Nevertheless, aldehyde levels in beer are usually low and increase over shelf life. In aged beer, 2-methyl propanal, 2-methyl butanal, 3-methyl butanal, pentanal, hexanal, furfural, methional, phenylacetaldehyde, and (*E*)-2-nonenal can be detected and are in some cases undesired (Vesely et al. 2003; Vanderhaegen et al. 2007).

Concluding remark

Branched aldehydes, such as 3-methyl butanal, are important flavour compounds in many food products, both

fermented and non-fermented (heat-treated) products. Knowledge gained about the generation pathways of these flavour compounds is essential for being able to control the formation of desired levels of these aldehydes. Currently, good examples of these are already implemented in fermented food products such as cheese, and there is a great potential to also implement this in other fermented and non-fermented food products.

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