


Review

Flavour Generation during Lactic Acid Fermentation of Brassica Vegetables—Literature Review

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Abstract: Fermentation is a method of food preservation that has been used for centuries. Lactic acid fermentation, apart from extending the shelf-life of vegetables, affects significantly the flavour of food products. In this review, the formation of flavour, including both taste and aroma, in fermented Brassica vegetables is summarized. The flavour-active compounds are generated in various metabolic pathways from many precursors present in raw materials used for fermentation. In Brassica vegetables, a unique group of chemicals, namely glucosinolates, is present, which significantly influence the flavour of fermented products. In this summary, we took a closer look at the flavour of two of the most commonly eaten worldwide fermented Brassica products, which are sauerkraut and kimchi. Finally, the needs and directions for future studies were addressed.

Keywords: lactic acid fermentation; flavour; aroma; taste; sensory analysis; cabbage



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1. Introduction

Many factors influence the everyday food choices of consumers, including the obvious ones like providing nutrition for the human body, but also bioactive values, appearance, taste, etc. [1]. The accessibility and choices of food products in the modern market are tremendous; however, according to statistical data, the flavour is the most important feature, which determines consumers' choices [2].

For ages, attempts to preserve food have been applied. One of the oldest methods to extend the shelf-life of perishable goods is fermentation. Apart from extending the shelf-life, fermentation also influences the sensory properties of final products. Many vegetables are fermented worldwide, but in this review, we would like to focus on a specific group of vegetables belonging to the *Brassicaceae* family. These vegetables contain a unique profile of bioactive compounds, which can contribute to the improved health of consumers. In this review, the formation of flavour, including both taste and aroma, in fermented Brassica vegetables is summarized and the biochemical pathways involved in their formation are described. Considering the wide range of Brassica-based fermented products, the more in-depth characteristic of two of the most popular products, which are sauerkraut and kimchi, is performed.

2. The Importance of Flavour

Flavour is a very complex sensation, composed mainly of taste, aroma, as well as tactile and temperature factors [3]. Taste receptors are located on the tongue and can distinguish four basic tastes, such as salty, bitter, sweet, and sour. However, the novel nomenclature also includes a fifth taste modality: umami. The sour taste might be caused by the presence of organic acids since its original role was to recognize spoiled or unripe food. A salty taste, similarly to sour, is triggered by ions, and thus these receptors are important in the assessment of electrolyte concentration, and their concentration determines whether the feeling is pleasant or not [4]. The role of savoury and sweet receptors is to

indicate the caloric content of food, since umami buds are triggered mainly by amino acids, and sweet receptors by saccharides [5]. Bitter receptors aim to recognize any potentially toxic components, such as bacterial metabolites or spoilage products. Bitter is the most complex among the human tastes, and according to many sources, it is the most important one [6]. Notably, many bitter compounds might have a highly beneficial influence on human health. This is often related to the fact that many toxic components in low doses might show positive effects on human health. However, the same compounds consumed in high doses can induce toxic or even mortal effects. It is also known that the level of bitterness perception differs between individuals, and to date, it was correlated with TAS2R genes. Although the research aiming to explain that phenomena are numerous, these complex relationships have not yet been fully elucidated [7].

While five tastes have been perceived in the mouth, it appears that an infinite number of chemical stimuli can be perceived in the nasal cavity [8]. The aroma molecules reach the nasal cavity in a few different ways, such as during exhalation, in the chewing of food, as well as in food swallowing [9]. Usually, odoriferous compounds are present in food products in low concentrations, such as mg/kg, or sometimes even lower, such as µg/kg or even ng/kg. That is because the olfactory system of humans can detect aroma-active substances present in extremely low concentrations [10]. Few definitions should be mentioned when food odorants are considered. First is the “odour threshold”, which defines the lowest concentration of the compound in a specific medium, which is sufficient to be recognized as a particular odour. Therefore, this parameter is necessary to characterize the aroma compounds and their contribution to food flavour [11]. Similarly, for taste, the concentration of taste providing the compounds which stimulate the receptors in the mouth is called the “taste threshold” [12]. However, the odour threshold is not easy to determine, since many factors influence its value, including mainly interactions of odorants with food macro constituents, such as proteins, lipids, or carbohydrates. These interactions influence the aroma binding and the release of compounds to headspace in various ways. Consequently, it influences the value of the odour threshold.

The flavour quality of fruit and vegetables is influenced by genetic, pre-harvest, harvesting, and also post-harvest factors [13]. Firstly, depending on the cultivar used in the plantation process, genetic traits have a significant impact on the flavour of the obtained plant. Furthermore, the conditions of the cultivation process, such as temperatures, humidity, and soil quality, influence the metabolomic profile of the plant and therefore have a crucial impact on the flavour components profile. Post-harvesting factors which influence the flavour are the storage of raw products, processing, including fermentation, thermal processing etc. and lastly the storage of the final product [14]. Heat treatment is pretty straightforward, since it involves only temperature influence on the non-stable precursors, as well as enzyme inactivation; it therefore, in the majority of cases, leads to the decrement of odour active substances. Fermentation, on the other hand, is a more complex issue because it involves the action of microorganisms, as well as the enzymatic processes the occur in the plant anyway.

3. Strategies Used for Aroma Analysis

In every food product, the number of volatiles is usually high, however, the number of aroma active compounds present in the concentration above their odour threshold is limited. The most common strategy dedicated to analyzing the dominant odour active substances—so-called key odorants—is the sensomic approach, which involves the application of gas chromatography–olfactometry (GC-O) [15]. GC-O combines the separation step (GC), with the human nose playing the role of the detector. Among the extraction techniques used for the sensomic approach is solvent assisted flavour extraction (SAFE), introduced by Engel et al. [16], which is considered the gold standard. SAFE is considered to be a method that allows for effective volatile isolation at low temperatures, avoiding potential flavour modification or artefact formation at high temperatures [17]. In a similar manner, for taste active components, liquid chromatography fractioning and later a taste test by the sensory

panel is applied. The flavour dilution factor (FD) is the dilution step in which the respective odorant is detected for the last time. A higher FD factor means a higher odour potency of the analyte [18]. However, the FD factor does not display the importance of the single aroma compounds appropriately. That is because the volatility of each odorant determines its available amount in the headspace, whereas during the GC-O, the entire amount of each compound is vaporized in the injection port, and the amount of analyte reaching the odorant receptors is larger. To solve this issue, an odour activity value (OAV) was suggested, which is expressed as the concentration divided by the odour threshold [19]. Analogically in taste analysis, a procedure aiming to describe the importance of the taste-active component in flavour creation is the taste dilution analysis (TDA). However, in taste, the analysis is more straightforward, since the effect of the matrix does not play a significant role. As concluded by Greger and Schieberle [20], the aroma profile might be reconstituted based on the results of quantitative data. The authors presented it based on the analysis of apricot, which was closely mimicked by the mixture of 18 identified volatiles of identical concentrations to those present in the apricot.

To better understand the flavour, after recognition of aroma-active compounds, their origin needs to be determined. This step involves multiple biochemical analyses. The application of radiolabelled compounds is one of the most common steps in providing information regarding potential biochemical pathways leading to flavour formation. The next steps aim to provide information regarding the enzymes involved in these pathways by their isolation and evaluation of the activity with dedicated precursors.

4. Lactic Acid Fermentation—State-of-Art

Among the types of fermentation, the application of lactic acid bacteria (LAB) is the most commonly used methods for preserving dairy and vegetables, due to its simplicity, low cost and sustainability to maintain [21]. In addition to extending shelf life, lactic acid (LA) fermentation also modifies the sensory properties of products and increases their health-promoting effects [22]. Preservation by LA fermentation occurs mainly due to the synthesis of a wide range of metabolites including organic acids, fatty acids, bacteriocins, carbon dioxide, diacetyl and others [23]. Notably, a low pH and a relatively high concentration of LA provide the conditions that help inhibit the growth of competing, pathogenic and spoilage microbes. Therefore, LA fermentation not only extends the shelf-life but also secures the microbial safety of fermented foodstuffs. Moreover, in addition to providing safety and extending the shelf-life, LAB generate various secondary metabolites which affect the texture and nutritional value [24] and also possess a range of flavour activities [25].

LAB ferment the products alone or in combination with other groups of microbes, including other bacteria, yeast or fungi. LAB constitute a group of Gram-positive, non-sporulating bacteria belonging to the *Firmicutes* phylum. Compared to other bacteria, LAB are characterized by rapid acidification due to the fast consumption of carbohydrates. LAB can be further divided into homo- and heterofermentative, depending on the main products of their fermentation. Homofermentative bacteria convert glucose into LA with around an 80% theoretical efficiency rate [26] and include genera such as *Lactococcus*, *Pediococcus*, *Streptococcus* and *Enterococcus*. In turn, heterofermentative LAB, which include genera *Leuconostoc*, *Oenococcus* and some *Lactobacillus*, except for LA also produce other acids, ethanol and carbon dioxide [27]. Additionally, some bacteria can be classified as facultatively heterofermentative depending on the environmental conditions [28]. It is worth underlining that a new taxonomic classification for the genus *Lactobacillus* was introduced in 2020 [29]. Based on the genome sequencing, the authors proposed the union of *Lactobacillaceae* and *Leuconostocaceae* and 23 new genera of *Lactobacillus*. However, in this study, the original nomenclature from cited articles was applied.

LA fermentation of vegetables can be carried out by means of two main approaches. The first is the spontaneous fermentation done by the autochthonous bacteria present in the raw material. In this case, fermentation takes place under favourable conditions of temperature, salt concentration and oxygen availability. This type of fermentation is

conducted in most of the fermented food consumed in developing countries. The second type is controlled fermentation, with the use of the starter cultures containing selected strains of bacteria, which secure the consistency, reliability and reproducibility of the final product [23]. Importantly, the area of the application of starter cultures in LA fermentation of vegetables is less developed compared to dairy and meat production, but some attempts have been conducted [30].

5. Flavour of Brassica Vegetables

Brassica vegetables are an important group of products and are commonly consumed in all parts of the world. Brassica vegetables are part of the genus *Brassicaceae* and include, among others, broccoli (*Brassica oleracea* var. *italica*), Brussels sprouts (*Brassica oleracea* L. var. *gemmifera* (DC.) Zenker), cabbage (*Brassica oleracea* L. var. *capitata* L.), cauliflower (*Brassica oleracea* var. *botrytis* L.), horseradish (*Armoracia rusticana*), rapeseed (*Brassica napus* subsp. *napus*) and many others. Another name used for Brassica plants is cruciferous, a name that is related to the shape of their flowers, the four petals of which resemble a cross.

The flavour of Brassica vegetables is unique and is not always tolerated by a significant part of society, which is caused mainly by genetic predisposition [14]. The compounds responsible for their characteristic bitter taste, as well as their sulfurous aroma, are mainly sulfur-containing substances, such as glucosinolates (GLS), their degradation products isothiocyanates, as well as other sulfur-containing volatile organic compounds (sulfides, thiols, etc.) [31]. Some Brassica vegetables, such as horseradish, are used mainly to flavour dishes due to the high content of allyl isothiocyanate [32]. Despite the multiple literature sources that describe GLS as bitter and isothiocyanates as pungent, it has been proven so far that this is not always true, since not all GLS and isothiocyanates impart the negative sensory traits [31,33]. As has been noticed in recent studies, some of the GLS were correlated with pleasant taste attributes, such as 4-hydroxyglucobrassicin in rocket salad [34]. On the other hand, in Brussels sprouts, the high GLS concentration caused consumer rejection. A major GLS in Brussels sprouts is sinigrin, which has a taste threshold of 106 mg/L [35], and in different cultivars of Brussels sprouts, the content of this GLS was higher [33]. To date, there has been no published record focused on the taste thresholds of other GLS than progoitrin and sinigrin [31,36], which is probably caused by the extremely high prices of their standards, which are dictated by their problematic extraction and purification. Therefore, the majority of research has rather focused on the correlation studies integrating data from sensory studies with the results of quantification of GLS and other phytochemicals, such as sugars, phenolic compounds, etc. [33,34]. Even though knowledge of the role of GLS in flavour creation is still under investigation and more experiments are necessary to fully explore this subject, their fate in the processing of the Brassica plant cannot be overlooked. GLS are not contributors to flavour by themselves, but they are also important flavour precursors. Products of GLS degradation are the main odorants in rapeseed oil, accounting for 80% of volatiles [10]. The enzymatic pathway leading to GLS hydrolysis involves an enzyme called myrosinase (EC 3.2.1.147). The enzyme usually associated with Brassica plants might also occur in certain insects, bacteria, or fungi [37,38]. Myrosinase and GLS are stored in different compartments in the plant cell [39] and the reactions between them occurs when the cell structure is broken. Possible products of GLS degradation are isothiocyanates, thiocyanates, nitriles, or epithionitriles [14] as presented in Figure 1. The biological role of the GLS-myrosinase system is to defend plants against being eaten by insects or herbivores, since products of GLS hydrolysis are toxic to predators [40]. In general, the direction of the GLS hydrolysis and the formed products depend on the structure of native GLS, the conditions of the reaction, as well as the species of the plant. All these possible products of GLS hydrolysis differ significantly in terms of flavour. Usually, isothiocyanates are the main products that influence the final aroma, due to relatively low odour thresholds, compared with nitriles or thiocyanates [41].

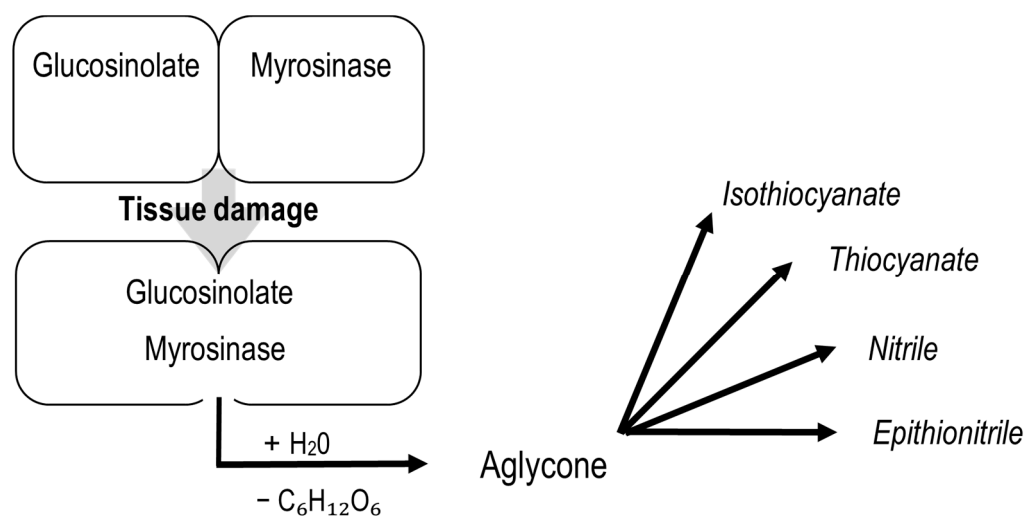


Figure 1. Degradation of glucosinolates in plant cells.

New topics such as thermal degradation of GLS in the model system are under investigation. Ortner and Granvogl [42] performed an experiment aimed at observing the odorants resulting from the thermal treatment of sinigrin itself without any potential reaction partners which might be present in the food matrix. GC-O was used to determine the aroma-active substances occurring after thermal treatment. A total of 29 aroma active spots were detected after using GC-O. The examples of odours detected on GC-O were: garlic-like (allyl methyl sulfide), onion-like (thiophene), sweaty-like (2/3-methylbutanoic acid), coffee-like (2-furanmethanethiol), caramel-like (4-hydroxy-2,5-dimethylfuran-3(2*H*)-one), butter-like (2,3-butanedione), popcorn-like (2-acetyl-1-pyrroline), etc. As seen, many pleasant aroma impressions were perceived, which suggests that thermal, non-enzymatic degradation of sinigrin might lead to the occurrence of different class components other than isothiocyanates, nitriles, or sulfides. Similarly, Zhang et al. [43] investigated the thermal degradation behaviour and volatile products of progoitrin. The authors observed 33 different compounds originating from the thermal degradation of progoitrin. Among others, there were, for example, 2,3-butanedione, dimethyl disulfide and 1,5-hexanediol, and the majority of identified components were aroma active.

Opposite to GLS, isothiocyanates are volatile substances, and therefore their role in flavour creation was mainly associated with the aroma. However, it was proven that due to the presence of thiocyanate moiety, this group has a predisposition to affect taste receptors as well [44]. So far, the majority of research has considered the impact of isothiocyanates on the aroma rather than on the taste of vegetables. Marcinkowska and Jeleń [41] determined odour thresholds, as well as odour quality descriptors, for 19 isothiocyanates that are frequently found in Brassica plants. They found that the odour thresholds of investigated isothiocyanates are within the range of 0.005–0.2 mg/L. The main descriptors for standard substances were: garlic, sulfur, and cabbage-like.

Not many papers involving a sensomic approach in the analysis of key odorants in Brassica plants were published to date. Marcinkowska et al. [45] analyzed key odorants in raw and cooked kohlrabi. The authors identified 55 odour-active compounds in raw and cooked kohlrabi by using GC-O. Twenty eight of these odorants were characterized with high FD values > 64; therefore, these compounds were quantified and their OAVs were determined. Based on the obtained results, it was concluded that the backbone of kohlrabi aroma constitutes: dimethyl trisulfide, 2-isopropyl-3-methoxypyrazine, hexanoic acid; 1-isothiocyanato-3-(methylsulfanyl)propane; and 1-isothiocyanato-4-(methylsulfanyl)butane. In another study, which aimed at the detection of odorants in raw and cooked broccoli, 33 aroma-active compounds were detected. The majority of those were sulfur components, and the highest FD (1024) values were related to methanethiol and 1-penthanethiol,

followed by methyl trisulfide, dimethyl tetrasulfide, 2-methyl methanethiosulphonate, 4-methylpentyl isothiocyanate and hexyl isothiocyanate (all with an FD of 256) [46].

Although GLS and their degradation products are important for the flavour of Brassica vegetables, other groups of non-volatile compounds are also necessary to consider, such as sugars, phenolic compounds, amino acids, or fatty acids. These substances were recognized widely as contributors to the flavour of many food products, as they both contain the taste and are also the precursors of multiple odorants. Sugars, which are components of the majority of food products, have a dual role in flavour creation. On the one hand, they introduce the sweet taste to the product, while at the same time they might be a masking agent for bitter molecules. The sweetness of sugars is expressed as a relative sweetness to sucrose in the following order: glucose (0.64) < sucrose (1.0) < fructose (1.2). In the study focused on the flavour of broccoli, Brussels sprouts, cauliflower, and kohlrabi, it was shown that sweetness and sugar concentration varied between these vegetables; however, in all of them, the sweetness was the most important trait in shaping consumers' desirability based on a sensory analysis [33].

Phenolic compounds are important phytochemicals in plants, including in the *Brassicaceae* family. They are classified according to their structure into simple phenols, phenolic acids, flavonoids, and hydroxycinnamic acid derivatives. The concentration of these components was determined in many Brassica vegetables, such as in cauliflower, kale, broccoli, white cabbage, turnip, pak choi, leaf rape, leaf mustard, etc. [47] However, only a few studies aimed to correlate their concentration with flavour attributes. Zabarar et al. [48] concluded that phenolic compounds are likely to contribute to the bitterness of broccoli, cauliflower, Brussels sprouts, and red cabbage, since GLS themselves did not explain the source of the bitter flavour. In contradiction to this, another study focused on the correlation of bitterness with phenolic composition in different cultivars of broccoli, cauliflower, kohlrabi, and Brussels sprouts but did not find any relation between bitterness and phenolic content [33]. Therefore, more data are still necessary if any unambiguous conclusions are to be made in this regard. This is especially the case if the strong impact of different factors, which might influence the composition of phenolic components in these vegetables, such as genetic and environmental factors, but also post-harvest treatment, such as thermal treatment, storage, or fermentation, are taken into consideration [47,49].

6. Changes in Flavour during the Fermentation of Brassica Vegetables

LA fermentation significantly modifies the aroma and sensory perception of fermented products. Compared to unfermented foodstuffs, LA fermentation leads to the diminishing of some compounds, forming new ones, while others remain unchanged, which finally leads to the unique flavour of fermented food. Flavour can be formed based on the composition of non-volatile and volatile compounds derived from the main nutrients in the fermenting matrix, including proteins, carbohydrates and fatty acids. In the case of Brassica vegetables, the important group of compounds generated during fermentation are derivatives of GLS and sulfur compounds, which are responsible for the unique taste and aroma of fermented products. Non-volatile fraction includes mainly amino acids and non-volatile acids, such as citric and malic acids, while volatile fraction refers to a wide range of chemicals, such as alcohols, aldehydes, acids, ketones, terpenes, hydrocarbons, esters, sulfur-containing compounds and others. The aroma compounds are formed in several metabolic pathways through fatty acid oxidation, Strecker degradation, glycosides hydrolysis and oxidation, as well as miscellaneous reactions caused by bacteria activity and through the change in environmental conditions [24]. Flavour compounds generated during fermentation include some end-products of primary metabolism (e.g., from the metabolism of carbohydrates) and in the majority the secondary metabolites, for which their physiological role for bacteria is often unknown and their formation is only partly understood [50]. Notably, the formation of flavour compounds is dependent on LAB species. For instance, branched-chain aldehydes formed from branched-chain amino acids could be formed only by *Lactococcus lactis* and not by *Lactobacillus* and *Leuconostoc* strains [51]. The

most obvious change in the fermented Brassica vegetables is an increased sourness, which is caused by LA formation. Since the majority of LA is produced from sugars, the sweetness of vegetables will likely be reduced. Importantly, lowering the pH during the progress of fermentation and a higher content of LA may affect the activity of plant enzymes that generate compounds responsible for flavour or their precursors.

In the following sections, the complexity of the formation of aroma-active compounds from different metabolic pathways occurring during the fermentation of Brassica vegetables will be presented.

6.1. Carbohydrate Metabolism

Sugars are primary substrates for LAB in fermented Brassica vegetables. Depending on the LAB species present in the raw material, sugars undergo homo- or heterofermentation with the formation of various products. Homofermentation, as mentioned before, results in the formation of mainly LA (Figure 2). Pyruvate is often an intermediate compound in many metabolic pathways that occur during fermentation, which can then be converted into many aroma-active compounds [52]. When carbohydrates are available in the fermenting matrix, the conversion of pyruvate into LA is the main metabolic pathway. Thus, the LA is quantitatively the most dominant acid in the fermented product. LA is, however, odourless and does not directly contribute to the flavour of fermented products. Heterofermentative LAB, except for LA, also produce acetic acid and other compounds, which can have aroma-active properties or can be precursors of aroma compounds. Ethanol formed by heterofermentative bacteria possesses a rather neutral aroma; however, its presence can affect the retention of other flavour compounds. An important product of LAB is acetic acid, which is responsible for its cider-vinegar flavour and can be formed by the oxidation of pyruvate. The ratio between LA and acetic acid decreases if there is an increase in the oxygen input during fermentation. The pool of acetic acid is usually built in the first 48 h of fermentation of cabbage [53,54], increasing the sourness of the sauerkraut. Both LA and acetic acid can be precursors for ester formation, ethyl lactate and ethyl acetate, respectively, which are responsible for the fruity aroma. The formation of these esters is highly dependent on the LAB strains. According to Yang et al. [54], the selection of specific LAB strains can result in a higher production of ethyl acetate, which can be useful in the formation of starter cultures for obtaining the products of certain sensory properties.

Another carbohydrate substrate is citrate, which can be co-metabolized with sugars by citrate-utilizing LAB, such as *Lactococcus lactis* and *Leuconostoc mesenteroides*. Fermentation of citrate leads to the formation of 2,3-butanedione, 2-hydroxy-3-butanone, acetaldehyde and 2,3-butanediol, which significantly contribute to the aroma of fermented vegetables [55]. Acetaldehyde can be directly formed by pyruvate decarboxylase or pyruvate oxidase and can be produced indirectly by the intermediate product acetyl-CoA with pyruvate dehydrogenase [56]. 2,3-Butanedione is produced by the metabolic intermediate α -acetolactate through oxidative decarboxylation and at the same time, α -acetolactate can be converted to 2-hydroxy-3-butanone by α -acetolactate decarboxylase or through non-oxidative decarboxylation [55]. Apart from their aroma activity, these compounds are important aroma precursors for a variety of odour compounds, which can be formed from Maillard and Strecker reactions with amino acids, similar to those observed in wine [57].

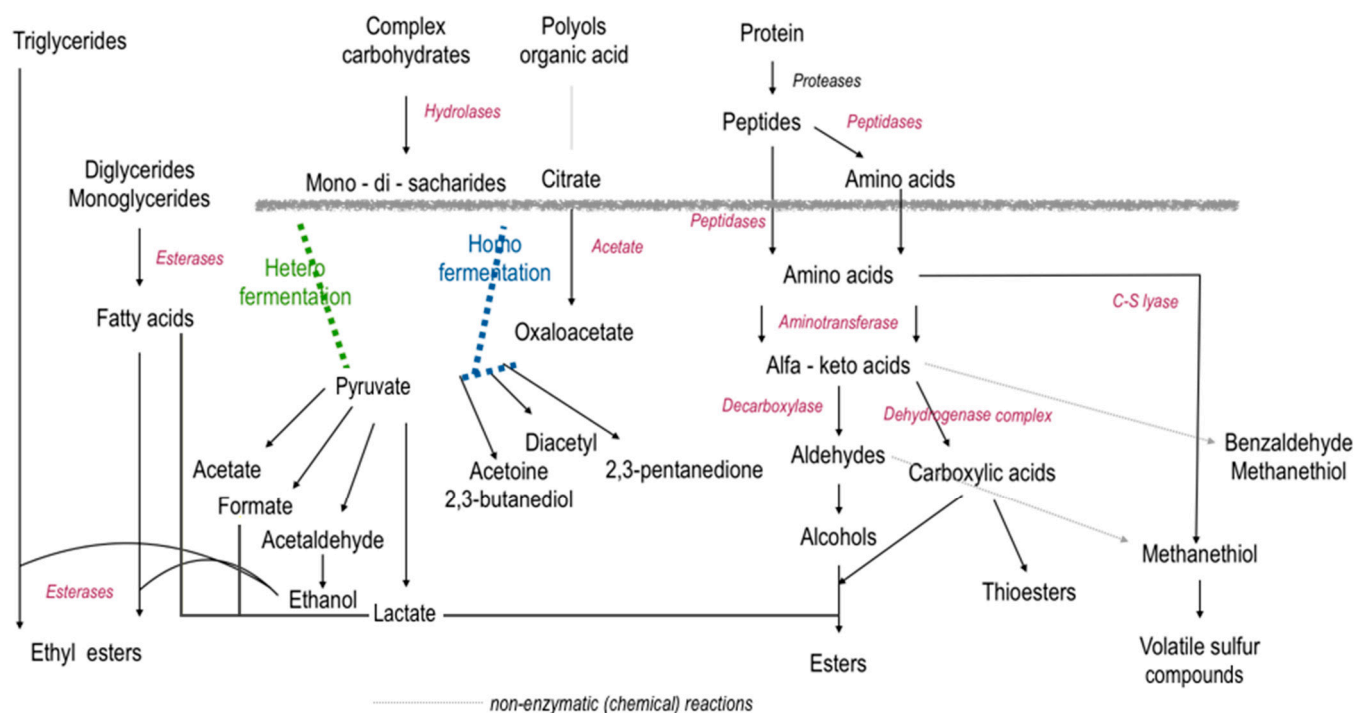


Figure 2. The metabolic pathways involved in the formation of aroma-active compounds during lactic acid fermentation.

6.2. Amino Acid Metabolism

During fermentation, extensive proteolysis occurs, which increases the pool of smaller peptides and free amino acids. Amino acids play a pivotal role in the formation of organoleptic features of fermented products. Amino acids themselves possess taste properties but are also substrates in the formation of aroma-active compounds. There are three main groups of amino acids that are distinguished by their taste: umami (aspartic and glutamic acid), sweet (alanine, serine, glycine, threonine, proline and asparagine) and bitter (valine, histidine, arginine, phenylalanine and leucine) [58]. In Brassica vegetables, the main amino acids are glutamine, asparagine, alanine and glycine in cauliflower [59], glutamic acid, aspartic acid, serine and alanine in broccoli [60], while in trochuda cabbage the main amino acids are arginine, followed by proline, threonine, glutamine, cysteine, and glutamic acid [61]. Glutamic acid, glycine and γ -aminobutyric acid were reported to be the main compounds responsible for the taste of paocai, the product obtained from LA fermentation of Chinese cabbage, cabbage, radish, mustard stems, long beans, peppers, daikon, carrots and ginger, in an electronic tongue study [62]. The authors underlined the role of LAB in the formation of flavour-active compounds.

Degradation of amino acids during fermentation is done via Ehrlich and Strecker pathways as well as others. For instance, leucine, valine, isoleucine, methionine and tyrosine are converted to fusel alcohols, which can be further oxidized to aldehydes, which can then be converted into the corresponding acid, which finally can be esterified [63]. The reaction starts with the formation of α -ketoacid from the amino acid, which can then be converted to aldehydes, carboxylic acid and alcohols (Ehrlich pathway). Next, aldehydes are subjected to further conversions into alcohols by dehydrogenation or into carboxylic acids by hydrogenation, while carboxylic acids can be esterified by specific esterases, leading to the formation of (thio)esters (Figure 2) [64]. Some important products of LA fermentation of amino acids are aldehydes (2-methyl-propanal, 2-methyl-butanal, 3-methyl-butanal, etc.) and corresponding alcohols (2-methyl-1-propanol, 2-methyl-butanol, 3-methyl-1-butanol, etc.), which are responsible for the fruity and alcoholic flavour [65]. Notably, during fermentation, the activity of many enzymes derived from LAB and the lysed LAB cells, including

the ones belonging to transaminase and lyase pathways, is observed, which contribute to the formation of a wide range of aroma-active compounds [64].

Among all of the amino acids, the metabolism of sulfur-containing amino acids such as methionine and cysteine is particularly important, as these are responsible for the formation of methanethiol, sulphides, thioesters and other sulfur-containing volatile compounds. Sulfur-containing aroma-active compounds are characterized by a very low odour threshold; thus, even their small concentration contributes to their cabbage-like, sulfury aroma [66]. It was repeatedly reported that these compounds are in the main responsible for the aroma of Brassica vegetables [45,46,67]. The formation of methanethiol from methionine can be achieved via a transaminase reaction with the formation of intermediate α -keto methylthio-butyric acid, which can undergo decarboxylation into 3-methylthio-propanal, which are then converted to methanethiol by a chemical reaction [24]. Methanethiol can then be oxidized into dimethyl disulphide or dimethyl trisulfide or can be converted into thioesters via esterase (Figure 3).

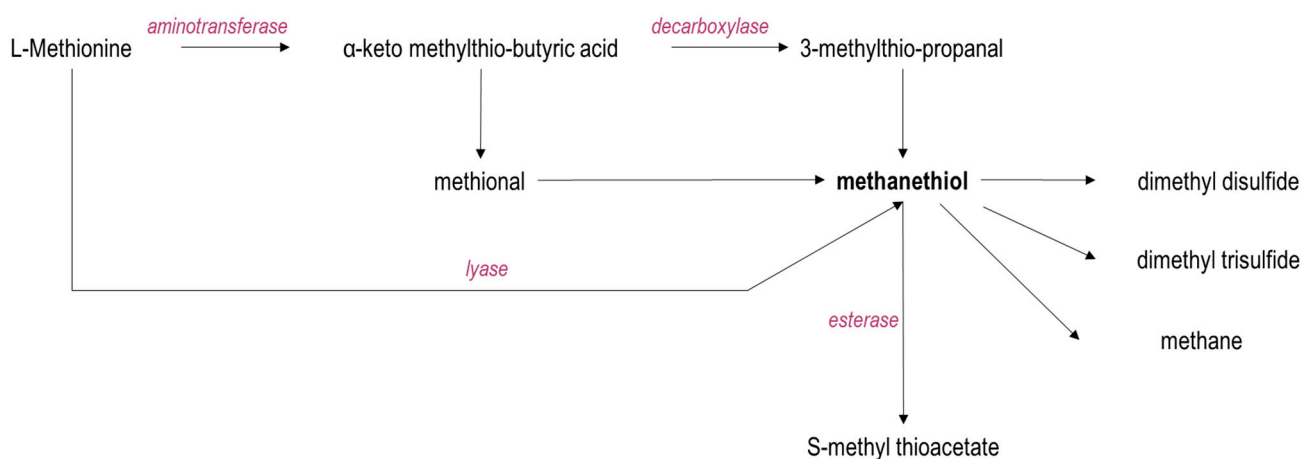


Figure 3. Schematic presentation of the formation of methanethiol.

6.3. Lipid Metabolism

Although Brassica vegetables are not a rich source of lipids (except the oil crops such as rapeseed and camelina), the products of their degradation during fermentation contribute to the aroma of fermented vegetables. Free fatty acids are precursors of esters, methyl ketones, aldehydes, secondary alcohols and lactones. The majority of LAB contain intracellular esterases [68], and thus they cannot hydrolyse lipids until they are released from the lysed cell. Esterases also have the ability to directly synthesize esters from glycerides and alcohols in alcoholysis occurring in an aqueous medium [25,50]. For instance, spontaneously fermented sauerkraut contains a higher content of ethyl octanoate, an ester derived from caprylic acid (C8:0), compared to the commercial product [54,69]. The authors explained that the formation of fatty acid-derived esters is highly dependent on the LAB used for fermentation. Many esters found in fermented Brassica vegetables possess pleasant fruity notes [70]. The compounds responsible for the green (some of them cabbage) aroma, such as 1-penten-3-ol, (Z)-3-hexenol, hexanol, and 1-octen-3-ol, can be formed during enzymatic oxidation of linoleic and linolenic acids and are commonly detected in fruits and vegetables, which were crushed or cut [71].

6.4. Glucosinolate Metabolism

GLS are an important group for Brassica vegetables which undergo degradation during fermentation. For sauerkraut, the time required for complete degradation of GLS varied from four days [72] to even two weeks of fermentation [73], and in the ready-to-eat product, the intact GLS are not present anymore. GLS are secondary plant metabolites with β -thiogluco moiety, which are derived from amino acids. Depending on the structure

and type of GLS, the degradation can lead to the formation of a broad range of compounds. Aliphatic and aromatic GLS are hydrolysed to corresponding isothiocyanates, nitriles but also thiocyanates and epithionitriles (Figure 1), and the direction of reaction depends on the reaction conditions (pH, temperature) and the presence of cofactors, such as epithio-specifier protein, nitrile-specifier protein, thiocyanate-specifier protein and ferrous ions [74,75]. Glucobrassicin, the main indole GLS detected in Brassica vegetables, undergoes hydrolysis to indole-3-acetonitrile and unstable isothiocyanate, which then converts to indole-3-carbinol, its product of oligomerisation (3,3-diindolylmethane, indolo[3,2-b]carbazole) or ascorbigens after reaction with ascorbic acid [72]. In the initial stage of fermentation, intensive respiration with the release of carbon dioxide and environmental acidification occur, which provides favourable conditions for myrosinase [76]. Importantly, the vegetables used for fermentation are usually shredded or cut, releasing myrosinase and GLS from the separated cell parts. The LAB were also found to be capable of hydrolysing GLS [77]. Thus, it cannot be excluded that these activities were combined from the beginning of the fermentation. The study of Mullaney et al. [77] showed that some LAB shifted the degradation of GLS into nitrile formation; however, the majority of studies reported that isothiocyanates are the main product of the GLS degradation during fermentation [78,79]. A recent study evaluating the changes of GLS showed that the direction of hydrolysis depends on the stage of fermentation [72]. Considering the final product, the content of isothiocyanates in sauerkraut, and also sauerkraut juice, was relatively high. This is of importance from the nutritional point of view [80], but also from the consumer perspective. Recent studies showed that isothiocyanates contribute to the taste and aroma of Brassica vegetables [45,46], which was summarized in excellent reviews [14,31]; however, the sensomic approach has never been applied to fermented Brassica vegetables, and thus the key odourants of fermented foodstuffs cannot be defined. Notably, the aroma and taste of ascorbigen, which is the main product in GLS degradation of sauerkraut, have not been elucidated to date, which we believe is worth consideration in the future.

7. Brassica Fermented Products

Many *Cruciferous* vegetables have been conducted in LA fermentation. Among them we can distinguish fermented cauliflower [81], broccoli [82], turnip [83], mustard [84,85], potherb mustard [86,87], nozawana [88] and watercress [89]. However, two of the most commonly eaten products from fermented Brassica vegetables are sauerkraut and kimchi, and therefore their closer characteristics will be presented.

7.1. Sauerkraut

Sauerkraut is a product that is obtained from fermented white cabbage (*Brassica oleracea* var. *capitata*), commonly consumed in Central and Eastern Europe. The word “sauerkraut” originated in the German language and means “sour cabbage”. To produce sauerkraut, the cabbage is shredded, mixed with salt brine and sometimes also with shredded carrot as an additional source of sugars. Salt is necessary for the development of anaerobic conditions during fermentation and for inhibiting the growth of spoilage microbes and the activity of endogenous pectinolytic enzymes responsible for cabbage softening [90]. Importantly, the amount of salt added influences the population and profile of LAB and the sensory quality of sauerkraut. Considering the WHO recommendations to reduce the consumption of salt, the attempts to replace sodium chloride with other minerals without compromising the sensory quality were conducted [91,92]. When the fermentation mixture is prepared, it is placed in fermentation vessels and kept from one week to several months for fermentation. After that, sauerkraut is packed in metal cans or glass jars, and is consumed as a fresh product or is pasteurized to extend its shelf-life [90]. Notably, even the type of package can affect the sauerkraut quality, as reported by Kapusta-Duch et al. [93,94]

From a microbiological point of view, in the course of fermentation, cabbage undergoes a sequential fermentation that is initiated by heterofermentative bacteria and is finished by homofermentative LAB [95]. The fermentation by LAB is crucial to obtain the unique

sauerkraut flavour because a study on the acidification of cabbage without the microbial fermentation did not result in a similar flavour to sauerkraut [96]. Notably, the study by Penas et al. [97] showed that the sensory perception is similar for sauerkrauts obtained via spontaneous LA fermentation and starter cultures. However, although insignificant, the highest overall acceptability was obtained for cabbage fermented with *Lactobacillus plantarum* and 1.5% of sodium chloride. Interestingly, this product had the highest score for an attribute described as raw cabbage-like, which was associated with green, immature sauerkraut [97]. In this study, the commercial sauerkrauts were characterized by the highest acid flavour and saltiness. In the same sauerkrauts, the authors analyzed the profile of breakdown products of GLS [98]. They found that fermentation induced the formation of iberin, iberin nitrile, allyl cyanide, allyl isothiocyanate and sulforaphane, which are derived from glucoiberin, sinigrin and glucoraphanine, respectively. Notably, in commercial sauerkrauts, the products derived from sinigrin, the main GLS in cabbage, were not detected. Interestingly, the salt concentration was found to influence the isothiocyanate and nitrile formation [98]. The products fermented with a higher concentration of salt (1.5%) were characterized by a higher content of isothiocyanates when fermented with *Lactobacillus plantarum* for iberin and with *Leuconostoc mesenteroides* for allyl isothiocyanate.

LA fermentation was reported to completely degrade GLS in sauerkraut, irrespective of the cabbage cultivation season, salt concentration and fermentation type [99]. The most comprehensive profile of GLS breakdown products was provided by Ciska et al. [72]. The authors detected the presence of 16 degradation products of GLS, including four derived from indole GLS (glucoiberin), one from aromatic GLS (gluconasturtin) and 11 from aliphatic GLS. Glucoiberin, glucoiberin, glucoiberin, glucoiberin, glucoiberin and glucoiberin were hydrolysed to both isothiocyanates and nitriles; gluconapin and gluconasturtin hydrolysed only to isothiocyanates; progoitrin degraded with the formation of 5-vinyloxazolidine-2-thione, the cyclisation product of the unstable isothiocyanate; and sinigrin hydrolysed to allyl isothiocyanate, which partly underwent cyclisation to epithiocyanate, 1-cyano-2,3-epithiopropene. In this study, at the beginning of fermentation, the formation of nitriles dominated. However, after two days of fermentation, their content decreased significantly, and the formation of isothiocyanates was favoured [72]. The high presence of GLS breakdown products can partly explain the unique aroma of sauerkraut, since many of them were reported to have an aroma activity, very high dilution factors and low odour thresholds [41,45].

An interesting study by Sarvan et al. [100] showed that blanching of cabbage to inactivate autochthonous microbiota in raw material and then fermentation with *Lactobacillus paracasei* LMG-P22043 can protect GLS from complete degradation. Unfortunately, the authors did not evaluate how this change would affect the flavour of the obtained product. In another study, the sensory properties of sauerkraut obtained with cabbage from organic and conventional farming were compared [101]. Interestingly, the sauerkraut from organic farming was characterized by higher overall acceptance and a better taste and aroma. The biggest difference was obtained for aroma; however, the author evaluated only the overall aroma and not individual attributes. Thus, it is difficult to guess which compounds were responsible.

The profile of metabolites responsible for the flavour of sauerkraut gained some attention very recently [30,54,69,102,103]. Satora et al. [102] evaluated the effect of different cabbage varieties on the chemical composition and sensory properties of sauerkraut. In this study, eight late cabbage varieties fermented spontaneously were compared. The varieties used for fermentation differed with the amount of sugars, specifically glucose, which could affect the properties of the final product. However, even if the initial content of glucose was low while the count of bacteria was high, the efficiency of LA production was also high. In the analyzed sauerkrauts, the presence of 61 volatile compounds was detected, including 5 esters, 15 alcohols, 9 carbonyl compounds, 12 sulfur-containing compounds (among them 4 isothiocyanates), 8 acids, 5 terpenes and 6 nitriles and 1,1'-oxybis-octane. The most abundant compounds were methanol and 3-hydroxybutanone.

In general, the concentration of individual compounds differed significantly between the varieties. The sensory analysis showed that the variety did not influence the appearance and texture of sauerkraut, which were suggested to depend on salt concentration [102]. On the contrary, the aroma and taste differed between varieties. The varieties characterized by a higher concentration of isothiocyanates, higher alcohols and volatile organic acids had higher scores for pungent aroma. The analysis of taste descriptors did not reveal any significant differences in saltiness, bitterness and off-tastes. The differences were observed for saueriness; however, surprisingly, they did not correspond to the content of organic acids. Spicy taste correlated with the sourness, and thus the same compounds could be responsible for these sensations. The sweet taste was the highest in the varieties in which the residue sugars were detected. In another, very recent study, the effect of the cultivar of sauerkraut in comparison to commercial sauerkraut was compared [69]. The presence of 32 volatile compounds, including 14 esters, 8 alcohols, 5 sulfur-containing compounds and 4 carbonyl compounds, was determined, and the differences in their abundance were detected among the investigated sauerkrauts. The most numerous and the most abundant in all varieties were esters and a significantly higher level was noted in the traditionally fermented products. Notably, compared to commercial fermentation, spontaneous fermentation can achieve a higher level of ethyl acetate, an ester derived from acetic acid. Likewise, the abundance of alcohols was higher in spontaneously fermented sauerkraut. Interestingly, the level of 2-isothiocyanatobutane and 4-isothiocyanatobut-1-ene was higher in the commercial sauerkrauts, while the abundance of allyl isothiocyanate was higher in traditionally obtained products. The partial squares-discriminant analysis (PLS-DA) revealed that compounds which contributed to the discrimination between the samples were ethyl hexadecanoate, hexyl acetate, neryl acetate, (Z)-3-octen-1-yl acetate, 1-pentanol, and 2-isothiocyanatobutane. The sensory analysis compared white colour, red colour, sulfur odour, fresh cabbage odour, fresh cabbage taste, hardness, crunchiness, juiciness, saltiness, sweetness, and overall quality, which were significantly different between the analyzed sauerkraut products. In general, the sensory properties of traditional cabbage cultivars were slightly better compared to commercially available sauerkraut products [69].

Studies by Yang et al. [54,103] evaluated the metabolic and microbial profile of northeast sauerkrauts, which are derived not from white cabbage (*Brassica oleracea* L. var. *capitata* L.) like European sauerkraut, but from Chinese cabbage (*Brassica rapa* L. *pekinensis*, cv. Wombok). In the first study [54], LAB were isolated from traditional northeast sauerkraut and single starter cultures were compared. In total, 99 volatile compounds, including 10 acids, 9 alcohols, 23 esters, 7 isothiocyanates, 13 aldehydes, 7 ketones, 2 hydrocarbons, 5 nitriles, 7 sulfides, 3 indoles, 9 terpenes, and 4 lactones were tentatively identified at the end of fermentation. The greatest abundance of esters (ethyl lactate, ethyl acetate and isoamyl acetate) was detected in sauerkraut obtained after fermentation with *Lactobacillus plantarum*, while the highest level of lactones was observed in sauerkraut inoculated with *Lactobacillus paracasei*. In turn, the fermentation with *Leuconostoc mesenteroides* and *Weissella cibaria* resulted in the formation of acids and ketones [54]. The highest increase of free amino acids was observed in sauerkraut inoculated with *Lactobacillus paracasei*. In the second study by Yang et al. [103], the sauerkrauts of different households were compared. In three different houses, the sampling was performed during the whole fermentation process. This time, 130 volatile compounds, including 11 acids, 16 alcohols, 24 esters, 22 aldehydes, 3 ketones, 6 nitriles, 5 isothiocyanates, 7 sulfides, 2 indoles, 5 hydrocarbons, 7 phenols, 6 lactones, and 16 terpenes, were detected. The principal component analysis showed that at the beginning of the fermentation, the profile of volatiles was quite similar in all households. However, with the progress of the fermentation, the volatilomes separated, and three clusters for each house could be separated. The correlation analysis showed that the main bacteria detected in sauerkrauts, namely *Pseudomonas*, *Chloroplast*, *Rhizobium*, *Aureimonas*, *Sphingomonas*, *Lactobacillus*, *Leuconostoc*, *Enterobacter*, *Clostridium* and *Weissella*, were statistically correlated with volatile compounds. At the same time, some negative correlations were observed, such as *Pseudomonas*, with 33 kinds of volatile compounds,

including nitriles, *Chloroplast* with 49 volatile compounds, including aldehydes, esters and nitriles, *Rhizobium* with 36 volatile compounds, including lactones and aldehydes and others [103]. These observations can help to design the starter cultures for sauerkraut with certain, favourable properties. In the next study [30], the volatilome of northeast sauerkrauts fermented with mixed starter cultures was evaluated using knowledge about the LAB strains obtained before [103]. This time, except for the metabolomic profiling, the in-depth sensory characteristic with sensory panel, e-nose and e-tongue was performed. Chemometric analysis with clustering found 82 volatile compounds which were attributed to 11 classes, including 2 organic acids, 17 alcohols, 19 esters, 9 terpenes, 2 lactones, 9 aldehydes, 6 ketones, 8 sulfides, 4 isothiocyanates, 3 polyphenols and 3 nitriles. All samples were clustered into five clusters according to the different stages of fermentation and type of mixed culture. Among the detected volatiles, 45 had an OAV above 1 and were considered to have made the biggest contribution to the aroma of obtained sauerkrauts. The e-tongue analysis showed that the sauerkraut with *Lactobacillus plantarum* and *Lactobacillus paracasei* mixed-culture had the highest sourness, while the lowest was detected for sauerkraut from spontaneous fermentation. Higher umami and lower bitterness values were revealed in sauerkraut with *Leuconostoc mesenteroides* and *Lactobacillus plantarum* as well as *Leuconostoc mesenteroides* and *Lactobacillus paracasei*. The results of the sensory panel evaluation showed the very big differences between the acceptance. The spontaneous fermentation had the lowest acceptability (1.07), while sauerkraut with *Leuconostoc mesenteroides* and *Lactobacillus paracasei*; *Leuconostoc mesenteroides* and *Lactobacillus plantarum*; *Lactobacillus plantarum* and *Lactobacillus paracasei* as well as *Lactobacillus plantarum* and *Weissella cibaria* had acceptance at around eight. The high sensory quality of sauerkraut can be associated with alcohols and aldehydes, which were detected in high abundance in sauerkraut with *Leuconostoc mesenteroides* and *Weissella cibaria* as well as in *Lactobacillus paracasei* and *Weissella cibaria*. Sauerkraut with *Leuconostoc mesenteroides* and *Lactobacillus plantarum*, which had the highest overall acceptance, was associated with the presence of esters (ethyl lactate, tetrahydrofurfuryl acetate, ethyl hexanoate, ethyl 3-phenylpropanoate and ethyl acetate), terpenes (geraniol, linalool, nerolidol and dihydrocarveol) and 3,5-ditert-butylphenol and this starter mixture was the most promising for northeast sauerkraut.

7.2. Kimchi

Kimchi is a product originating from Korea, made by the fermentation of vegetables, such as Chinese cabbage, radish or cucumber with multiple seasonings, such as salts, red pepper, ginger, garlic and leek [104]. There are more than 50 different types of kimchi, depending on the raw ingredients and preparation methods. Traditionally, fermented and salted kimchi was prepared to preserve fresh plants during the winter months in Korea. Although nowadays there is no problem with vegetable supplies during the winter season, kimchi is still consumed daily by Koreans and is gaining popularity in other parts of the world [105].

The major microbial group involved in the fermentation of kimchi are LAB. LAB present in the raw materials mediates the fermentation and is a key indicator of kimchi quality [106]. The flavour of kimchi is a result of the composition and content of microbiota-related metabolites, such as organic acids and free sugars, which affect the acidity and sweetness, the two most important taste attributes of kimchi. The degradation of carbohydrates, which are major components of cabbage, results in the formation of multiple organic acids, which are responsible for the unique sour taste of kimchi [107]. It was observed that the optimum pH range for the most desirable flavour of kimchi is 4.0–4.5 [108]. pH values can be affected by multiple factors, such as type of cabbage, additives, storage conditions, or the amount of initial inoculated cells [109]. Five organic acids were identified in fermented cabbage: LA, citric acid, malic acid, acetic acid, and succinic [110]. It was reported that in the early stages of fermentation, the content of citric acid, malic acid and succinic acid increased, then later decreased with a simultaneous rise in LA and acetic acid [107]. The composition of organic acids in kimchi might be affected by different changes in the

fermentation process, such as the salinity [111] or the type of starter culture [110]. Some of the major sugars detected in this kimchi were mannose, fructose, glucose, and galactose. Their concentration is reduced with fermentation time, whereas mannitol appeared in the middle stage of fermentation and is reduced slowly. Mannitol is produced through the reductive action of glucose, fructose, and sucrose and it influences the sharp umami taste of kimchi [112]. Lee et al. [110] studied the effects of combining two LABs as a starter culture on model kimchi fermentation. The authors described in detail how this affected the final composition of volatile and non-volatile metabolites. Based on the obtained results it was concluded that a combination of different LAB starter cultures might help modulate the composition of kimchi significantly. Therefore, the flavour formed mainly by metabolites can also be changed by using appropriate LAB strains.

The sensory profile of kimchi is described as heat burn, fermented, and related to the seasoning (fishy, garlic, red peppers) [113]. The terminology regarding kimchi flavour is large and reflects ingredients, processing, fundamental tastes, and trigeminal sensations. The most intense odorants in kimchi have included diallyl disulfide, diallyl trisulfide, dimethyl trisulfide, and methallyl disulphide. Less intense examples presented in the samples were 3-(methylthio)propanal, (*E,Z*)-2,6-nonadienal, phenylacetaldehyde, linalool, (*E,E*)-2,4-decadienal and 2,3-butanedione [114]. Sulfur compounds are known to be the most important volatile substances that determine the aroma of kimchi and are formed during the fermentation process of the kimchi ingredients [115].

The specific flavour of kimchi may affect consumers' liking, especially non-Koreans who are not used to its sharp, specific notes. It is challenging for food industries to launch this kind of product into foreign markets since the acceptability of the quality might differ across cultures. Jang et al. [116] analyzed the influence of the fermentation stage on sensory perception. Korean and American consumers compared kimchi samples fermented to three pH degrees: 3.9 (HFK), 4.2 (MFK), and 5.9 (LFK). Americans preferred HFK and LFK variants, whereas Koreans did not show any significant preferences between these samples.

It should be highlighted that, opposite to sauerkraut (composed of cabbage and salt), other kimchi ingredients such as red pepper, anchovy sauce, garlic, ginger and onion play a crucial role in the formation of the unique aroma of the final product. Therefore, the flavour of kimchi may differ significantly between individual kimchi types.

8. Summary and Future Perspectives

To date, the effect of LA fermentation on the formation of flavour in dairy products was fully elucidated and understood. Much less is known about the LA fermentation of plant-based food, including Brassica vegetables. As presented in this review, metabolic profiling has gained some scientific attention, and the fate of primary and secondary metabolites during fermentation can be predicted. However, more still has to be done regarding the effect of individual LABs on the formation of flavour-providing compounds. Importantly, more studies using the sensomic approach with GC-O have to be performed to understand which compounds are responsible for the unique aroma of fermented food. This, together with microbiological studies, can help to design the fermented products for individual preferences.

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