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Lactofermentation of vegetables: An ancient method of preservation matching new trends

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A R T I C L E I N F O

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A B S T R A C T

Background: Fermented foods have been an important part of the human diet for millenaries all over the world. Regarding fermented vegetables, they belong to traditionally consumed products in Asian countries, but now benefit from a renewed interest in Western countries.

Scope and approach: This review first gives a panorama of the large diversity of fermented vegetables, their history and the renewed interest they spark in the society and the scientific community. It summarises the advancement of knowledge on the spontaneous microbial community that develops during the fermentation process, which contains a large diversity of lactic acid bacteria including a few keystone species, but also other bacteria and yeasts. It details the consequences of microbial activities on the composition, safety aspects and the organoleptic, nutritional and health properties of fermented vegetables.

Key findings and conclusions: Fermented vegetables meet many societal expectations. The microbiological and biochemical changes that occur during fermentation are increasingly known. In many areas however, further investigations are necessary to better document the potential sanitary issues and the ways of controlling them, the potential health benefits of these "microbial foods", the interest of selected starters to bring specific organoleptic and/or health properties, and thus better exploit the potential of innovation in this area.

1. Introduction

Fermented foods have been consumed for millenaries and are still important components of the human diet all over the world. Fermentation was first empirically used to increase the shelf life of varied foodstuffs and to detoxify some of them, and is nowadays mainly used to diversify food flavours and to bring other benefits to consumers. A large variety of animal and plant foods can be fermented, such as milk, meat, fish, legumes, cereals, vegetables, among which dairy-based fermented products are the best described (Moss Maurice & Adams Martin, 2008; Tamang et al., 2020). During all these fermentations, the foodstuff undergoes a biochemical process driven by microorganisms, which results in the production of various metabolites. Fermented foods undergo different types of fermentations: lactic fermentation by lactic acid bacteria (LAB), which occurs in many fermented foods, alcoholic fermentation by yeasts in alcoholic drinks and sourdough, and acetic "fermentation" by acetic acid bacteria in vinegar and kombucha – even if, strictly speaking, acetic acid results from an oxidation and not a fermentation. In fermented vegetables and yoghurt, LAB are the main microorganisms and fermentation can thus also be referred to as lacto-fermentation, with lactic acid as the main metabolite produced. In some other fermented foods, LAB contribute to fermentation at some point but other microorganisms are required to perform the expected fermentation. In various cheeses for example, other bacteria, yeasts, and/or filamentous fungi are involved in the formation of flavour and aspect. Lactic fermentation is essential because the production of lactic acid, among other metabolites also produced by LAB, results in a pH decrease, thus inhibiting pathogens.

Concerning vegetables, a few flagship fermented products have been the focus of interest among the scientific community, *e.g.* sauerkraut (Buckenhuekes, 2015; Siddeeg et al., 2022), kimchi (Cha et al., 2023; Lee, Whon, et al., 2020), and juices (Garcia et al., 2020). Recent reviews

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also address the microorganisms of spontaneously fermented products and their applications (*e.g.* Wuyts et al., 2020; Mota-Gutierrez & Cocolin, 2021). In the past few years, fermented vegetables have benefited from an increase in the consumer's and media's interest in Western countries. The aim of this review is to present a panorama of fermented vegetables, their origin, the renewed interest they generate, and to highlight the main advances on our understanding of their microbiology, biochemistry and potential nutritional and health benefits. This review will neither cover olives, for which the production process is quite different and recent reviews are available (Perpetuini et al., 2020, Anagnostopoulos & Tsaltas, 2022), nor other plant products, *e.g.* cereals and legumes (Medina-Pradas et al., 2017; Oguntoyinbo et al., 2016), including plant-based dairy alternatives (Harper et al., 2022; Tangyu et al., 2019), and beverages such as water kefir (Lynch et al., 2021) and kombucha (Laureys et al., 2020).

2. Lacto-fermentation of plant-based raw materials: an ancient practice all around the world

Lacto-fermentation of plant-based raw materials has been documented in virtually all periods of history and is still used all over the world (Buckenhuskes, 2015). For example, contemporary Amazonian populations living in forest areas consume wild plants, *e.g.* leaves, roots, and bulbs, in a fermented form (Hladik et al., 1996). In this case, fermentation is used either to detoxify raw material sources, *e.g.* cassava root, and/or to improve products organoleptically. It can be assumed that the way of life of these Amazonian populations is not so far from that of the hunter-gatherers. Unfortunately, no archaeological traces of plant fermentation have been found, which could be explained by the fact that the containers used to manufacture them were not long-lasting. According to the study of Paleolithic hunter-gatherers, fermentation was part of the traditional technical processes for preserving meat, with food being deposited in shallow caches, under piles of stones or by maceration in wineskins (Soulier & Costamagno, 2018). Accessing the methods of preserving foodstuffs in these ancient times is a challenge because soft raw materials such as meat and vegetables do not leave any traces, which makes it difficult to accurately determine the first evidence for fermented foods. In this context, the oldest factual evidence of fermented foods concerns fermented milk and dates back to the Neolithic period, with the discovery of pottery used as dishes for draining fermented milk (Salque et al., 2013). For plant-based products, the first evidence of the fermentation process dates to 4000-2000 BC with abundant iconography illustrating the making of wine and bread by Egyptians and Sumerians (Ross et al., 2002) and to 2000 BC for vegetables with kimchi, a traditional Korean dish. Kimchi is the most important traditional fermented food in Korea and the *Kimjang* making and sharing culture registered in the Intangible Cultural Heritage of Humanity in 2013 (Kim et al., 2014). Historically, the tradition of making kimchi among Koreans started as a necessity of storing and preserving vegetables during the long harsh cold winters when many people died of starvation. Kimchi has been suggested to be invented 4000 years ago according to the *Sikyung* (Book of Odes) published *circa* 500 BC (Surya & Lee, 2022). It would seem, however, that the consumption of fermented vegetables can be traced back much further in history. According to Buckenhuskes (2015), Stone Age people probably ate the fermented stomach content of ruminants they hunted and also probably used animal pouches to store plant products which very likely underwent fermentation, consuming by this way a sort of sour cabbage.

Among plant-based raw materials, cabbage is the vegetable for which we found the most evidence of its consumption through the ages. As previously mentioned, in Asia the first traces of its consumption date back to 2000 BC with kimchi. Fermented cabbage was then introduced in Western countries by Genghis Khan 1000 years later in Europe after invading China (Wacher et al., 2010). Consumption of fermented cabbage has early been associated with health benefits. Thus, 400 BC, Hippocrates recommended its consumption against overweight and Romans consumed it to prevent intestinal infections (De Grijs, 2021). Four centuries later, Pliny the Elder was the first who described the production of sauerkraut by preservation of salted cabbage in earthen vessels (De Grijs, 2021). In the 18th century, Captain James Cook noticed that sauerkraut stored in wooden barrels prevented scurvy among sailors, due to vitamin C deficiency, and used it for his lengthy voyages (Buckenhueskes, 2015; De Grijs, 2021).

Through a large review of ethnographic literature and archival sources in seven Eastern European countries, Sõukand et al. (2015) documented a large bio-cultural diversity of traditional fermented plant-based foods and beverages. More than a quarter of the products listed are pickles, i.e. lacto-fermented vegetables, which have been until recently or are still currently transformed. These pickles are made from plants of 47 botanical taxa belonging to 14 families, used as main or additional ingredients. Among these taxa, *Solanum lycopersicum* (tomato), *Cucumis sativus* (cucumber), and *Brassica oleracea* (cabbage) are the most frequently cited. The lack of scientific documentation on the diversity of plant-based foods and beverages was also underlined (Sõukand et al., 2015) in the field. In its exhaustive review on all traditional fermented foods, Tamang and Kailasapathy (2010) dedicated a chapter to fermented vegetables. Many vegetables are used in the world according to the local resources, as for example cabbage with sauerkraut (literally ''sour cabbage'' in the German language) in Western Europe, kimchi in Asia, *gundrunk* in Himalayas, but also *inziangsang* made from mustard leaves in India, *pak-gard-dong* in Thailand, *jeruk* made from cucumber and *sayur asin* in Indonesia, *sink* made from radish in Himalayas, *sunki* made from leaves of red turnip in Japan (Wacher et al., 2010). In China, *paocai* is made from Chinese cabbage, cabbage, radish, mustard stems, long beans, peppers, daikon, carrots, and/or ginger with spices and seasonings such as cloves, garlic, and onion generally added to improve the flavour and is still fermented at domestic scale (Swain et al., 2014; Zhang et al., 2021).

Some fermented vegetables are now widely manufactured at artisanal and/or industrial scales and not only at domestic scale. The industrial production of sauerkraut began as soon as 1830 (Buckenhueskes, 2015). The artisanal and industrial production in Germany from 2004 to 2021 was fairly stable with an average of 80,000 tons per year (https://www.statista.com). In Korea, kimchi has for a long time been only produced on a household scale, but a growing part is now industrially produced, which accounts for 37% of the total kimchi produced in Korea (Kim et al., 2014). With the steadily increasing popularity of Korean cuisine in the world, kimchi's total export value in 2021 represented 140.8 M USD in 2021 (Statista, https://www.statista.com). The manufacture of *paocai* in China on an industrial scale has rapidly grown, mainly in the Sichuan region, and has been accompanied by a phenomenon of standardisation with the publication of an international standard specific to the production of *paocai* (ISO 24220:2020).

3. A renewed interest in vegetable fermentation among the scientific and citizen communities for a decade

3.1. Publication survey

To investigate the scientific interest in fermented vegetables, we built a query to search the Web of Science Core collection database (Clarivate Analytics), by combining i) a list of about 40 vegetables with II) terms such as fermented/fermentation/lacto-fermentation/lactic acid bacteria/lactobacillus and other LAB genera, and iii) by adding terms that design fermented vegetables such as kimchi, sauerkraut, suncai etc, while excluding some unrelated terms such as rumen/faecal/wine etc, in the 'title' field. The full request is detailed in Supplementary Table 1. The query generated about 4000 results since 1990. The number of publications in the past decade increased fivefold compared to the previous one (Fig. 1). An average of 350 scientific publications including ~12 reviews were published per year over the past 3 years. A large part (30%) of studies concern fermented cabbage (kimchi,

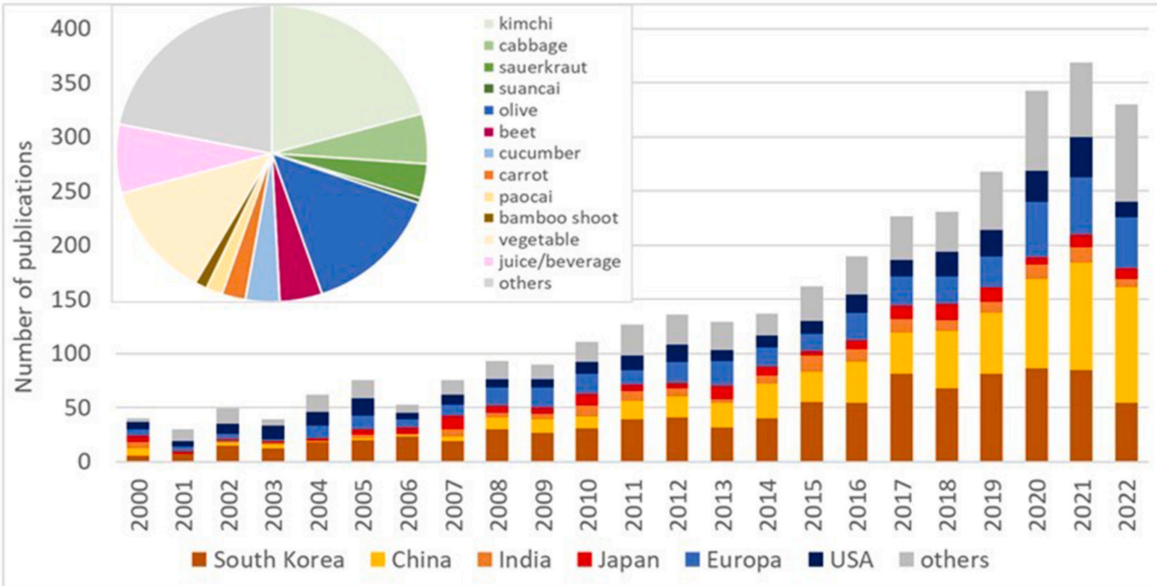

Fig. 1. Number of publications on fermented vegetables since 2000 and proportion of each vegetable in the total number of publications, according to the WoS query described in Table S1.

sauerkraut, suncai and other fermented cabbage products). The second vegetable studied is olive (14%), followed by beet, cucumber, and carrot (~3% each) (Fig. 1). Mixtures of vegetables are often used but the related studies were not specifically quantified here.

The first publishing country is South Korea (27% of the publications in the past decades, including 12 review papers), followed by People's Republic of China (23%), whose number of publications has jumped up over the past decade (15-fold increase compared to the previous decade). Two other Asian countries, Japan and India, account for more than about 4% each, illustrating the prominent place of Asian countries in research on fermented vegetables, with more than half of the publications in the field. In the same period, five European countries (Spain, Poland, Italy, France, and Germany) published 13% of papers and the USA 8% (Fig. 1).

Some geographical specificities of the vegetables studied are clearly noticeable, in relation with its importance in the culinary traditions of each country. As expected, kimchi is mainly studied in South Korea, whereas *paocai* is exclusively studied in People's Republic of China, and bamboo shoot fermentation in India and People's Republic of China (50 and 30% of publications on this vegetable, respectively). Concerning the fermented vegetables the better known in Western countries, sauerkraut is studied in the People's Republic of China, followed by the USA and Poland (31%, 22%, and 7% of sauerkraut-related publications, respectively); publications concerning olive fermentation mainly come from Spain and Italy (32% and 22% of publications, respectively), while cucumber is mainly studied in the USA, with 58% of publications on this vegetable.

3.2. Web-based analysis of trends in vegetable fermentation

Household and artisanal fermentation has become one of the hottest trends in culinary circles worldwide (Katz, 2012; Redzepi & Zilber, 2018). To investigate which type of knowledge a citizen can find on the web when he seeks information on fermentation and fermented vegetables, a non-exhaustive search was performed on the web using google search tool, by using simple words like 'fermented food', 'fermented vegetables', 'fermentation', in three languages, English, Spanish, and French, in all geographic areas.

All types of internet media echo vegetable fermentation: blogs, websites, videos (YouTube, Vimeo …), podcasts, and of course social media (FaceBook, Twitter, Pinterest, Tumbler). They offer a large variety of contents, with some sites being very specialised, whereas others deal with many fermentation-related topics. We suggest a site typology with the four main items found: preparation and accommodation', 'activists or engaged', 'commercial', and 'events', the most numerous being sites focusing on recipes and cooking.

Specific traits of 'activists or engaged' web sites are that they cover a wide part of the fermentation-related themes, not only cooking but also history of fermentation, origin of fermented foods, some scientific or more often pseudo-scientific explanations about fermentation and health allegations. They often appear to be resource sites and some are used as reference sites for a large part of the other web contents.

3.3. Hypotheses about such an increase of interest in Western countries for plant-based fermented foods

Fermented foods have many intrinsic qualities that make them attractive: minimal requirement of technical training or knowledge, low cost, diversified flavours, textures and appearance, they are of cultural and social importance and often recognized as nutritional and healthy. In particular, plant-based fermented foods have become part of the new food trends in the last 10–15 years, and the growing interest in such products may be explained by the conjunction of several societal trends, some of which are mentioned in recent reviews (Lavefve et al., 2019; Medina-Pradas et al., 2017).

(1) Consumers' demand for more natural foods: Consumers have been looking for simplicity and naturalness on their plates for several years now (Garin, 2022). Many scandals have indeed affected the citizens' confidence in industrial foods. In this respect, fermented foods, because of their simplicity of realisation, absence of additives (clean label), and age-old tradition are reassuring (Medina-Pradas et al., 2017). In addition, they benefit from a very positive image in social networks with the claim of multiple nutritional and health benefits; some of which are proven but others remain quite hypothetical (see chapter 5 below). A recent consumers survey show that most consumers remain interested in foods that promote health and wellness, and 50% of consumers prioritize healthy eating (Wilson, 2022).

(2) Consumers' demand for more food autonomy through different strategies: i) the appropriation of ancestral know-how - "do it yourself", for example the bread making at home during the Covid-related shut-downs was very evocative of this tendency-; ii) the capacity to store vegetables in simpler forms than canned, dried, or frozen food; iii) the growing development of amateur and shared gardens; and/or iv) a better knowledge of wild edible plants, with 20 new books published on the subject for example in France during the past five years, *i.e.* as many as the number published from 2000 to 2018 (Lortal, personal communication).
(3) Social demand for a soberer and more sustainable lifestyle, in the context of climate change and explosion of energy costs. Consumers overwhelmingly about buying environmentally and ethically sustainable products (Am et al., 2023) and have shown an increase of conscious eating, even if their interest in sustainability is lower than in health, according to a recent Mc Kinsey survey of some 8000 consumers in the United States, the United Kingdom, France, and Germany (Grimmelt et al., 2022). The total absence of energy needed to ferment, especially vegetables in jars, is attractive, as well as the possibility to store fermented vegetables for several months without refrigeration. Thus, being part of the local supply chain and spreading the consumption of vegetables over the winter months without consuming energy nor generating wastes fully meets the growing citizen expectations of sustainability (Lavefve et al., 2019).
(4) The growing proportion of vegetarian or vegan diets: currently the consumption of meat continues to decrease in France as it does throughout Europe. Only 2.2% of French people are vegetarians today, but 12% of young people aged 18 to 23 follow a vegetarian/vegan diet, mainly for ethical and environmental reasons. In January 2022, on a world scale, more than 629,000 people participated in Veganuary, a global campaign led by a British non-profit organisation with the same name, which aims to get people to only eat vegan food during the month of January. The number of participants tripled between 2018 and 2022 (Statista, https://www.statista.com). In 2019, the share of people declaring themselves vegetarian or vegan in the total population of Spain, France, Germany, and the United Kingdom was 2.8%, 5.2%, 5.6%, and 8%, respectively (Interbev, 2019). Nearly 40% of French people want to consume more vegan products, according to a recent study (BPI France, 2023). Wishing to diversify their diet and motivated by having a lower environmental impact, 22% of French people, in a 2022 study, said they had changed their eating habits in the past year and consumed more plant proteins (Onav, 2023).

Accordingly, fermented plant-based foods benefit from this interest. The potential for innovation and the market is such that many SMEs and even existing food industry majors have launched into this sector and are multiplying their product offers. The global 'sauerkrauts market', i.e. fermented cabbage, cucumber and kimchi, was USD 8.7 billion in 2022 and predicted to reach USD 14.1 billion by 2029, with an annual growth rate of 6%, according to Adroit Market Research (2022).

At the same time, the research effort on fermented vegetables dramatically increased even if much lower than that devoted to dairy products. One of the most promising is the European COST network PIMENTO, the objective of which is to federate the scientific community and other key stakeholders working on fermented food (https://fermentedfoods.eu/). Noted also French project "Ferments of the Future" Grand Challenge and financed to the tune of €48.3 million with the aims to accelerate research and innovation in the field of ferments and fermented foods including vegetal fermentations (INRAE, 2022).

There is a consensus on the huge innovation potential of fermented vegetables and, more generally, plants, which is of interest for citizens, SMEs, large enterprises, and even restaurants, such as the famous Noma restaurant in Copenhagen, which answer consumers' demand for foods with original and creative organoleptic properties. However, the specific organoleptic properties of fermented vegetables can also be a barrier to their consumption in Western countries that do not possess a culinary tradition in the field. According to a recent quantitative study performed on 1093 French persons over 20 years of age representative of the French vegetable-eating population, interviewed on their consumption of fermented vegetables, the main factors that discourage them from eating fermented vegetables were, in descending order of citation, their taste, salt content, acidity, and odour (Ronan Symoneaux, unpublished results).

4. A spontaneous fermentation due to endogenous LAB communities

4.1. A large variety of vegetables can be fermented

The better-known fermented vegetables are, in Asia, the famous cabbage-based Korean *kimchi* and Chinese *paocai*, and the Chinese *suancai* made from different vegetables. In Western countries, sauerkraut, cucumbers, and olives are by far the most common fermented vegetables, while a wide variety of fermented vegetables are commonly consumed in Eastern countries (Tamang et al., 2020). Their microbial communities have been explored for decades. Their manufacture mainly results from spontaneous fermentation, even if the use of lactic starters has been recommended in the production of cucumbers, sauerkraut, and olives (Medina-Pradas et al., 2017; Peñas et al., 2017), and generally results in an acceleration of the fermentation process in different vegetables (Montet et al., 2014).

Besides these well-known products, many traditional products have been listed all over the world, which shows that many, if not all, vegetables can be fermented (Gänzle, 2022; Tamang et al., 2016). They cover all the vegetable organs: roots (beetroot, carrot, horseradish, parsnip, radish, sweet potato, turnip, …), bulbs (onion, garlic, fennel), fruit (eggplant, zucchini, tomato, French beans …), leaf/stems (bamboo shoots and a variety of leafy vegetables), flower (cauliflower), depending on the locally available resources (Irakoze et al., 2021; Montet et al., 2014). For example, in a recent citizen science study in which 75 French homemade fermented vegetables were collected, 23 different vegetables were used alone or in mixture, the most frequently used being cabbage and carrot (Thierry et al., submitted).

4.2. Culture-dependent and independent approaches provide complementary information on microbial communities

The microbial communities of fermented foods were historically characterised using culture-dependent approaches only, generally focused on the microorganisms considered as the main involved in fermentation, *i.e.* LAB. Since, an increasing number of studies based on culture-independent approaches have been published, thus completing the view of the microbiota of fermented foods (Chen et al., 2017; De Filippis et al., 2018). Both culture-dependent and -independent approaches have their interests, limits, and biases and thus bring complementary knowledge (Boers et al., 2019; de Filippis et al., 2018). For example, in an elegant study that characterised the dynamic changes during carrot juice fermentation, Wuyts et al. (2018) showed by combining both approaches how *Enterobacteriaceae* were outcompeted by LAB during the first 13 days of fermentation. Culture-independent approaches are required in particular to get an idea of the whole microbiota in fermented products when a kinetic monitoring is not feasible.

4.3. Lactic acid bacteria dominate the microbial community of spontaneously fermented vegetables

In a wide panorama of the microorganisms isolated for different fermented foods all over the world, more than 30 traditional fermented vegetables were listed (Tamang et al., 2016). LAB form the dominant population of all these vegetables, and 37 species were isolated, of which 20 species of the former *Lactobacillus* genus (Fig. 2). The most frequently isolated LAB species were *Lactiplantibacillus plantarum*, *Levilactobacillus brevis, Leuconostoc mesenteroides,* and *Pediococcus pentosaceus,* as well as, to a lower extent, *Limosilactobacillus fermentum*, *Lactococcus lactis* and other pediococci and leuconostocs species (Fig. 2). In agreement, the four main species listed in another list of 18 traditional fermented vegetables were *L. plantarum* and *L. brevis,* followed by *P. pentosaceus* and *Leuc. mesenteroides* (Montet et al., 2014). The prevalence of *L. plantarum* is likely due to the nomadic lifestyle and large metabolic capacity of this species (Duar et al., 2017). The key molecular features responsible for LAB adaptation to the niche of plant-based fermented foods were recently detailed (Gustaw et al., 2021).

Besides LAB, other microorganisms can grow and survive in fermented vegetables, e.g. yeasts, other bacteria, and bacteriophages. The presence of yeasts has been reported for example in kimchi and other vegetables (Tamang et al., 2016), Chinese sauerkraut (Liu et al., 2021), Chinese *paocai* (Wang et al., 2022), and diverse home-made French and Moldavian fermented vegetables (Thierry et al., submitted). The main genera reported are *Candida, Saccharomyces*, *Pichia, Kazachstania, Issatchenkia*, *Torulopsis* and *Zygosaccharomyces.* (Liu et al., 2021). Some yeasts are considered as potential spoilage agents (Ballester et al., 2022), but their role in the fermentation of vegetables has to be further investigated. In Chinese paocai, for example, the yeasts *Debaryomyces hansenii* and *Kazachstania exigua* were shown to produce esters and alcohols and were considered as contributors to the formation of *paocai* key odorants (Wang et al., 2022), while in *shalgam*, a fermented black carrot juice, *Pichia kudriavzevii* was shown as the most prevalent species and some actively growing strains selected as autochthonous starters (Kahve et al., 2022). Yeasts have also been proposed as starter cultures to reduce too high concentrations of organic acids (Lee et al., 2015). Regarding non-LAB bacteria, the isolation of several *Bacillus* species (*B. subtilis*, *B. licheniformis*, *B. coagulans*, *B. cereus*, *B. pumilus, B.firmus, B.circulans, B. sphaericus*) was also reported in *soibum* and *tuaithur*, two traditional Indian products made from fermented bamboo shoots (Tamang et al., 2016). Many other microbial species can be isolated from vegetables at the beginning of fermentation, in particular *Enterobacteriaceae* isolates, but LAB are normally the main microbial group in fermented, acidified fermented vegetables. Bacteriophage DNA has also been found in kimchi, of which they could affect the quality (Jung et al., 2014). Interestingly, viral communities better differentiated Korean and Chinese kimchi than bacterial communities (10 samples of each origin) (Jung et al., 2018).

Table 1 gives a panorama of the main bacterial taxa identified using 16S metabarcoding analyses in 44 fermented vegetables, according to their abundance and to the type of vegetables. Among LAB, the most abundant taxa are members of the former *Lactobacillus* genus*,* which has been detected in all the vegetables studied. The main other LAB genera widely detected are *Weissella* (1%–50% of total bacterial taxa), followed by *Leuconostoc, Pediococcus*, and *Lactococcus*, generally represented at a lower abundance (1%–10% of total) (Table 1). Some non-LAB Gram-positive bacteria were also detected in about 10% of samples: staphylococci, bacilli, and clostridia. Various environmental Gram-negative bacteria were identified, including *Pseudomonas,* a group of ubiquitous bacteria, detected at abundance generally <10% in one third of samples, different genera of *Enterobacteriaceae (Serratia, Enterobacter, Erwinia, Pantoea, Citrobacter, Raoultella and Pectobacterium),* and *Halomonas,* a group of halophilic bacteria likely brought by the salt added to vegetables. Other genera were detected at a low abundance <10% in about 10% of samples (Table 1). The occurrence of taxa was similar for the tree groups of vegetables. The abundance of non-LAB taxa, however, tended to be more frequent in vegetables other than cabbage and cabbage mixtures (Table 1). For example, in fermented carrot, pepper, and radish, *Enterobacteriaceae* taxa accounted for about 80%, 60% and 50% of total taxa after 4 days fermented vegetables (Raghuvanshi et al., 2019).

As illustrated by Fig. 2 and Table 1, both culture-dependent and -independent approaches highlight the pivotal role of (formerly) *Lactobacillus* members in fermented vegetables.

4.4. Lacto-fermentation relies on a succession of LAB species: the example of sauerkraut

LAB are present only at low abundance (<0.1%) on the surface of vegetables, since the plant environment is not suitable for their growth (Montet et al., 2014; Yu et al., 2020). For example, LAB made up less than 0.9% of the bacterial community of the Napa cabbage phyllosphere

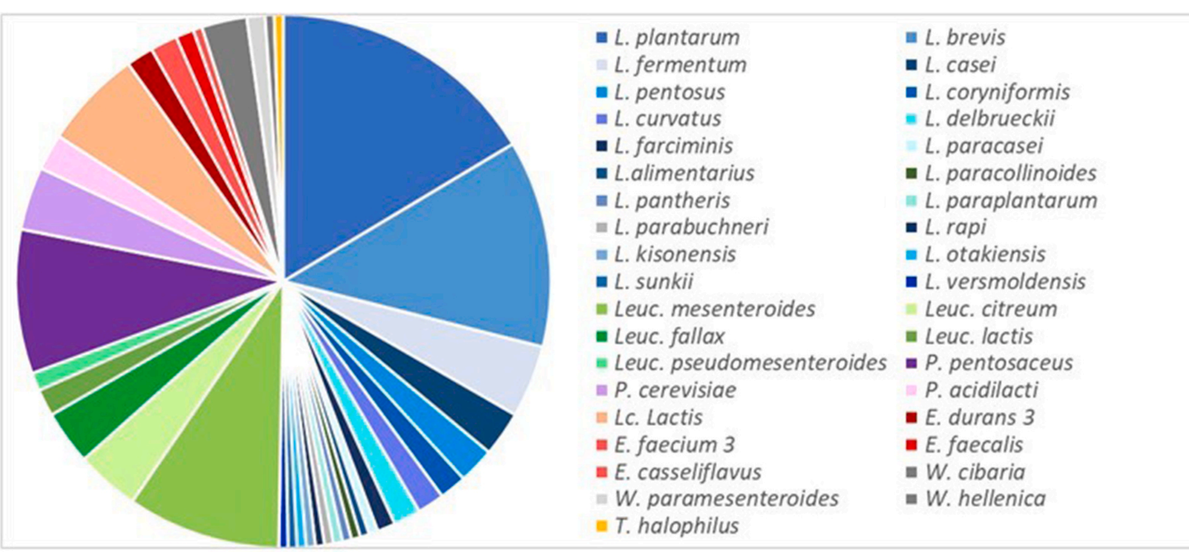

Fig. 2. Main species of lactic acid bacteria isolated from fermented vegetables (figure designed from the occurrence of LAB species cited in Table 3 in Tamang et al., 2016). LAB genera: L.: *Lactobacillus* (in different shades of blue); Leuc. *Leuconostoc* (in green)*;* P.: *Pediococcus* (in purple); Lc.: *Lactococcus* (in orange); E.: *Enterococcus* (in red); W.: *Weisselia* (in grey); T.: *Tetragenococcus* (in yellow). *'Lactobacillus'* is used here to refer to the former large *Lactobacillus* group before the recent taxonomic changes that split this genus (Zheng et al., 2020). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Main bacterial genera identified in fermented vegetables through 16S-amplicon based metagenomic analyses published over the past 10 years.
[1] formerly *Firmicutes*; [2] formerly *Proteobacteria;* [3] formerly *Epsilonproteobacteria.* The *Lactobacillus* term here refers to all the species that have been recently reclassified in other genera (Zheng et al, 2020).
A total of 44 products were analysed in 25 selected publications, the number corresponding to each type of vegetables is indicated in brackets. Only the taxa found in at least three different products are listed in this table.

		Number of fermented vegetables in which each genus was identified						
		According to the relative abundance of taxa in the product			According to the type of vegetable			Total number
Phylum	Genus	> 50%	10%-50%	1%-10%	Cabbage, alone (n=17)	Cabbage, mixed (n=10)	Other vegetables (n=17)	(n=44)
Bacillota [1]	*Lactobacillus* [3]	23	16	2	17	10	14	41
	Weissella	4	8	8	8	4	8	20
	Leuconostoc	1	3	12	10	2	4	16
	Pediococcus	0	4		6	4	5	15
	Lactococcus	0	5	11	10	2	4	16
	Streptococcus	0	0	3	1	1	1	3
	Enterococcus	0	1	3	0	0	4	4
	Bacillus	0	2	2	0	0	4	4
	Clostridium	0	4	0	3	0	1	4
	Staphylococcus	0	1	3	1	0	3	4
Pseudomonadota [2]	*Pseudomonas*	1	2	13	4	3	9	16
	Halomonas	0	1	7	2	1	5	8
	Chromohalobacter	1	1	1	1	0	2	3
	Serratia	0	2	7	7	2	0	9
	Enterobacter	0	2	6	7	1	0	8
	Citrobacter	0	1	2	1	1	1	3
	Raoultella	0	0	3	3	0	0	3
	Pectobacterium	0	1	2	2	0	1	3
	Erwinia	0	0	4	4	0	0	4
	Pantoea	0	2	1	1	1	1	3
	Vibrio	0	2	4	2	1	3	6
	Salinivibrio	0	1	3	0	0	3	3
	Acinetobacter	0	0	3	2	0	1	3
	Comamonas	0	0	4	1	0	3	4
Campylobacterota [3]	*Arcobacter*	0	0	5	1	1	3	5

The 25 selected papers used to build Table 1 are: Cao et al (2017), Du et al. (2018), Guan et al. (2020), He et al. (2020), Huang et al. (2020), Jung et al. (2011), Jung et al (2022), Liang et al (2016), Liang et al (2018), Liu & Tong (2017), Liu et al. (2019), Liu et al (2021), López-García et al (2021), Medina et al. (2016), Müller et al (2018), Paramithiotis et al (2014), Tomita et al. (2020), Wang et al. (2022), Weldemichael (2019), Wuyts et al (2018), Yang et al (2020), Yu et al (2022), Zabat et al (2018), Zhao et al (2018), Zhao et al (2022).

(Miller et al., 2019). The epiphytic microbial population of vegetables depends on many factors, e.g. the type of vegetable, the harvesting season, the geographical origin, their shelf life, but essentially consists of aerobes, as pseudomonads, enterobacteria, and coryneforms (Di Cagno et al., 2013). According to Yu et al. (2020), the selective conditions (e.g. high salt, moderate temperature, and low oxygen availability) applied in fermentations are likely more influential factors of LAB growth, compared to the initial numbers and diversity of LAB on plant tissues. LAB grow during fermentation, by converting vegetable carbohydrates into organic acids and other metabolites. The drop in pH caused by acid production inhibits the growth of pathogenic and spoilage microorganisms. It favours then the growth of the most acid-tolerant LAB species, inducing a succession of different LAB species during fermentation and further storage.

Major microbial changes occur throughout the fermentation process of vegetables. Environmental aerobic or facultatively anaerobic microorganisms first grow and are progressively replaced by a succession of heterofermentative and then homofermentative LAB, as illustrated in Fig. 3. The prevalence of LAB are the main microorganisms alive in all fermented vegetables, with *Leuc. mesenteroides, L. plantarum* and *L. brevis* as keystone species, as shown by both culture-dependent and -independent approaches. These conclusions seem to be extendable to most fermented vegetables, *e.g.* bamboo shoots (Behera & Balaji, 2021), and radish (Pardali et al., 2017). These three bacteria are categorised into generalist LAB (Duar et al., 2017), which are the most frequently LAB found in plants and dominate plant fermentations (Yu et al., 2022).

Schematically, lactic fermentation occurs in sauerkraut in two stages (Pederson & Albury, 1969; Wacher et al., 2010). Heterofermentative LAB, typically *Leuc. mesenteroides* and/or *Weissella* initiate fermentation. They are followed by homofermentative LAB, mainly *L. plantarum*, as described in sauerkraut as early as in the 1930's (Pederson & Albury, 1969). Similar LAB dominant species and succession were observed on 38 domestic fermented carrot juices (Wuyts et al., 2018).

In greater detail, four successive stages have been described in kimchi (Patra et al., 2016) and sauerkraut (Buckenhueskes, 2015) fermentation. The following lines detail sauerkraut fermentation. During the first three days, fermentation is initiated. It is made possible by the anaerobic atmosphere created by the compaction of the vegetable that eliminates air pockets, and by the consumption of the remaining oxygen by plant cells and aerobic or facultative anaerobic microorganisms. The pH begins to drop as a result of the fermentation of sugars into organic acids. The 2nd stage is characterised by the development of facultative anaerobic LAB, due to the drop in pH and the anaerobiosis. The main LAB at this stage are *Leuconostoc* spp., mainly *Leuc. mesenteroides*, a fast-growing species under the initial conditions, i.e. microaerophilic and low acid concentration (Moss Maurice & Adams Martin, 2008). They produce lactic and acetic acids, thus leading to a further drop in pH, and CO_2, which replaces the residual air and further

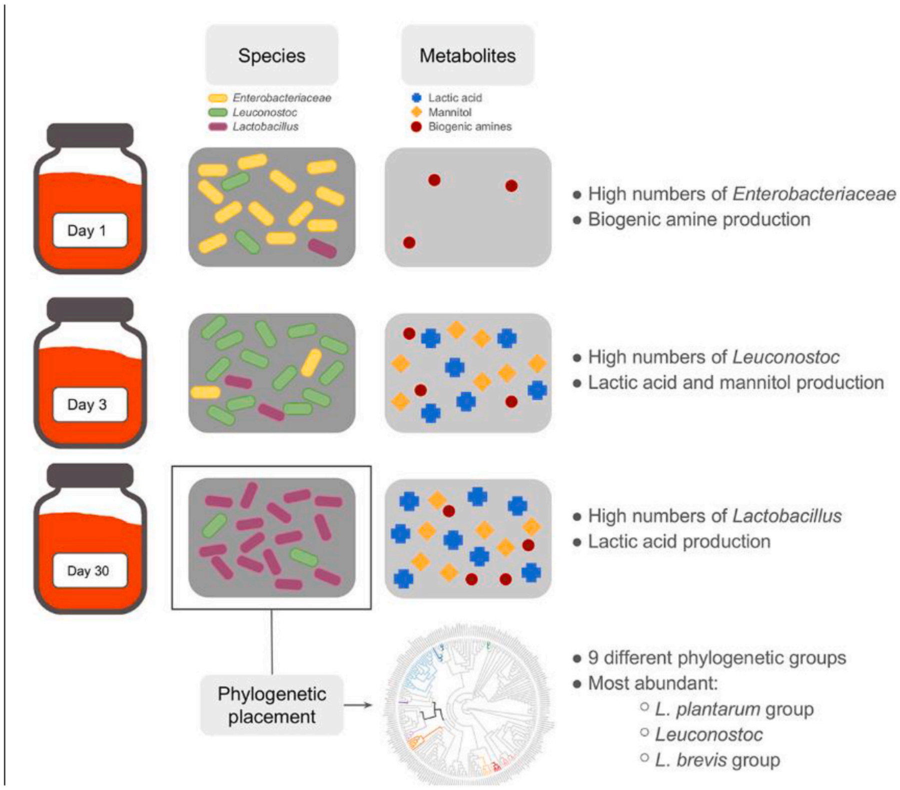

Fig. 3. Graphical summary illustrating the bacterial community dynamics and metabolite production during vegetable lacto-fermentation. Example of spontaneous fermented carrot juices. Figure reproduced from Wuyts et al., 2018, with author and editor authorizations.

increases anaerobic conditions. This prevents vitamin C oxidation and colour loss (Moss Maurice & Adams Martin, 2008). Early predominance of heterofermentative LAB is considered to be essential in the production of good quality sauerkraut (Pederson & Albury, 1969). *Leuc. mesenteroides* is among the less acid- and salt-tolerant LAB and other heterofermentative LAB with a greater tolerance to acidic pH, e.g. heterofermentative LAB then start to grow (Moss Maurice & Adams Martin, 2008). During the 3rd stage, more acid-tolerant homofermentative LAB grow and become dominant, such as *L. plantarum*. At this stage, LAB populations can reach up to 10^9 CFU/g. The pH continues to decrease, reaching around 4.1 to 3.8 due to a lactic acid concentration around 2%. The sauerkraut can be pasteurised at this stage, although it is not systematic. In the case of unpasteurized sauerkraut, a 4th stage is reached, during which fermentation continues to proceed, with the growth of heterofermentative LAB capable of fermenting pentoses, such as *L. brevis*. Lactic acid concentration reaches then around 2.5%, resulting in a further pH decrease up to 3.4. In some vegetables, a pH increase can be observed, as in bamboo shoots where pH increased from about 4 at 7 days to 4.5 at 3 months (Chen et al., 2022).

4.5. Safety concerns

Biological hazards include the presence of pathogens and undesirable metabolites that result from microbial metabolism during fermentation, such biogenic amines and nitrites.

Pathogen growth is considered as inhibited at the low pH encountered in fermented vegetables and the antagonistic effects of LAB. However, several recent outbreaks linked to kimchi consumption contaminated by *Listeria, Escherichia coli* or norovirus have shown that such generalisations are not necessarily accurate, for reasons referring to both the raw materials and the pathogen adaptability (Kim et al., 2021; Lee, Whon, et al., 2020; Patra et al., 2016). Contrasted results were observed in studies that investigated either the survival of artificially contaminated fermented vegetables, or analysed pathogens in household or commercial products. For example, a low probability of foodborne illness due to pathogenic *E. coli* or *Clostridium perfringens* in kimchi was found by using quantitative microbial risk assessment (Choi et al., 2020; Nam et al., 2021). Pathogenic *E. coli* were detected in only one sample of the 877 kimchi samples analysed. Similarly, no pathogens (*E. coli*, coagulase-positive staphylococci, *Salmonella*, and *Listeria monocytogenes*) were detected in the 75 homemade French fermented vegetables characterised in a recent study (Thierry et al., submitted). In contrast, *L. monocytogenes* inoculated in kimchi was shown to survive (Lee, Whon, et al., 2020), as did *L. monocytogenes* and *Salmonella* Typhimurium inoculated in a traditional Greece fermented cauliflower (Paramithiotis et al., 2012). Several factors may be involved in these apparently conflicting observations, such as the acidification rate. It is commonly recommended that the final pH of the product be around 4.0. For example, the specifications for the protected geographical indication (PGI) "Sauerkraut of Alsace" (Ministère de l'agriculture et de l'alimentation, 2018) indicate target values < 4 and 1% for pH and titratable acidity, respectively, so as to fulfil both safety and sensory (not too high acidity) property requirements. Ideally, the pH should drop below 4 within 3–4 days. The Codex Alimentarius standard for pickled fruits and vegetables stipulates that the product has to be prepared and packed "to ensure an equilibrium pH of less than 4.6" (FAO, 2007). It is important to underline here that not only the pH but also the titratable acidity is important. For example, in an interesting study in which the fermentation of whole heads and shredded cabbage were compared, inoculated *E. coli* O157:H7 and *L. monocytogenes* declined faster for shredded cabbage, in which the total titratable acidity was twice as high as in the whole head cabbage (Niksic et al., 2005).

As for biogenic amines, the consumption of food containing high concentrations of these amines can have toxicological consequences. Amines can be produced by many *Enterobacteriaceae* and some LAB species (Halász et al., 1999). Their formation results from amino acid decarboxylation, e.g. histamine, tyramine, putrescine, and cadaverine derive from histidine, tyrosine, ornithine and lysine decarboxylation, respectively. The microbial activity is highly strain-specific (Barbieri et al., 2019). In kimchi, biogenic amines can also come from some specific ingredients commonly used, such as *jeotgal* or *aekjeot*, Korean fermented seafoods (Park et al., 2019). The subject generated controversial debates, as underlined by Patra et al. (2016). Globally, however, high biogenic amine contents are generally associated with poor hygienic quality of the products (EFSA Panel on Biological Hazards (BIOHAZ), 2011). Histamine and tyramine are the most frequently responsible for food incidents among the eight biogenic amines found in foods (Gao et al., 2023). The regulation regarding the content of biogenic amines in foods is not unified and depends on the country and the type of food (Gao et al., 2023).

Nitrite is formed, accumulated, and partially degraded during the fermentation process of many fermented vegetables. Its formation has been mainly studied in Asian products (Liu et al., 2017; Song et al., 2021), in which the nitrite average concentration can be high, e. g. an average concentration about 30 mg/kg but up to 88 mg/kg in Chinese northeast sauerkraut (Liu et al., 2017). Nitrite mainly results from microbial reduction of nitrate to nitrite by environmental bacteria such as *Pseudomonas* and *Erwinia*.

Even if generally considered as safe, fermented vegetables may present health risks, especially those manufactured under poor hygienic conditions, thus suggesting the need for further investigations and a range of measures, i.e. good manufacturing practises and use of high-quality raw materials, as detailed by Tamang et al. (2020).

4.6. Main factors influencing microbial community dynamics

Many biotic and abiotic factors can influence the establishment of the fermenting microbial community that progressively replaces the vegetable community. Microorganisms are brought by the raw materials used, but also by the equipment and the environment (Mudoor Sooresh et al., 2023). The microorganisms present on vegetable surfaces vary according to many factors, as mentioned above.

The dynamics of activity of the first growing LAB results in a progressive decrease of oxygen levels and of pH, which further influence the succession of LAB species, as detailed above. It is important to notice that the pH value depends both on the buffer content of the medium and on the relative proportion of acetic and lactic acids, thus explaining why the final pH is not directly proportional to the total titratable acidity (Pederson & Bagg, 1944).

Technological factors (NaCl concentration, incubation temperature, oxygen availability, cutting type, …) are essential since they determine the start up speed of the fermentation process and shape the microbial community.

Salt is added as dry salt or as brine, depending on the capacity of vegetables to release juice. In both cases, vegetables have to be tightly packed and totally covered with juice or brine, so as to prevent any air pocket and favour anaerobiosis conditions. The main role of salt is thus to withdraw water and nutrients from vegetable tissue, which also provides microorganisms with the substrates (carbohydrates and other nutrients) they need for growth. The final concentration of NaCl recommended varies according to the fermented vegetables, but generally ranges from 1% to 3% of the final product. The recommended concentration for industrial sauerkraut is 2%–2.2% (Pederson & Albury, 1969). Many LAB species are able to grow in this concentration range, even if salt differently influences them. The pioneer studies of Perderson et al. on sauerkraut demonstrated that salt concentration, fixed at 1%, 3.25% or 3.5%, determined the rate of acidification and the growth dynamics within the four main LAB species (Pederson & Albury, 1969). Notably, *Leuc. mesenteroides* is less salt-tolerant than other, homofermentative, LAB species and consequently, sauerkraut contains less acetic acid at salt concentrations higher than 2%. Yeast growth was also noted more prevalent at high salt concentrations. It can be mentioned also that some traditional fermented vegetables in China are manufactured with a very high NaCl content, e.g. 6–7% in radish and up to 17% in potherb mustard and tuber mustard (Zhao et al., 2022).

Temperature has also a key role in shaping the bacterial community and its dynamics. Fermented vegetables are generally incubated at ambient temperature for domestic and artisanal productions and at 15–18 °C for industrial production of sauerkraut (Pederson & Albury, 1969). For example, the bacterial community of *paocai*, a Chinese traditional fermented cabbage, was explored at 10, 15, 25 and 35 °C. Lc. lactis initiated lactic fermentation at the lowest temperatures, whereas Enterobacteriaceae first dominated at higher temperatures. L. plantarum dominated at the end in all conditions but its impact was observed sooner at higher temperatures (Wang et al., 2020). The acidification rate was markedly slowed down at low temperatures, with a pH value of 4.0 reached at 1, 2, 12 or more than 20 days according to the decreasing temperatures.

The presence of oxygen favours the growth of undesirable aerobic microbiota, potentially responsible for spoilage. Therefore, vegetables should be compacted to avoid any air pocket. Some other strategies can be combined, such as N_2 purging in commercial production of fermented cucumber to prevent bloated defect (Zhai et al., 2018) or covering the brine surface by olive oil, according to a traditional Greek recipe to prepare fermented radish (Pardali et al., 2017).

The use of starters has been proposed to improve and standardise the quality of fermented vegetables and promote functional properties (Lee et al., 2015). However, commercial starters are far less used than for the production of other fermented foods, such as dairy products. Potential starters have to be selected based on many criteria (Gaspar & Crespo, 2016; Peñas et al., 2017). Their capacity to ferment specific raw materials and outcompete the autochthonous microbial community is essential, and autochthonous starters have been recommended for this purpose (Di Cagno et al., 2013). Many additional criteria can be considered, such as starter antimicrobial activity against pathogens such as pathogenic *E. coli*, *Salmonella*, or more globally *Enterobacteriaceae* (Choi et al., 2021; Li et al., 2022). Starters have also been tested for other purposes, for example to reduce biogenic amine formation in sauerkraut (Halász et al., 1999) and in fermented beets (Choińska et al., 2022), improve sensory properties (Di Cagno et al., 2008), reduce bloating defect in fermented cucumbers (Zhai et al., 2018), or promote health properties (Lee et al., 2015). However, high inoculation levels are generally required to induce the expected benefits, thus increasing the cost, and creating a dependence of citizens and SMEs to starter providers. The use of efficient starters may also lead to a standardisation of the products and a decrease of the microbial diversity.

4.7. Main biochemical transformations during vegetable fermentation

Vegetables typically contain about 90% water, 0.3–6.8% carbohydrates, 0.5–4.3% proteins, 1–4% fibres, 0.5% minerals, the main being potassium, and some other components, e.g. organic acids, polyols, and lipid traces, depending on the vegetable (Anses, 2020). The main carbohydrates are glucose and fructose, except in a few vegetables rich in sucrose, e.g. beetroot, carrot, and celeriac (Fig. 4).

Nutrients are progressively released in the brine by lixiviation, at a rate that depends on the salting step and on the type of cutting: entire vegetables, roughly, or finely chopped vegetables. The main biochemical transformations during vegetable fermentation are the conversion of carbohydrates into organic acids (lactic and acetic acids), mannitol, and carbon dioxide. A decrease of pH until 3.4–3.8 is observed simultaneously to carbohydrate consumption and metabolite production. Homofermentative LAB convert carbohydrates into lactic acid as the main product, while heterofermentative LAB produce several end-products, summarised in Table 2. The reduction of fructose by heterofermentative LAB leads to the accumulation of mannitol (Martínez-Miranda et al., 2022). Mannitol can also result from the conversion of glucose or fructose by yeast such as *Candida* strains.

Plant proteins can be hydrolysed by plant and/or microbial proteases during fermentation, thus releasing peptides and amino acids. LAB have

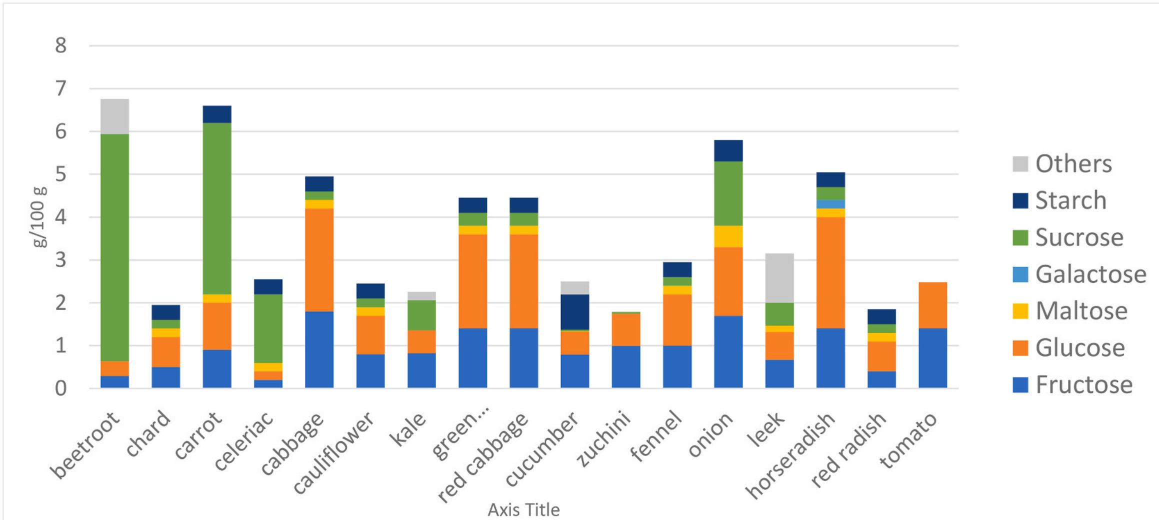

Fig. 4. Carbohydrate content of some raw vegetables commonly used in the manufacture of fermented vegetables. This figure was prepared using raw data from Ciqual (Anses, 2020) and https://www.aprifel.com/en/nutritional-sheet/beetroot/.

Table 2
Main fermentation products and examples of lactic acid bacteria species involved in carbohydrate fermentation.

Fermentation type	Examples of LAB species	Main fermentation products	Main pathways/ enzymes involved
heterofermentative	*Leuconoctoc mesenteroides* *Levilactobacillus brevis* *Limosilactobacillus fermentum*	lactic acid mannitol acetic acid ethanol carbon dioxide	phosphoketolase pathway lactate dehydrogenase mannitol dehydrogenase
homofermentative	*Lactiplantibacillus plantarum* *Lactococcus lactis* *Pediococcus pentosaceus*	**lactic acid** (mannitol) (acetic acid) (ethanol) (carbon dioxide)	glycolysis lactate dehydrogenase mannitol 1-phosphate dehydrogenase

a complex proteolytic system, which is essential to meet their nitrogenous requirements (Juillard et al., 2022). Some of the amino acids released are further converted into other amino acids or other compounds. These phenomena have been extensively documented in dairy fermented foods, but the information is scarce for fermented vegetables. For example, in cucumbers, the total amount of free amino acids decreased from 2.4 mg/kg to 0.6 and 1.3 mg/kg in acidified and fermented products, respectively (Moore et al., 2022). Amino acids can be used as nutrients or be converted, e.g. glutamic acid is converted to γ-aminobutyric acid (GABA), and arginine is converted into ornithine (Moore et al., 2022). During fermentation of a mixture of cauliflower and white beans by *L. plantarum,* a slight (3–4%) increase of the total amount of some amino acids essential in the human diet (histidine, leucine, isoleucine, valine) was observed (Thompson et al., 2020). In fermented chayote, a drastic decrease of protein content and increase of free amino acid content occurred during the first week of fermentation Shang et al., 2022). Such changes may have implications for both sensory and health properties of the final product, as detailed below.

Biochemical changes impact the sensory properties of fermented vegetables. The main flavour changes induced by lactic fermentation is an increased sourness, caused by the organic acids produced, likely associated with a decreased sweetness due to sugar consumption. Besides the acidic taste, salty, sweet, and savoury tastes and sour, spicy, and green or mouldy odours have been described for kimchi (Cheigh et al., 1994). The flavour of sauerkraut was early described as depending on its content in acids, alcohol and esters (Pederson & Albury, 1969). The microbial flavour compounds include some end-products of primary metabolism, *e.g.* organic acids produced from carbohydrate fermentation, and many secondary metabolites. Regarding the latter, their pathways of formation are only partly described and the physiological role for microorganisms often unknown (Wieczorek & Drabińska, 2022).

Plant components can be converted by LAB during fermentation, generating varied flavour compounds, as schematised on Fig. 5. Lactic acid is the main compound produced by homofermentative LAB (Fig. 5). It contributes to the acid taste but not directly to the aroma of fermented foods. Pyruvate, an intermediate compound in carbohydrate catabolism, can be converted into many flavour compounds. Several flavour compounds are produced by heterofermentative LAB, which also can be precursors of other flavour compounds. Acetic acid, which results from pyruvate oxidation, is responsible for a desirable, vinegar-like flavour, and increases the sourness of the sauerkraut. There is also an increase in free fatty acids in sauerkraut, compared to their content in cabbage, while other lipid classes decrease (Pederson & Albury, 1969). Esters generally associated with fruity aroma, can be synthesised from the esterification of acids and ethanol (Wieczorek & Drabińska, 2022).

In fermented *Brassica*, i.e. cabbage-related, vegetables, such as sauerkraut and kimchi, the typical flavour is associated with varied sulphur-containing compounds, including glucosinolates and their degradation products, *i.e.* isothiocyanates, and other volatile organic sulphur compounds (Wieczorek & Drabińska, 2022). Glucosinolates are hydrolysed by myrosinase, a plant enzyme that is active on glucosinolates after the disruption of vegetable tissue induced by the cutting before process or/and to microbial enzymatic activity. The unstable aglycones derived from glucosinolate hydrolysis are then converted into a range of isothiocyanates, thiocyanates, and nitriles, depending on the biotic and abiotic factors, *e.g.* salt content and pH.

In kimchi, several key odorants have been identified including several sulphur-containing compounds and aldehydes derived from amino acid catabolism (3-methylthiopropanal (baked/boiled potato-like), phenylacetaldehyde) and from lipid catabolism ((E,Z)-2,6-nonadienal (cucumberlike), (E,E)-2,4-decadienal (fatty and/or sweet)) and 2,3-butanedione (buttery) from carbohydrate and/or citric acid fermentation by LAB (Cha et al., 1998). The abundance of volatile (flavour) compounds have been correlated with the abundance of specific taxa in many studies, *e.g* for sauerkraut (Yang et al., 2020) or *suancai* (He et al., 2020).

The use of metabolomics approach by combining both untargeted metabolite profiles and targeted metabolomics to identify functional

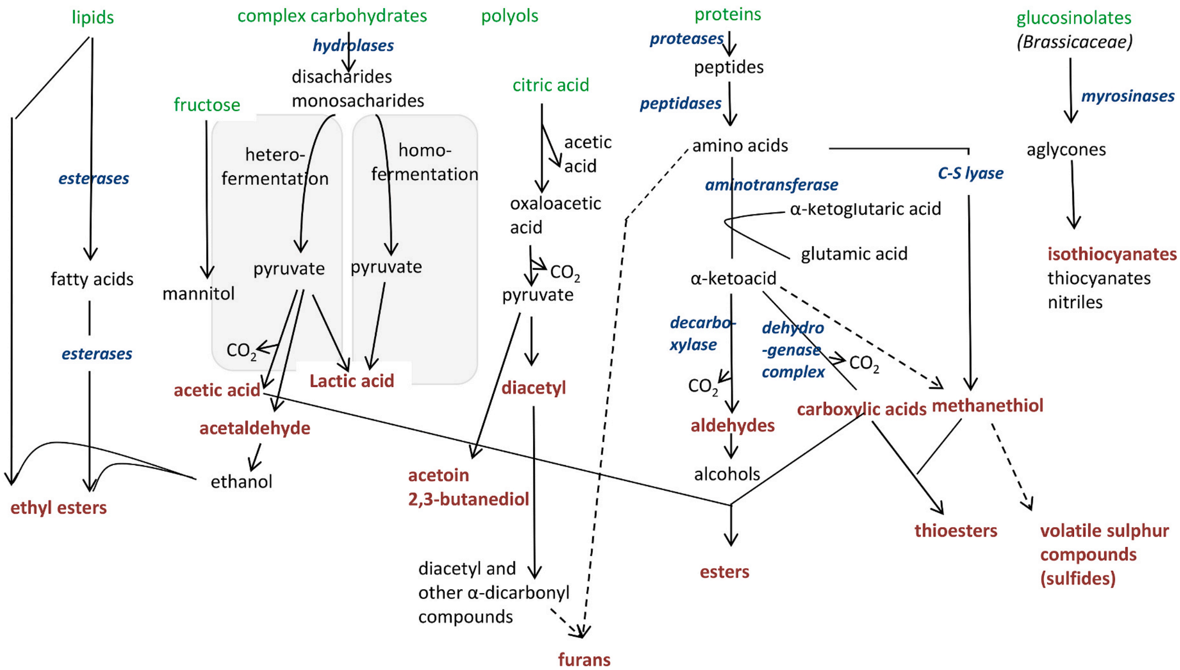

Fig. 5. Schematic overview of conversion pathways leading to the formation of the main flavour compounds by lactic acid bacteria in fermented vegetables; black arrows: metabolic pathways; broken arrows: chemical (non-enzymatic) reactions; green: plant components; **red, bold**: flavour compounds; *blue, italics*: main enzymes involved. Adapted from Thierry et al. (2015). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

metabolites and food safety biomarkers opens further perspectives to better understand the complexity of fermented food metabolome (Singh et al., 2017).

5. A nutritional and health interest especially supported *in vitro*

5.1. Fermented vegetables share nutritional and health interests with other fermented foods

Fermented vegetables share with other fermented foods three generic characteristics of interest: i) many and varied live microorganisms that have grown in the raw material, ii) many unique microbial metabolites of interest produced by these microorganisms, such as large concentrations of organic acids that are present only when a microbial process has taken place, and iii) (pre)hydrolysed macromolecules from the raw material, which often increase their digestibility, reduce their toxicity and/or improve mineral bioavailability. Moreover, fermented indigenous vegetables have a potential in combating malnutrition in some parts of the world, as for example in Sub-Sahara Africa (Irakoze et al., 2021).

The question of fermented foods, including fermented vegetables, as a “dietary source of live microorganisms” was strongly put forward by Rezac et al. (2018), providing a literature review of microbial species ingested through varied kinds of retailed fermented foods. Most fermented foods are good sources of living LAB, including species that are reported to provide human health benefits (Bell et al., 2017). This has led to the notion of ‘probiotic food’, as recently discussed for kimchi (Cha et al., 2023). However, the term ‘probiotic food’ should be used with care. The International Scientific Association for Probiotics and Prebiotics (ISAPP) recently clarified the distinction between fermented foods and probiotics (Marco et al., 2021). Thus, to label a product as probiotic requires demonstrating both the beneficial effects of specific strains from an intervention study with proof of safety and the presence of these strains in sufficient numbers in the final product at the time of consumption.

Recent findings clearly provide evidence that fermented foods are a major source of LAB for the gut microbiome (Butler et al., 2020; Cha et al., 2023; Pasolli et al., 2020). In a large American cohort study, Taylor et al. (2020) recently demonstrated that the consumption of fermented foods is associated with systematic and consistent differences in the gut microbiome and metabolome. Similarly, in a study in humans investigating the longitudinal effects of specific diets, the fermented-food diet increased the gut microbiota diversity and decreased markers of inflammation (Wastyk et al., 2021). However, most of the studies regarding what happens in the human gut after ingestion of these ‘microbial foods’ concern dairy products and not fermented vegetables, except kimchi (Cha et al., 2023). As they are rich in an undefined microbial biodiversity, fermented vegetables are promising vectors to preserve microbial diversity in our diet, and more generally in all aspects of our life as suggested by Dominguez-Bello et al. (2019).

5.2. Fermented vegetables, a source of various micronutrients

Raw vegetables intrinsically contain various micronutrients, in particular minerals and vitamins. They are known to be a good source of vitamin C, folate (vitamin B9) and other B-group vitamins, vitamin K, carotenoids, and some minerals *e.g.* potassium. Fermentation can affect the micronutriment content of vegetables in different ways: production, degradation, release in the brine, and concentration or dilution according to the amount of water added. From a nutritional point of view, it is important to examine the content of nutrients expressed per quantity of fresh matter of fermented vegetables without brine, *i.e.* as they are consumed. The content of minerals, vitamins, and antinutritional compounds of fermented vegetables depend, first of all, on their content in raw vegetables. It is noteworthy to underline the lack of reports that draw a mass balance to support robust conclusions about how the fermentation process affects micronutrient concentrations. Almost all published studies on this matter report that mineral and vitamin contents decrease during fermentation.

Regarding minerals, for example, in broccoli, beet, carrot and cucumber, calcium, iron, magnesium, potassium, and phosphorus decreased to 5%–40% of the initial concentrations (Kiczorowski et al., 2022). These minerals are released with water in brine, regardless if they were soluble or bound to the plant cell wall. It can be noted that it is also the case for heavy metals, and fermentation could thus be considered as a detoxification process of vegetables contaminated with heavy metals. Fermentation also increases the bioavailability of minerals. In kimchi, it decreased by 72% the insoluble oxalate-bound calcium (Wadamori et al., 2014). The bioavailability of iron was also reported to be improved by LAB fermentation in carrot, beet, and sweet potato (Montet et al., 2014).

As for vitamins, a decrease of their content during fermentation has most generally been reported, as stated above. It is the case for example for vitamin C (ascorbic acid) in French beans and marrow (Di Cagno et al., 2008), in beet, broccoli, carrot, pepper and cucumber (Kiczorowski et al., 2022), and in cabbage (Martinez-Villaluenga, et al., 2009; Peñas et al., 2010), with final contents from 11 to 97% compared to that of the corresponding raw vegetable. Similar conclusions have been made for other vitamins, e.g. thiamin (vitamin B1) and riboflavin (vitamin B2) in French beans (Mnkeni et al., 1995), and folate (vitamine B9) in a mix beet/turnip/onion (Jägerstad et al., 2005). In contrast, some other studies reported an increase in vitamin content. In cabbage, vitamin C may increase due to the action of microorganisms and the degradation of ascorbigen (Berger et al., 2020). In a mix of cauliflower/beans, the increase of riboflavin and folate depended on the *L. plantarum* strain used as starter (Thompson et al., 2020). Carotenoids, *e.g.* provitamin A and beta carotene, are liposoluble, so their lixiviation in brine is limited. Fermentation thus does not induce important losses of these compounds (Mappelli-Brahm et al., 2020). The evolution of vitamins initially present in vegetables is difficult to predict. In the case of vitamin B12, a vitamin not synthesised by plants, the production of small amounts has been reported after addition of *Propionibacterium freudenreichii* in a vegetable mixture of white cabbage/carrots/onions/red and green pepper (Jägerstad et al., 2004), and of a particular strain of *L. plantarum* in cauliflower/beans (Thompson et al., 2020).

Although the nutritional aspects of fermented vegetables are addressed by some studies, comprehensive studies with mass balance considering the composition of raw vegetable, brine, and fermented products are lacking to understand the role of LAB metabolism, lixiviation or dilution factors in the final composition of fermented vegetables.

5.3. Hydrolysis of some plant components during lacto-fermentation

Complex metabolic and functional pathways are used by LAB during plant foods fermentation, as comprehensively reviewed by Filannino et al. (2018). Together with plant enzyme activities, some LAB metabolic features of LAB can decrease the content of undesirable compounds, improve the bioavailability and bioactivity of phytochemicals, e.g. polyphenols and glucosinolates, and/or produce metabolites of interest for human health that result from plant components.

FODMAPs (*i.e.* fermentable oligo-, di-, monosaccharides and polyols) are present in some vegetables like artichoke or onion (FOS, *i.e.* fructooligosaccharides, inuline, …). They can have beneficial effects on colon health, but also increase gastrointestinal symptoms in people who suffer from functional bowel disorders such as irritable bowel syndrome (Nyyssölä et al., 2020). The FODMAP content of vegetables can be reduced by some LAB strains, even if other LAB strains are producers of these compounds. FODMAPs are soluble in water and can thus be lost in the brine (Ispiryan et al., 2022).

Fermentation also acts to detoxify undesirable components present in the raw material. Cyanogenic glucosides like linamarin and lotaustraline can be catabolised by the galactosidases of certain LAB species (Oguntoyinbo et al., 2016).

Polyphenol composition and bioactivity is qualitatively and/or quantitatively modified depending on the bacterial strains during fermentation (Sharma et al., 2022). The more complex polyphenols are especially affected, increasing the content in low molecular mass ones (Šalić & Šamec, 2022).

Glucosinolates are present in *Brassicaceae* vegetables and their content decreases during fermentation. For example, in broccoli, total glucosinolates decreased from 73 mg/kg to 11 mg/kg fresh weight after 12 days (Salas-Millán et al., 2022). Only traces of glucosinolates were reported in cabbage after 7 days of fermentation (Martinez-Villaluenda et al., 2009). Glucosinolates degradation products, *e.g.* isothiocyanates, are not only involved in the typical flavour of vegetables containing them as mentioned above, but also in the beneficial effects on health including anti-inflammatory, antioxidant, and chemo-protective effects (Connolly et al., 2021).

Bioactive peptides can also be released during the fermentation process, as mainly studied in cereals and legumes, but also shown in some vegetables (Rizzello et al., 2016).

5.4. Health effects explored for a few highly consumed fermented vegetables, in particular kimchi

Amongst fermented vegetables, kimchi is by far the most studied regarding its nutritional and health properties, likely because of its place in the Korean diet. It was ranked as the most frequently consumed food by Koreans in 2020, with a daily adult consumption of 50–200 g (Moss Maurice & Adams Martin, 2008; Cha et al., 2023). The health properties of sauerkraut have also been studied, but other fermented vegetables have been only scarcely studied (Lavefve et al., 2019).

The health properties of kimchi and other cabbage-based fermented foods can be due to several factors, that include: the nutritive content of raw material itself (Chinese cabbage and other raw ingredients), the presence of some phytochemicals such as thiocyanate and polyphenols; the presence of diverse LAB in high numbers; and to some of the metabolites that LAB produce during fermentation. The intake of *Brassica* vegetables reduces the risk of certain chronic diseases, such cancer and cardiovascular diseases, as shown by many reports based on epidemiological and clinical studies (Peñas et al., 2017). The consumption of fermented cabbage may also be helpful in mitigating COVID-19 severity, by enhancing Nrf2-associated antioxidant effects (Bousquet et al., 2021).

Kimchi has been assigned many health-related properties, based on *in vitro* and *in vivo* animal and human studies, including anti-inflammatory, antibacterial, antioxidant, anticancer, antiobesity, probiotic properties, cholesterol reduction, and anti-aging properties (Patra et al., 2016). The properties that appear as the most documented are the beneficial effects of kimchi and kimchi LAB on human gut environment, as shown by clinical studies on human cohorts of healthy participants or patients with colon adenoma, metabolic disorders, or *Helicobacter pylori* infection, according to a recent review that addresses a potential probiotic status for kimchi (Cha et al., 2023). Interestingly, fresh kimchi and fermented kimchi differently influenced the gut microbiota and gene expression profiles in obese women, suggesting that the effect of kimchi does not only result from the raw materials (Han et al., 2015). The health benefits of kimchi consumption, demonstrated in recent studies using experimental animal models, also include anticancer activity, anti-obesity activity, antidiabetic activity, and alleviation of obesity-induced neuroinflammation (Cha et al., 2023). The role of a specific compound, 3-(4-hydroxyl-3,5-dimethoxyphenyl)propionic acid, has been highlighted in the anti-atherosclerotic effects of kimchi, based on its lipid-lowering, antioxidant, and anti-inflammatory activities (Kim et al., 2018). As a result, kimchi has been recognized worldwide for a high number of health claims and was considered as one of the world's five healthiest foods (Patra et al., 2016).

As for sauerkraut, the potential health benefits of its consumption have been less extensively studied, but anticarcinogenic and anti-inflammatory properties have been documented and attributed to its high levels of phytochemicals, as recently reviewed (Peñas et al., 2017).

5.5. Sodium issue

As detailed above, NaCl has a major role in vegetable fermentation, by favouring the release of juice from the vegetables, thus promoting the development of the expected microbial community. Since the publication in 2012 of the WHO guidelines to reduce sodium intake to 2 g/day (*i.e.*, 5 g of salt/day, World Health Organization, 2012), there is a trend to reduce the salt level added in sauerkraut manufacture (Peñas et al., 2017). However, the consumption of fermented vegetables is highly variable according to the region. Kimchi, as a staple of the Korean diet, highly contributes to the sodium content in the Korean diet. In contrast, in Western countries, the consumption of fermented vegetables is quite low and so is thus its contribution to the total sodium content in the diet, compared to foods that are generally targeted to efficiently decrease the diet sodium content, e.g. breads, meat products, cheese. It can be underlined, however, that kimchi consumption is not associated with hypertension prevalence (Song & Lee, 2014).

Alternatives to reduce salt in fermented vegetables have been studied (Bautista-Gallego et al., 2013). As in many fermented foods, salt reduction may alter the sensorial and microbiological properties of the products, and also increase sanitary risks. NaCl substitutes like KCl, $CaCl_2$ and $ZnCl_2$ show promising perspectives, according to Bautista-Gallego et al. (2013). The same authors concluded however that the first step 'would simply consist of using the recommended concentration in each product'.

6. Conclusions and perspectives

Fermented vegetables benefit from an undeniable growth of interest from scientists, companies, and citizens, which can be explained by many factors that include the progressive 'veganization' of the diet in Western countries, the increasing expectations of sustainability, and the question of potential health benefits. This review highlights several important points to keep in mind. We now have a good view of the lactic bacterial community of fermented vegetables, which develops as a succession of species consisting of both a few keystone LAB species and a wide diversity of other LAB. There is far less knowledge on the *Enterobacteriaceae* that predominate at the beginning of fermentation and on the potential risks associated with their presence, as well as on the yeasts that are also commonly observed in these products. Sanitary issues with fermented vegetables do not appear to be of the same importance compared to fermented animal products, even if some conflicting data have been reported. The use of good quality raw materials, the respect of good manufacturing practices that favour a rapid acidification by LAB, and the control of the processing parameters are a prerequisite, even if this topic still deserves further verifications. In addition, the potential of selected starters to diversify flavour and/or nutritional and health properties and also to further decrease possible sanitary risks, need to be further investigated.

The additional nutritional benefits brought to vegetables by fermentation has been clearly demonstrated for the detoxification and digestibility improvement aspects, at least for cabbage. However, robust biochemical studies are still lacking on micronutrients like vitamins to precisely evaluate the effects of fermentation since raw vegetables are already rich in these compounds. The potential health benefits of fermented vegetables are not sufficiently documented, except for kimchi, to consider specific diet recommendations regarding fermented vegetables. Some changes in public policies to better include fermented foods - in general - during Food Guide revision have been suggested (Chilton et al., 2015), which may indirectly benefit the consumption of fermented vegetables. Even if they do not fulfil the definition of probiotic foods, as stated above, fermented vegetables are "microbial foods", whose positive effects on microbiota diversity and inflammation reduction was recently highlighted.

The potential of innovation related to fermented vegetables is obvious due to the variety of vegetables that can be fermented and combinations of them. Nevertheless, cultural aspects, and related organoleptic preferences, remain important and can limit the increase of their consumption. Information campaigns, role of collective catering, and school canteens, chef of gourmet restaurant, social media, and influencer roles can certainly ultimately overcome this limitation.

Author statement

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Declaration of competing interest

Nothing to disclose.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tifs.2023.07.009.

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