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**Review**

# Flavour in black garlic: a comprehensive review

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**Abstract**

Black garlic has grown in popularity over the last few years and is now widely used, particularly in fine dining restaurants, but it is also available in shops. After several days to weeks of treatment of the entire bulbs at temperatures between 60 °C and 70 °C, its flavour bears little resemblance to the fresh clove. It has an non-crunchy, soft consistency, a slight acidity and a caramel-like sweetness. It can be used in a variety of applications, including desserts and even chocolate. The purpose of this review is a deeper understanding of the fermentation processes. It is argued that this knowledge provides a basis for systematic temperature controlled process during preparation, which improves the flavour of black garlic better.

**Keywords**

black garlic, flavour, heat treatment, enzymatic reactions, glycation

## 1. Introduction

Black garlic has an distinct flavour and has little in common with fresh, white garlic (*Allium sativum* L.). For example, black garlic shows a caramel odour with braised beef-saucy flavour, and has a spicy, caramel-like, occasionally slightly burnt, slightly mushroom-like odor and even a touch of dried fruit and nuts (Kilic-Buyukkurt *et al.*, 2023). Although the sulphur notes are reminiscent of its origins, it is by no means pungent or even lachrymatory (Hu *et al.*, 2024). The irritating, cold trigeminal irritation of white garlic has disappeared, as has the prolonged "garlic breathe". Instead, the flavour of black garlic dominates, with a savoury flavour and a taste combination of sweet, sour and umami.

On the other hand, Mediterranean, Indian and Asian cuisine would be inconceivable without fresh, white garlic. Whether raw, *e.g.* in aioli, roasted or cooked, *e.g.* in stews, curries or wok recipes, garlic contributes significantly to the final flavour of many traditional dishes. Fresh garlic contains about 63 % water, 28 % carbohydrates (main component storage carbohydrates are fructans), 2.3 % organosulphur compounds, 2 % proteins (including the enzyme alliinase), 1.2 % free amino acids, and 1.5 % lipids (Santhosha *et al.*, 2013). Due to sulphur compounds, fresh garlic is characterised by strong trigeminal

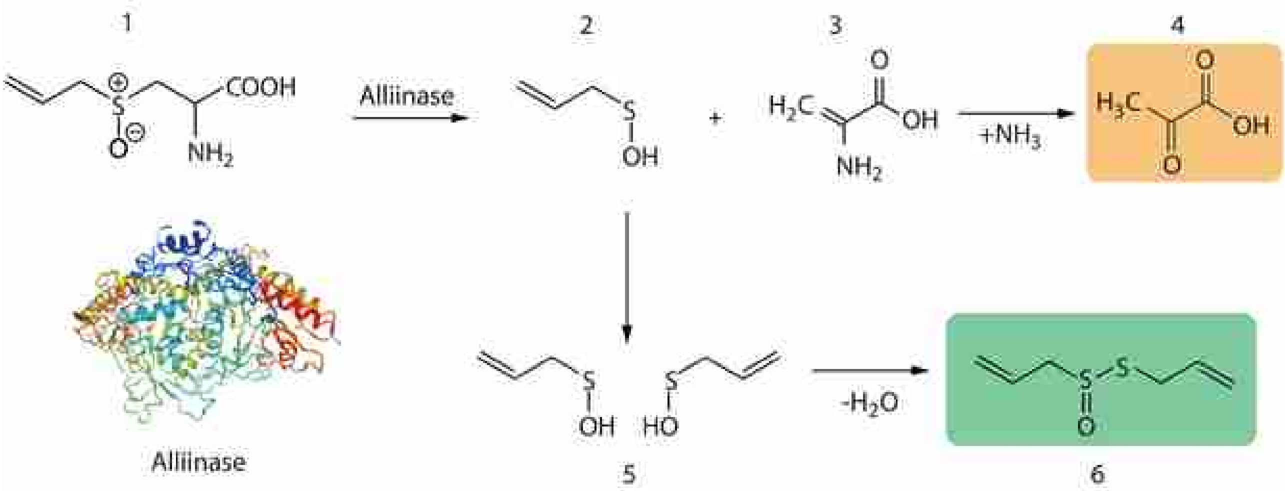


*Figure 1. Schematic representation of the chemical processes involved in the formation of the odourant compound allicin (6) from alliin (1). An important by-product is pyruvate (4), which is responsible for a large part of the initial (fermentation) processes in black garlic. The structure of alliinase is shown at bottom left (from Databank Swiss-Model).*

stimuli associated with orthonasal and lacrimatory irritation, and a painful cold sensation on the tongue, similar to onions (Bautista *et al.*, 2005; MacPheroson *et al.*, 2005). Fresh garlic is dominated by the intense, pungent, sulphur-like odour of the Allioideae subfamily, which many people dislike. It persists for a long time after consumption. The degradation products of the sulphur compounds can be detected in the breath for hours after consumption and are even excreted through the skin (Taucher *et al.*, 1996).

Most of these irritations are caused by sulphur compounds that are produced when the plant cells are damaged during cutting and oral processing. Garlic contains both alliin and the alliin-cleaving enzyme alliinase (also known as alliin lyase), but not in the same cells or cell spaces. Alliin, which is polar and therefore water soluble (Figure 1), is stored in the cytoplasm of intact plant cells. The enzyme alliinase is located in the vacuoles.

The biological function of alliin in garlic and onion plants is a defensive mechanism: when garlic cells are destroyed by herbivores, alliin and alliinase interact, which results in chemical reactions sketched in Figure 1. Water-soluble alliin (1) is converted by alliinase to allylsulfenic acid (2) and dehydroalanine (3). Dehydroalanine is unstable and decomposes to ammonia $NH_3$ and pyruvic acid. In the next step, allylsulfenic acid (4) reacts in an aqueous medium to form the volatile molecule allicin (5), which is responsible for the typical garlic odour. The degradation of dehydroalinin (3) is important in the context of fermentation, since the anion of pyruvic acid is pyruvate.

When exposed to heat, such as during steaming, cooking or frying, most pungent trigeminal sensations disappear. The strong trigeminal cold pain stimuli are also lost. The alliinases are inactivated when exposed to heat, which prevents the process shown in Figure 1. The strong trigeminal stimuli and the typical garlic odour are also significantly reduced if garlic is first blanched and then pickled. The blanching temperature plays a decisive role. Blanching at 60 °C up to 30 min has only a minor effect, whereas the pungent sensations decrease significantly with increasing blanching temperature (Rejano *et al.,* 2004). This is due to the increasing inactivation of the allinase enzyme with increasing blanching temperature (and blanching time), as shown in Figure 2. The maximum activity is between 30 °C and 40 °C. In

this range, garlic can be fermented by the classical lactic acid process. Above 80 °C, the enzyme is irreversibly inactive and no significant concentrations of pungent odours are formed. The odour is then largely determined by the odorants that can form under the particular thermal conditions (Bi *et al.*, 2023).
The transition range between 50 °C and 70 °C is therefore of interest for black garlic processing, as the activity of alliinase is still present in this temperature interval, while the higher thermal energy allows the flavour development of black garlic. The strong changes occur as a result of several weeks of ageing, maturing, or accelerated curing at higher temperatures between 60 °C and 70 °C.

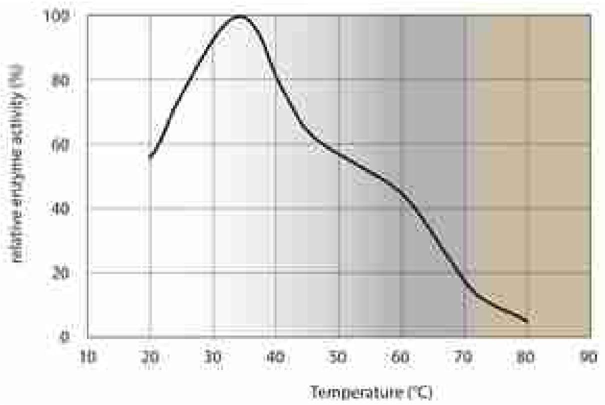


*Figure 3. The activity of the enzyme alliinase shows a maximum between 30 and 40 °C (Wang* et al.*, 2011).*

## 2. Phenomenology of black garlic

The production of black garlic involves the ageing (curing) of entire bulbs at temperatures between 60 °C and 90 °C, under conditions of sufficient high humidity (between 70 % and 90 %). The changes that occur during the heat treatment of garlic are visible to the naked eye at a macroscopic level, as shown in Figure 3. In addition, the garlic cloves have shrunk considerably, indicating a corresponding loss of water. Even at this stage, the cloves are no longer crunchy but rather leathery (Choi *et al.*, 2014). As the process continues, the loss of water decreases and the individual cloves have a rubbery, almost elastic texture. In the early stages, only a small proportion of the free water evaporates; the remaining water is required for the enzymatic conversion of alliin to alliciin, as shown in Figure 1. If the moisture content, and thus the water activity $a_w$ becomes too low, enzymatic reactions are no longer possible, because most of the remaining water is largely bound (Troller and Christian, 2012). However, the colour changes continue, indicating significant changes in taste and odour. A glycation reaction is possible even at temperatures around 70 °C if enzymatically reactive precursors with sufficient water activity are formed, as shown in more detail in section 3. These are likely to react over time to form corresponding brown and odourant substances even at comparatively low temperatures. Choi *et al.* (2024) have measured the colour intensity accordingly.

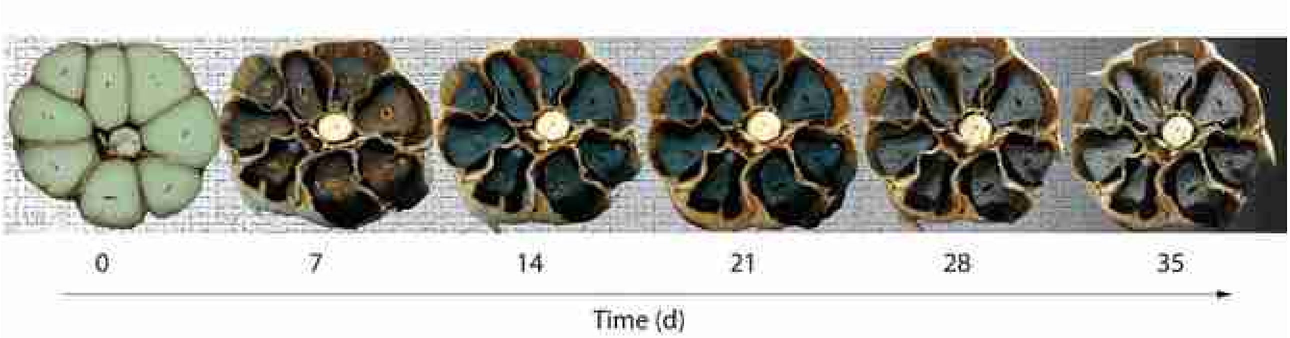


*Figure 2. Change of colour of garlic during an experiment conducted over 35 days at a temperature of 70 °C under 90 % humidity (Choi* et al.*, 2014).*

*Table 1. Water and acid content, pH and reducing sugar concentration during garlic ripening at 70 °C (data from Choi* et al.*, 2014). Values indicated by different letters in the same row are significantly different by Duncan's multiple range test (*$p < 0.05$*).*

| | Time (d) | | | | | |
|---|---|---|---|---|---|---|
| Component | 0 | 7 | 14 | 21 | 28 | 35 |
| | | | | | | |
| Water content (%) | 64.21 ± 1.48 a | 32.72 ± 0.97 b | 31.77 ± 2.60 b | 31.12 ± 0.17 b,c | 29.55 ± 0.39 c | 29.88 ± 0.49 c |
| Acidity (mg/kg) | 0.40 ± 0.01 e | 1.30 ± 0.01 d,e | 1.50 ± 0.02 c,d | 1.70 ± 0.03 c | 2.30 ± 0.06 b | 2.60 ± 0.03 a |
| pH value | 6.33 ± 0.07 e | 5.49 ± 0.09 d,e | 4.41 ± 0.17 c,d | 4.22 ± 0.08 | 4.07 ± 0.02 b | 3.74 ± 0.062 a |
| Reducing sugars (g/kg) | 1.52 ± 0.01 d | 2.73 ± 0.32 c | 12.42 ± 0.85 b | 15.96 ± 0.29 a | 15.98 ± 0.23 a | 16.07 ± 0.38 a |

The data from Choi *et al.* (2014) shown in Table 1 support these subjective perceptions. The water content decreases the most in the first 7 days; the pH value drops from values just above 6 to values below 4. The clearest differences can be seen in the content in reducing sugars, which increase by a factor of 10 during temperature induced curing. The reducing sugars are essentially mono-, di- and oligosaccharides, which contribute to the perception of sweetness, albeit with varying degrees of sweetness. Black garlic therefore dominates in the basic taste qualities of sweet and sour (Choi *et al.*, 2014).

The flavour of garlic changes significantly during the thermal process. Recently, the taste of black garlic has also been investigated using electronic tongues, in particular the sour, bitter and umami tastes (Najman *et al.,* 2022). The results of a flavour comparison between fresh garlic (0 days) and thermally processed garlic (in this case 45 days at 70 °C and 80 % humidity) are shown in Figure 4. Electronic tongues work with well defined sensors, but the results are not always directly transferable to sensory experiments with (trained) panels (Najman *et al.,* 2022). For example, salty compounds are detected in fresh garlic, which have less to do with the salty taste and more to do with free cations dissolved in the cell plasma, such as sodium and calcium (Najman *et al.,* 2022). Nevertheless, the differences are visible and correspond to the impressions given by the analytical data in Table 1. The changes can be clearly assigned to sour, bitter, sweet and umami flavours (Najman *et al.*, 2022). The sour taste correlates with the changes in pH and acid concentration in Table 1, as does the increase in sweetness due to the increasing concentration of low molecular weight sugars, while the increase in umami flavour corresponds to protein hydrolysis. The pronounced bitter taste of fresh garlic is mainly due to the glycosinolates (Jones *et al.*, 2004) and other sulphur compounds (Kubec *et al.*, 2018), which react and degrade during garlic processing. The increase in acidity is reminiscent of lactic acid fermentation inside the gloves during the very first stage of processing, as indicated by the decrease in pH according to Table 1. These mainly phenomenological observations suggested a taste spider diagram to illustrate the pronounced differences between fresh and black garlic as shown in Figure 4.

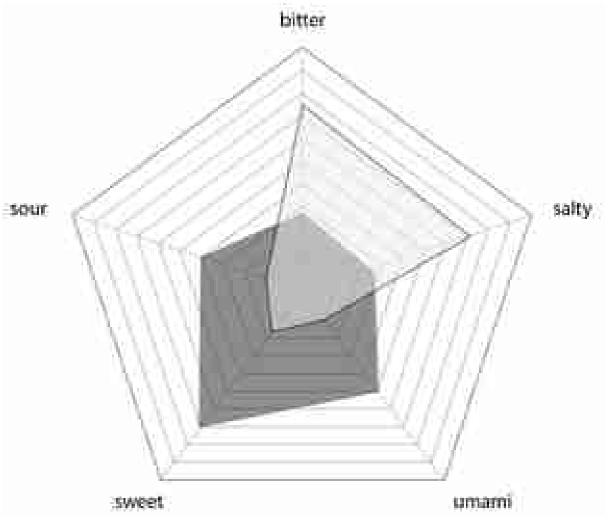


*Figure 4. Schematic phenomenological taste to visualize the differences between fresh garlic (*light grey*) and thermally processed garlic (45 d / 70 °C,* dark grey*) shows significant differences (based on the descriptions by Jones* et al.*, 2004; Kubec* et al.*, 2018).*

## 3 Molecular aspects of flavour changes

### 3.1 Sugars and sweetness

Fresh garlic contains free, low molecular weight sugars with varying numbers of carbon atoms, such as arabinose, galactose, glucose, fructose, sucrose and maltose. There is also a high proportion of fructans, which is the main storage carbohydrate (Baumgartner *et al.*, 2000) in garlic. Fructans consist of fructose residues, usually with a sucrose unit at what would otherwise be the reducing endpoint (Figure 5).

The repeating number *n* in Figure 5 defines the chain length (degree of polymerisation) and the molecular weight. It can take values between 10 and 200; in garlic, chain lengths around $n = 9$ dominate (Baumgartner *et al.,* 2000). This means that both short, oligomeric structures and longer, polymerised chains can be present in fresh garlic. These polymerised fructan chains are too large to be recognised by sweet taste receptors and do not trigger a taste stimulus. However, if the fructan chains are cleaved during the thermal process due to prolonged exposure to temperature and sufficient humidity (Lu *et al.*, 2018), more and more sugars are released, and the sweet taste increases. Chua *et al.* (2022) performed a detailed analysis on mono- and disaccharides (Table 2). This study is limited to 12 days of garlic ripening at 75 °C and 85 % humidity, as the sugar content changes significantly only during the first 14 days (Table 2).

During the first 8 days, there is a sharp increase in fructose. After that, however, the fructose content drops sharply. Glucose, on the other hand, only increases after 10 days, then decreases slightly and remains at a high level of just under 20 %. The decrease in fructose content indicates participation in the glycation and amino-carbonyl reactions. It is also interesting to note that sucrose, which is present in practically all fructans, largely disappears. The explanation for this is in line with everyday experience: even if the sucrose is cleaved from the end group fructan, it is still split into glucose and fructose because the pH drops at the same time during the process. The high humidity prevents the water content from dropping too low, so in the acidic environment the sucrose forms inverted sugar, a mixture of glucose and fructose (Thavarajah and Low, 2006).

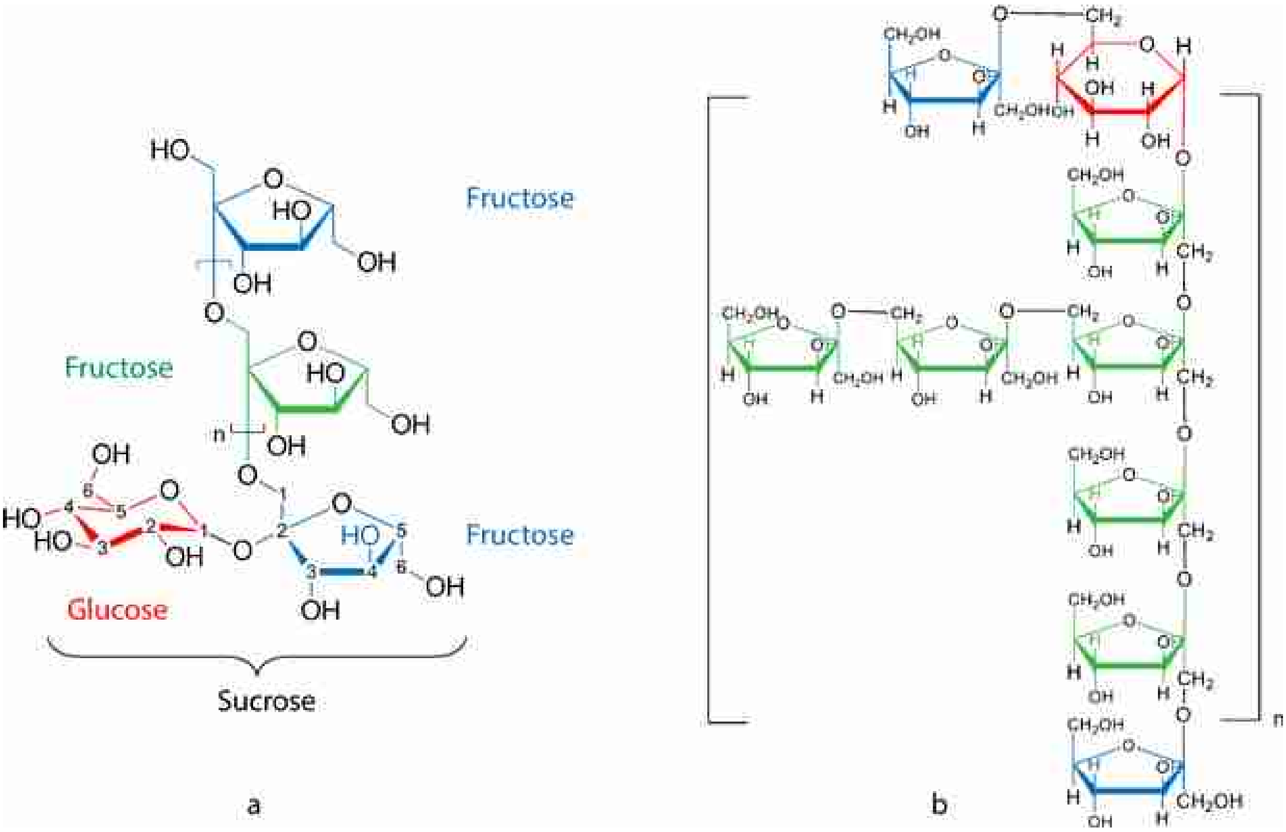


*Figure 5. Two typical examples of (a) inulins and (b) garlic fructans are shown. The fructose-based repeating unit (monomer) is shown in green. The reducing end contains sucrose, at the other end closes with fructose (alpha-D-glucopyranosyl-[beta-D-fructofuranosyl](n-1)-D-fructofuranoside). Garlic contains other similarly structured polysaccharides that are not shown here (Jiang* et al.*, 2022).*

*Table 2. The development of glucose, fructose and sucrose (% per weight) during the first 12 days of temperature-induced garlic ripening (from Chua* et al.*, 2022).*

| | Time (d) | | | |
|---|---|---|---|---|
| Sugar (%) | 0 | 8 | 10 | 12 |
| Glucose | < 0.1 | 1.5 | 23.3 | 19.8 |
| Fructose | < 0.1 | 20 | < 0.1 | < 0.1 |
| Sucrose | < 0.1 | 0.3 | < 0.1 | < 0.1 |

*Table 3.The development of free amino acids over time during the thermal process (rounded values only, from Choi et al. 2014) recorded not all 20 amino acids. Values followed by different letters in the same row are significantly different by Duncan's multiple range test (p < 0.05); -: non detected.*

| Amino acid (mg/100g) | Time (d) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | 35 |
| Leucine | 58.62 ± 3.37 b | 73.44 ± 1.79 a | 62.81 ± 1.51 b | 62.12 ± 5.64 b | 61.23 ± 4.32 b | 59.19 ± 3.94 b |
| Isoleucine | 50.04 ± 13.47 b | 89.25 ± 18.93 a | 86.45 ± 8.23 a | 83.79 ± 2.37 a | 79.44 ± 1.14 a | 71.07 ± 2.25 a |
| Valine | 47.74 ± 0.19 a | 47.68 ± 0.64 a | 36.71 ± 4.64 b | 35.23 ± 5.61 b | 34.73 ± 8.61 b | 33.91 ± 6.61 b |
| Methionine | 31.56 ± 1.40 b | 82.51 ± 4.70 a | 80.73 ± 6.37 a | 78.11 ± 2.33 a | 73.59 ± 9.71 a | 71.11 ± 3.55 a |
| Cysteine | 81.06 ± 0.95 a | 69.43 ± 0.94 b | 49.20 ± 5.42 c | 46.90 ± 8.98 c | 43.44 ± 3.38 c | 42.14 ± 7.18 c |
| Phenylalanine | 55.64 ± 0.74 c | 82.38 ± 8.35 b | 70.20 ± 4.41 b | 135.16 ± 7.10 a | 136.25 ± 12.76 a | 143.07 ± 6.32 a |
| Tyrosine | 449.95 ± 6.29 a | 109.13 ± 26.09 b | 102.33 ± 0.38 b | 82.28 ± 7.41 b | 78.35 ± 4.34 b | 77.31 ± 7.54 b |
| Aspartic acid | 90.12 ± 2.55 b | 117.50 ± 9.07 a | 64.53 ± 5.84 c | 62.43 ± 4.34 c | 61.65 ± 9.12 c | 60.19 ± 8.16 c |
| Glutamic acid | 286.60 ± 8.09 a | 128.87 ± 9.09 b | 112.81 ± 3.02 b | 108.11 ± 9.12 b | 101.88 ± 7.71 b | 100.11 ± 6.09 b |
| Arginine | 409.05 ± 3.00 a | 340.20 ± 75.68 b | 119.30 ± 0.52 d | 208.71 ± 51.36 c | 71.92 ± 0.65 d,e | 40.34 ± 1.10 e |
| Lysine | 61.68 ± 5.29 a | 57.96 ± 4.93 a | 47.39 ± 2.24 b | 42.50 ± 2.27 b | 41.53 ± 3.67 b | 40.50 ± 9.22 b |
| Histidine | 89.12 ± 1.88 b | 191.69 ± 18.89 a | 58.76 ± 13.02 c | 58.76 ± 13.02 c | 57.62 ± 5.51 c | 57.89 ± 8.34 c |
| Threonine | 81.25 ± 13.59 a | 46.30 ± 0.87 b | 53.53 ± 8.36 b | 57.54 ± 2.22 b | 58.36 ± 11.17 b | 59.36 ± 5.22 b |
| Serine | 38.53 ± 0.82 a | 25.72 ± 0.87 b | 25.84 ± 0.33 b | 24.78 ± 1.12 b | 24.78 ± 1.12 b | 23.71 ± 5.41 b |
| Glycine | 21.50 ± 2.51 b | 37.41 ± 8.54 a | 9.60 ± 1.78 c | 8.63 ± 1.18 c | - | - |
| Alanine | 89.72 ± 11.44 b | 239.13 ± 17.84 a | 67.01 ± 2.39 c | 83.59 ± 5.71 b | 47.38 ± 1.74 d | 32.74 ± 5.37 d |

A detailed analysis of free amino acids provides clues to the umami flavour of black garlic. A detailed analysis of the amino acid spectrum is necessary because the main trigger for the umami flavour is free glutamic acid, which is present to a greater or lesser extent in most proteins. Choi *et al.* (2014) found considerable differences in the temporal changes of free amino acids, which are summarised in Table 3. For reasons of readability, only rounded values without statistical and systematic deviations are given here, which can be viewed in the original publication if interested. The table, which at first glance appears confusing, nevertheless provides some useful information on flavour formation.

For example, it is noticeable that the concentrations of some free amino acids such as leucine, isoleucine, aspartic acid, histidine and alanine initially increase over time, but then decrease again. Proteins are partially broken down in the first few days, and amino acids are released in the process.

The decrease in these amino acids over time indicates reactions to aminocarbony products, which leads the odour of black garlic. Other amino acids, such as methionine, increase and remain at a high level.This is surprising at first glance, as this amino acid is known to react to form the odourant methional (Methven *et al.*, 2007) also known as 3-(methylsulfanyl)propanal (sulphurous, cooked vegetables, cooked eggs). The water required for this reaction is lacking for periods of more than 7 days, and the temperatures are too low for strong methional formation.

Glutamic acid, which triggers umami taste, also decreases significantly over time, which would contradict the increase in umami taste shown in Figure 4. On the other hand, it is possible that glutamic acid (1) is converted into pyroglutamic acid (2), also known as 5-oxoproline, under certain conditions, as outlined in Figure 6.

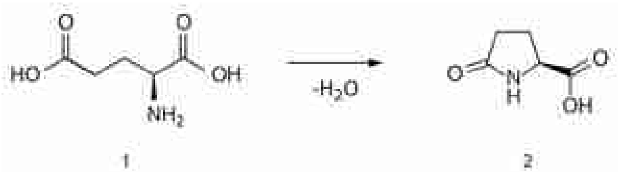


*Figure 6. The conversion of glutamic acid (1) to pyroglutamic acid (5-oxoproline) (2).*

Pyroglutamic acid contributes strongly to the umami taste. For instance, pyroglutamic acid has been found as a taste component in long cooked meat broths (Schieberle *et al.*, 1998), which is attributed to the umami taste (Zhang *et al.*, 2017). It is also found in long fermented products such as fish sauces (Park *et al.*, 2002). In particular, the presence of heat-resistant (thermophilic) lactobacilli seems to favour the formation of pyroglutamic acid. This has been studied in cheese ripening and provoked with appropriate microorganisms (Mucchetti *et al.*, 2002). It was also confirmed for the classic lactofermentation of kimchi (Eom *et al.,* 2023): when thermophilic lactobacilli are inoculated, more polyglutamic acid is produced. It is also known for its salty, umami and sour taste (Gazme *et al.*, 2019). High concentrations of polyglutamic acid can also be clearly detected in black garlic (Liang *et al.,* 2015).

The molecular mechanisms of salty and umami tastes through the activation of individual taste receptors has also been confirmed (Frerot and Chen, 2013; Yao and Udenigwe, 2018). These examples already show the importance of pyroglutamic acid for thermal and fermentative processes, especially when they are prolonged.

Thus, as Table 3 suggests, the strong degradation of glutamic acid is not a mandatory criterion for the final umami flavour of black garlic. Although glutamic acid, the umami tastant, is usually formed by proteolysis during the fermentation process, it can also be formed enzymatically (by glutaminase activities) from glutamine by certain lacatobacilli (Zhao *et al.*, 2016).

Almost all fermentation processes produce the umami aromatic pyroglutamic acid as well as certain tripeptides that may contain pyroglutamic acid, proline and another hydrophobic amino acid. Interestingly, even threshold concen-trations, *i.e.* individual components of various pyroglutamyl peptides and precursors such as

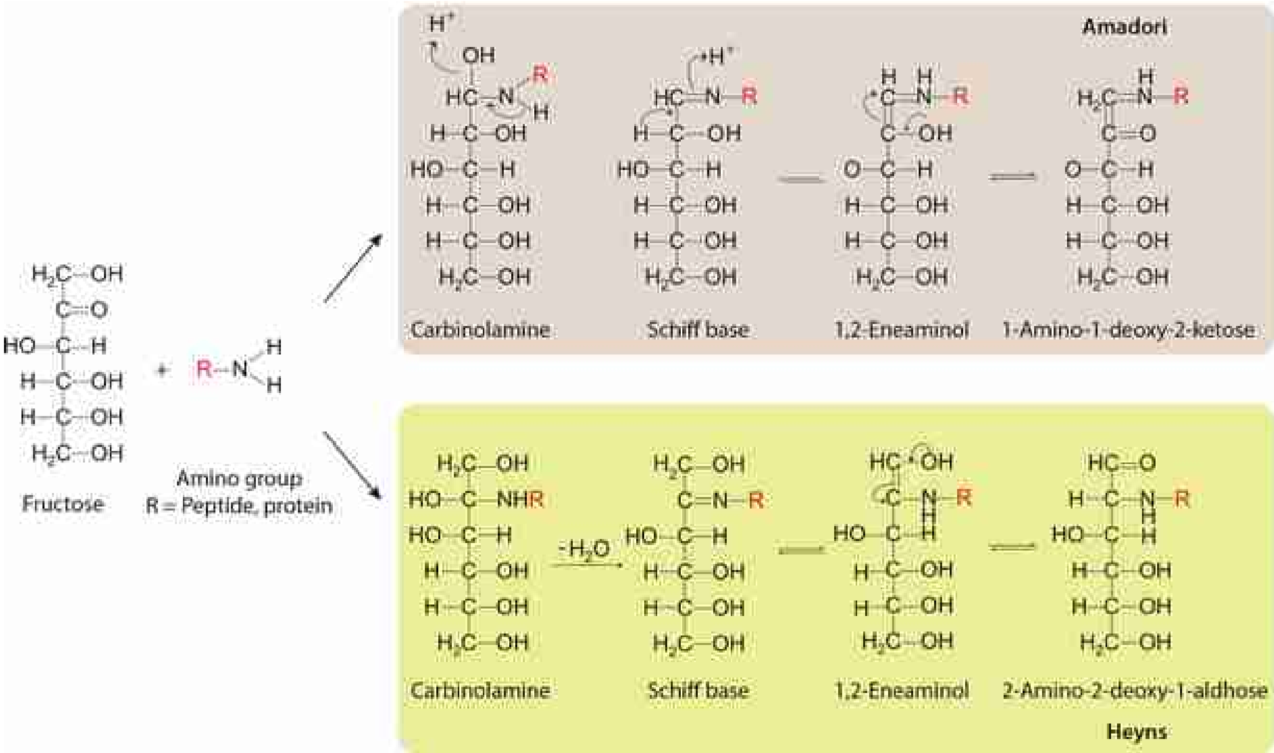


*Figure 7. Amadori and Heyns rearrangements between a fructose and any amino group -$NH_2$, which can be part of a protein or peptide, described by the residue R (after Ríos-Ríos* et al.*, 2019).*

Amadori products, enhance the umami taste of soy sauce (Kaneko *et al.,* 2011).

### 3.3. Amadori-Heyns rearrangement and glycation

Black garlic is produced neither by fermentation nor by a pure non-enzymatic browning reaction, but by a mixed form during the different stages of thermal treatment. At the relatively high temperatures between 60 °C and 90 °C, alliinases are partially inactivated, as shown in Figure 2. On the other hand, the water content decreases and the cell walls become unstable (Wei and Lintilhac, 2003). Protein-cleaving enzymes, proteases, stored there are released and cleave the proteins into peptides of different lengths at a very early stage, as the simultaneously high humidity prevents the water activity from decreasing. Over time, the proteases are also inactivated, while the fructan are thermally cleaved. The free fructose that is formed (Baumgartner *et al.,* 2000) can react with proteins and peptides to form Amadori and Heyns products, which play a central role in flavour formation both during heating (frying) and

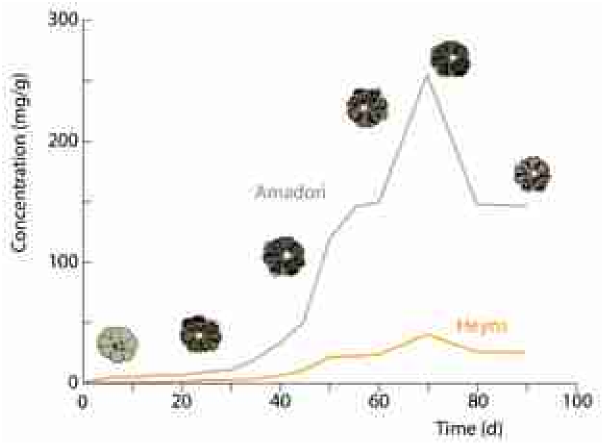


*Figure 8. Time course of the formation of Amadori and Heyn products at 55 °C and 80 % humidity (Yuan* et al.*, 2018).*

fermentation, as they serve as chemical precursors and starting compounds for taste and odours (Ríos-Ríos *et al.*, 2019). The respective reactions and rearrangements are summarised in Figure 7.
The respective concentration of Amadori and Heyns products at a given point in time depends strongly on the temperature and humidity. Yuan, *et al.* (2018) carried out a systematically controlled heat treatment. Garlic was processed in a humidity-controlled room at 80 % humidity and 55 °C for 90 days. The garlic was analysed every 5 days during the first 60 days and every 10 days during the last 30 days. The comparatively low temperature leads to slower reaction times and thus to a more complete analysis of the individual compounds formed during the process. The results are shown in Figure 8.
The concentrations of these precursors reach a maximum after a certain time (depending on temperature and humidity), in this example after approx. 70 days, at higher temperatures after a correspondingly shorter time. Sufficient fructose must first be formed before the rearrangement to Amadori and Heyns products takes place. The drop in concentration after the maximum indicates the formation of odorous and flavour-relevant substances under degradation of Amadori and Heyns products. After a certain time, which depends on the temperature, a glycation - and thus a non-enzymatic browning - happens. This takes place much more slowly than in the oven or in a frying pan, which is why the smell and flavour of black garlic differs significantly from fried and roasted garlic.

### 3.4 Acids and pH value

In contrast to lactobacilli induced fermentation processes, anaerobic fermentation may takes place to a very limited extent in black garlic, even at low temperatures in the first few hours. The pH value nevertheless drops sharply, as shown in Figure 11. Increasing concentrations of browning reaction products are associated with the synthesis of organic acids resulting from the

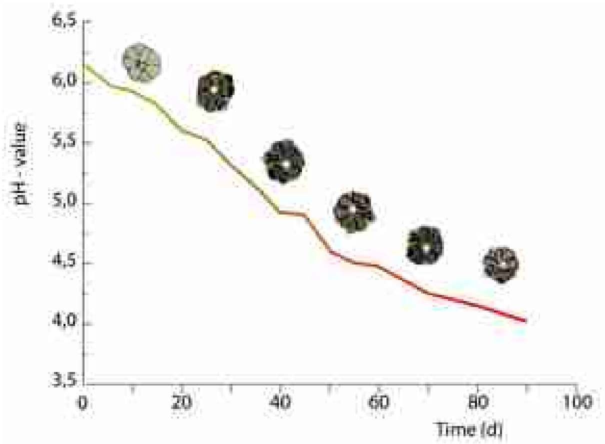


*Figure 9. The decrease of the pH-value at 55 °C and 80 % humidity. The colour change of garlic is shown for better visualisation (Yuan* et al.*, 2018).*

oxidation of aldehyde groups in aldoses, *e.g.*, from the Heyns precursors shown in Figure 7. (Najman *et al.,* 2022). However, the pH-value decreases significantly, as has can be seen for example in the investigations from Yuan *et al.* (2018), shown in Figure 9.
In addition, there are indications of how thermostable microorganisms support the formation of organic acids. It appears that garlic endophytes, *i.e.* microorganisms that live in symbiosis with the fruiting body, are involved. Qiu *et al.* (2018) identified seven bacillus species in fresh and black garlic. Further investigations showed that the total number of bacteria and endophytes initially decreased sharply during the temperature treatment of black garlic, then quickly increased again, remained at a certain level and finally decreased again. Dominant strains were *e.g. B. subtilis, B. methylotrophicus* and *B. amyloliquefaciens*. These strains are capable of fermenting glucose, lactose, sucrose to acids but, unlike conventional lactofermentation, no carbon dioxide. Some of these isolated bacterial strains showed high heat resistance up to 70 °C. Some microorganisms also show significant resistance to low pH values, remaining active even at pH values

around 3. The acidic flavour is based on acetic acid, succinic acid, formic acid, fumaric acid and its isomer maleic acid in different concentration ratios (Chua *et al.*, 2022).

It should also be remembered that pyroglutamic acid, as mentioned above, stimulates both umami and acid receptors and contributes to the sour taste.

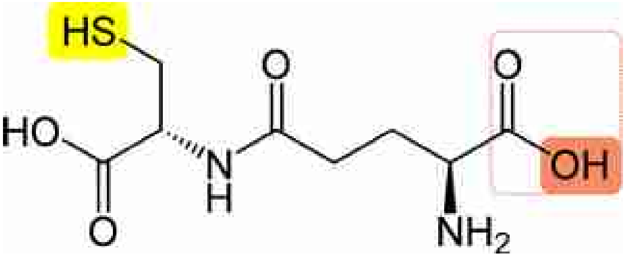


*Figure 10. The structure of γ-glutamyl cysteine. The hydrogen sulphide group of cysteine is highlighted in yellow and the carboxylic acid group COOH of glutamic acid is highlighted/framed in red.*

### 3.5 Mouth fullness (kokumi)

Mouth fullness (kokumi) is associated with a strong development of the umami taste, *e.g.* during fermentation (soya and fish sauces), ripening, maturing, curing (cheese, ham), or prolonged cooking (Tolstede *et al.*, 2009; Kuroda *et al.*, 2013; Araya-Morice *et al.*, 2021; Heres *et al.*, 2023; Li *et al.*, 2023).

This sensation is caused by γ-glutamyl peptides, the best known of which is gluthatione, GSH, γ-L-glutamylcysteinylglycine, a tripeptide consisting of the three amino acids glutamic acid, cysteine and glycine, which is found in most cells (Li *et al.*, 2022; Dutta *et al.*, 2022; Forde and Stieger, 2024). However, the kokumi sensation itself is not a taste perception, because mouth fullness is not perceived via the known taste receptors, but *via* special calcium channels, the so-called calcium-sensitive receptors (Maruyama *et al.*, 2021).

Liu *et al.* (2022) showed that a precursor of gluthathione, namely γ-glutamyl cysteine (Figure 10), is mainly responsible for the kokumi sensation in black garlic. However, first, the two amino acids must be linked and second the proteinogenic α-bond enzymatically transformed into a α-bond, by the two enzymes γ–glutamyl cysteine synthetase and γ–glutamyl transpeptidase. Therefore, kokumi intensity in black garlic depends strongly on the rate of these enzymatic processes during the process temperature and time. The maturation of garlic at high temperatures will therefore contain less kokumi than at low temperatures, such as the 50 °C just mentioned, at which the enzymes are still active for some time. Lui *et al.* (2022) have determined the temperature-time-dependent activity of the relevant enzymes and showed that at 50 °C their activity drops within the first 8 to 10 hours, where enzymes γ–glutamyl cysteine synthetase, showed the largest resistance with highest activity within 12 to 14 hours. Moreover, it seems that the activity of the enzymes is reduced by half between 5 and 6 h. After 18 h, no significant activity remains for all relevant enzymes. At higher temperatures, the decrease in activity is significantly faster. However, the systematically higher activity of γ-glutamyl cysteine synthetase compared to the other enzymes is striking. This underlines the formation of significant concentrations of the kokumi-stimulating γ–glutamylpeptides, provided the process temperature is not much higher than 50 °C. As an important result, this means that the processes that are relevant to kokumi take place at lower temperatures and in the early stages of garlic processing.

### 3.6. Formation of odorants

A striking feature of the odorant spectrum of black garlic is a deep, caramel-like flavour, which in most cases can be attributed to furans as reaction products from fructose (Leng *et al.*, 2020). For example, 5-hydroxymethylfurfural (1), often abbreviated as 5-HMF, whose odour attributes are caramel-like, buttery, musty and reminiscent of hay and tobacco in the mouth. In

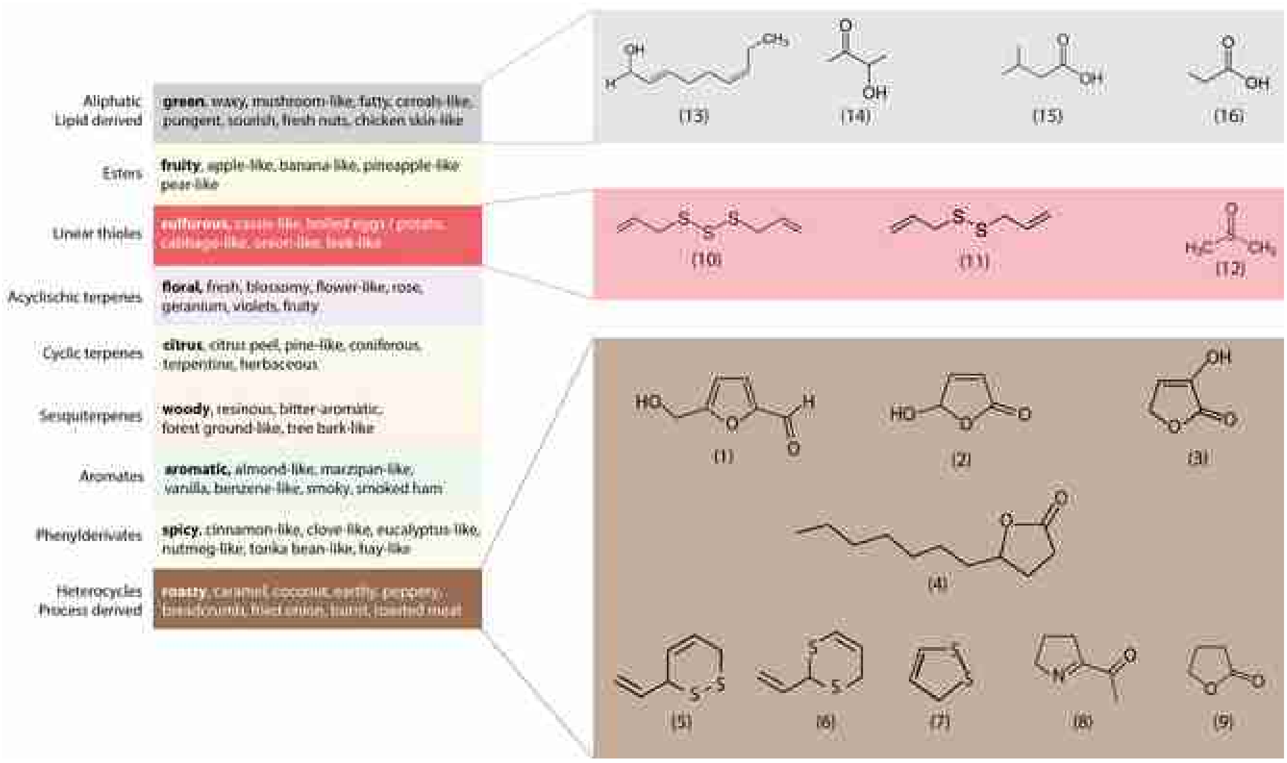


*Figure 11. Most important part of the aroma spectrum of black garlic (Vierich and Vilgis, 2021). The numbers under the structures of the most odour-active odorants correspond to the numbering in the text.*

addition to 5-HMF, furaneol (2), 2(5H)-furanone (3) and 5-heptyldihydro-2(3H)-furanone (4) stand out among the caramel flavours with high odour activity. They add odours ranging from dried fruit, caramelised fruit to burnt odours, such as those that occur when roasting onions and indicate the origin of fructose, too. Roasty odours are also formed, which are caused by 3-vinyl-1,2-dithiacyclohex-4-ene (5) and 2-vinyl-4H-1,3-dithiin (6) and 3H-1,2-dithiol (7), *i.e.* heterocyclic sulphur compounds that are reminiscent of sauces, roasted meat and rotten eggs. Similarly, 2-acetyl-1-pyrroline (8) adds the odour of fried rice and popcorn, and γ –butyrolactone (9) contributes a creamy impression of caramelised, burned milk.

The sulphurous character of black garlic is enhanced by linear sulphurous odorants such as diallyl trisulphide (10), allyl ditrisulphide (11) and dimethyl sulphoxide (12), with odour attributes such as cooked garlic, boiled potatoes and boiled eggs.

Sulphurous odorant compounds are of course also characteristic of black garlic, albeit much less pungent. These compounds are also widespread in allium vegetables such as garlic, onions, leeks, etc.

Alliin and its derivatives (isoalliin, methiin and propiin) are the most important precursors of these sulphur-containing compounds (Vierich and Vilgis, 2021). Alliin and its derivatives are cleaved by alliinase to form allicin, which in turn is a precursor of allicin. Alliin and its derivatives are cleaved by alliinase to allicin, which is rapidly converted to all types of sulfur compounds, including diallyl disulfide, diallyl trisulfide and diallyl sulfide. These compounds are responsible for the strong, often painfully cold and tear-irritating odour of fresh garlic. Compared to fresh garlic, this strong odour, accompanied by trigeminal stimulation, is much less pronounced in black garlic due to the much lower concentrations of diallyl disulfide and diallyl trisulfide.

Yang *et al.* (2019) found a high odour activity of diallyl disulfid in black garlic. The equally high odour activity of allylmethyltrisulfide also contributes to the "fresh", sulphurous aroma spectrum of black garlic. Fresh garlic contains an average of 13 % proteins and 75 % carbohydrates (mostly fructans). These two components form browning substances during the glycation reaction at a later stage, the garlic discolours and forms typical glycation reaction products during ripening (Najman *et al.*, 2022; Kilic-Buyukkurt *et al.*, 2023). Thus, heterocyclic compounds such as thiophenes, alkylpyrazines, furanones and furans are identified in black garlic, which are formed by the glycation during ripening in the later stages. Furaneol and other furans are derived from sugars via Amadori intermediates (Schwab, 2013). In fresh garlic, as mentioned above, fructan has a high proportion as a storage carbohydrate, but free, low-molecular sugars with different numbers of carbon atoms, such as arabinose, galactose, glucose, fructose, sucrose and maltose. From these, caramelisation reactions take place, which lead to various furans and furaneol.

The amino-carbonyl reaction can be followed directly using examples: 2-acetylfuran, which smells of roasted nuts, is formed by cyclisation of 1,4-dideoxyosone through dehydration from glucose and glycine. The odour-intensive compound 2(5H)-furanone is also formed by glycation. 3-(Methylthio)-propioaldehyde, which smells like boiled potatoes, is also a typical product, as it is formed from the amino acid phenylalanine by Strecker degradation (Gijs *et al.*, 2000) and also plays an important role in malting and during the brewing process in beer, for example.

The organic acids responsible for the sour taste are formed *via* both glycation reactions and lipid oxidation (Martínez-Casas *et al.*, 2017). Acetic acid and propionic acid are formed from hexoses or pentoses during thermal processing by further cleavage (Yang *et al.*, 2019). In addition, lipid oxidation in combination with the glycation and amino-carbonyl reactions contributes to the overall flavour of black garlic by providing intermediates from different reactions. The structure of the compound 5-heptyldihydro-2(3H)-furanone is reminiscent of a lactone (see (9) for the γ-butyrolactone formed here), and is therefore a odorant compound derived from a saturated fatty acid. The typical cucumber odor (E,Z)-2,6-nonadien-1-ol, on the other hand, is oxidised directly from unsaturated C18 fatty acids.

For a better understanding, it is important to know the origin of the odourants. The most odour-active compounds are summarized in Figure 11. They have been included in the flavour scheme developed by the author to provide a better overview. Obviously, fresh garlic occupies the same aroma groups, but with a significantly different weighting. This shows that no new odorant types, such as esters, terpenes, or aromatics are formed during garlic maturation at temperature, with the exception of the process induced odorants due to caramelisation and the glycation.

Most of the compounds formed by the glycation reaction contribute to the distinct roasted flavour of black garlic. Further improvement of the flavour could therefore be achieved by enhancing the glycation, *e.g.* through a selective temperature profile during ripening. This is discussed further below.

### 3.7. Influence of temperature, humidity, and time

Temperature, humidity and process time are the main factors that determine the sensory properties of the black garlic. This can already be observed in the formation of the caramel odour 5-HMF. Leng *et al.* (2020) presented experiments on this, which are summarised in Figure 13. Relatively short process times of up to 15 days were defined, but samples were taken every other day to determine the 5-HMF concentration. Maturation was analysed at

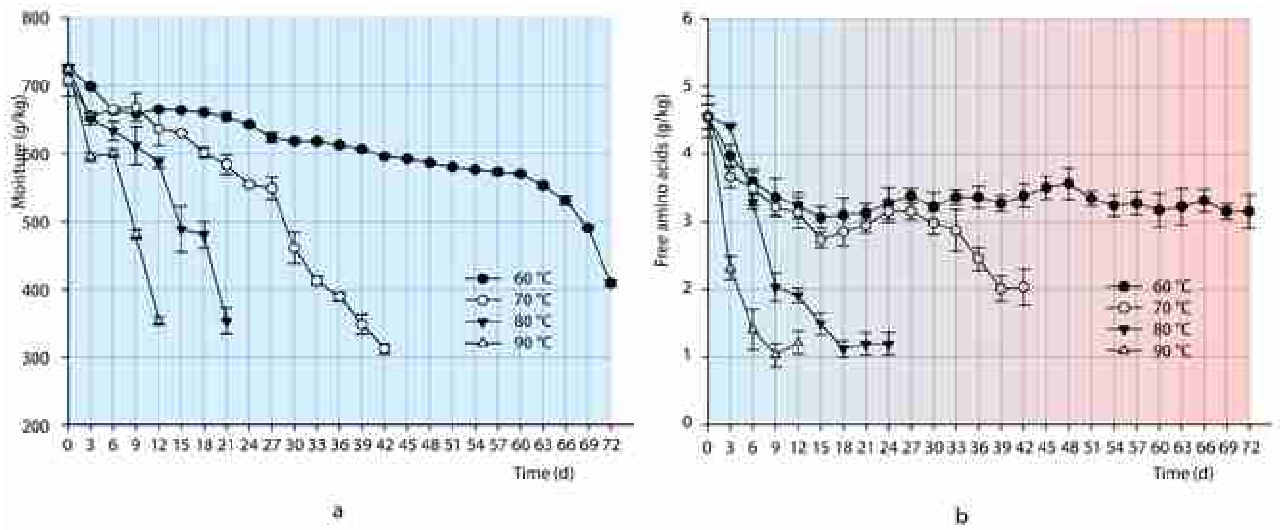


*Figure 12. The residual moisture (*a*) and the total concentration of free amino acids (*b*) as a function of the process time (from Zhang* et al.*, 2016).*

process temperatures of 50 °C to 90 °C, and the humidity was varied from 30 % to 70 %. Leng *et al.* (2020) found that at high process temperatures, the formation of the concentration of 5-HMF increases sharply between two and four days and also reaches its highest values. At low temperatures, the concentration remains lower and the increase is less. High humidity also favours rapid formation of the odorant compounds. However, it is noticeable that the concentration of 5-HMF decreases again at high temperatures and higher humidity, as the compound can continue to react.

Zhang *et al.* (2016) have performed a deeper and more thorough investigation, but limited themselves to the process temperature, albeit with process times of up to 72 days. Unfortunately, the air humidity was not controlled during these experiments, as the experiments were carried out in a drying oven and no further details of the prevailing humidity inside were reported. Nevertheless, especially as they confirm the results already mentioned. However, their results can used to optimise black garlic processing for better culinary solutions, which can be seen during the following discussion.

Figure 12 shows the residual moisture in the garlic and the number of free amino acids at the respective process time. At high temperatures of 90 °C or 80 °C, the residual moisture content drops by a factor of almost 2 in the first 12 or 21 days respectively. The garlic cloves quickly acquire a leathery to tough consistency.

At the same time, the concentration of free amino acids decreases rapidly during this time. They react relatively quickly to form aromatic substances with the risk of an unbalanced flavour. At 70 °C, both the residual moisture and the free amino acids concentrations decrease to a lesser extent. Even after 30 days, the residual moisture is still high enough to guarantee a pleasantly pasty, still not too leathery texture. At a process temperature of 60 °C, there is still enough residual moisture after 72 days to ensure a pleasant texture. The amino acid concentration also remains constant between 15 and 72 days. The low temperature reduces the glycation, resulting in less roasted and bitter products, while the remaining amino acids support the flavour with their balanced sweet, sour and umami taste.

The progression of the colour change during browning over time, objectively measured by colorimetry (Anzalone *et al.*, 2013), and the associated concentration of the caramel ingredient and indicator 5-hydroxymethylfurfural

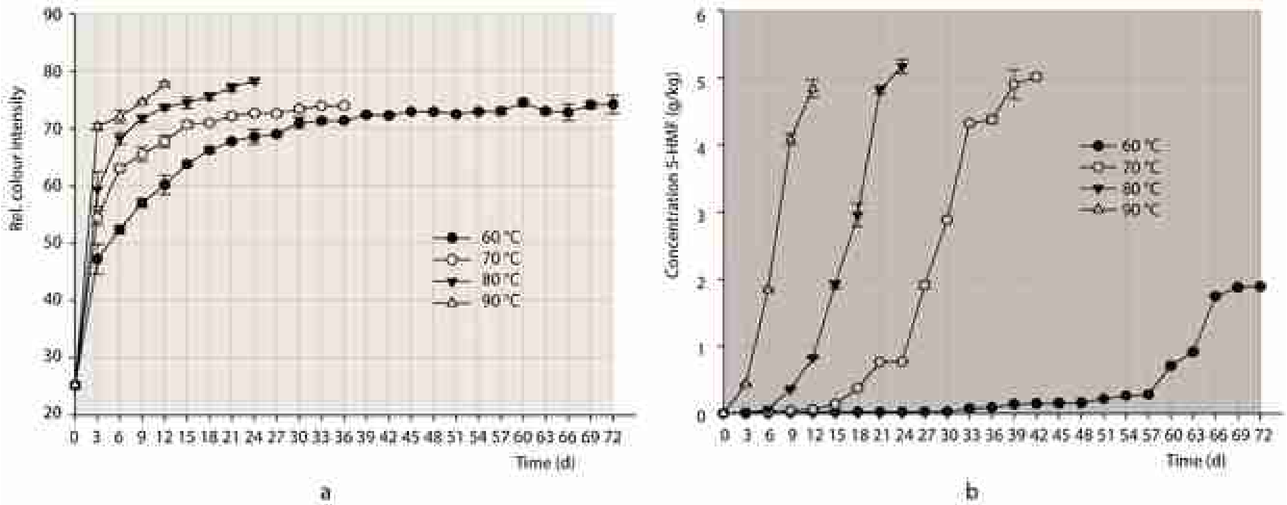


*Figure 13. Colour intensity (*a*) and concentration of 5-HMF (*b*) in the long-term process (Zhang* et al.*, 2016).*

are compared over time in Figure 13a and 13b. It can be seen that browning increases rapidly at all temperatures in the first three days.

However, the colour intensity at 90 °C is quantitatively higher by a factor of 2. The colour intensity then increases slowly at 60 °C and reaches saturation after approximatively 40 days. At 70 °C, saturation is reached after 20 days, and at 80 °C and 90 °C, maximum browning is reached after 24 and 12 days respectively. Similar ratios can be seen in the course of the 5-HMF concentration. At 70 °C, the concentration also rises sharply between 15 and 30 days, while at 90 °C the maximum concentration is already reached after 12 days. At 60 °C, however, the concentration remains within an acceptable range. Together with the above-mentioned results on flavour formation, it can be concluded that caramel flavours perceived as "burnt" develop less intensity at lower process temperatures.

These experiments show clearly: neither too low nor too high temperatures brought a sensory advantage. The evaluation at 70 °C was significantly better than at the other temperatures, which shows that the temperature of 70 °C promotes the development of good quality and flavour of black garlic during treatment (Kilic-Buyukkurt *et al.*, 2018). At temperatures of 80 °C and 90 °C, the appearance of the garlic sample was white to dark brown in the initial phase. The inside of the garlic sample showed some white spots. The colour of the garlic sample ranged from dark brown to completely black in the later stages.

Sensory testing revealed that the quality of the matured black garlic was better and its colour was uniformly black at 70 °C and 80 °C. At 90 °C, the garlic discoloured. At 90 °C, the garlic turned dark brown to black more quickly, but was more bitter and had an unpleasantly acidic flavour. At 60 °C, the garlic did not show uniform blackening, although the texture, flavour and aromas were more balanced.

## 4. Conclusion

It is clear from the above that many of the enzymatic processes that determine flavour appear to be determined in the first few hours of garlic ripening at temperature, whereas glycation reactions are effective at higher temperatures. It is therefore advisable to ripen garlic under graduated temperature and humidity conditions. Similar to the different resting times of malt mashing in beer brewing (Caviezel and Vilgis, 2018), the final flavour of


*Figure 14. A possible temperature (*red curve*) and humidity profile (*blue curve*) for black garlic in five zones: I: activation of enzymes, II: promotion of enzymatic processes, III: glycation zone, promotion of non-enzymatic browning reaction, IV: cooling phase with stopping of reactions, V: drying phase for safe storage and longer shelf life.*

black garlic can be controlled much more precisely than if temperature and humidity are kept constant for weeks.

At low temperatures between 50 °C and 60 °C, allinases and other enzymes are responsible for breaking down the pungent and bitter flavour components. These must first be activated, which happens at temperatures around 50 °C. However, this requires more time and it is necessary to keep the garlic at this temperature for a certain amount of time to allow sufficient time for these processes to take place. Heating the garlic too quickly would quickly inactivate the enzymes. At the same time, the humidity must be kept high during this phase, as drying out reduces the water activity of the garlic and restricts the enzyme activity.

Therefore, two temperature levels are required in the first phase, which lasts around 5 to 7 days: around 50 °C for activation and just under 60 °C for the enzymatic processes, as well as high humidity. At temperatures around 60 °C and higher humidity, however, the fructans also begin to decompose.

The number of reducing sugars increases, which is intrinsic to the rapid formation of Amadori and Heyns products as precursors for flavour and aroma formation. Higher temperatures are favourable for the glycation reactions. The subsequent glycation zone can therefore be extended at 80 °C over a longer period of time. During this time, the caramel flavours and glycation products are formed. Even browning (blackening) is also complete. These chemical reactions also require a high level of humidity, which can be up to 95 %. The flavour yield is then also possible with a shorter total ripening time despite relatively low temperatures for good flavour formation. Excessively long process times are counterproductive anyway, as indicated by the experiments of Zhang *et al.* (2016).

Figure 14 summarises these conclusive considerations. However, the times and temperatures shown in this figure do not necessarily have to be adhered to. This is merely an illustrative example of possible flavour optimisations. The drying phase V could be omitted when the black garlic does not to be strored for a long time. The advantage of such a staggered process control was already shown in the experiments of Liu *et al.* (2022). The concentration of the kokumi relevant peptides increased by employing lower initial activation temperatures and enzymatic processes for the enzymes during phases I and II.

As suggested from the results summarized in Figures 12 and 13, the variation of temperatures may have strong impact of the variation and composition of aroma compounds. Lower temperatures at the glycation phase III may pronounce more caramel type aroma compounds, higher temperatures and times could strengthen the savoury flavours. These experiments could be conducted by varying the humidity also, since quite some influence on the aroma formation is expected.

Further analytic research certainly helps to understand the processes in more details. Moreover, the processes described here are universal and can be applied straight forward to other bulbous plants from the onion family (Alliaceae), as has been shown with onions recently (Moreno-Ortega *et al.,* 2020).

## Conflict of Interest

The author declares no conflicts.

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