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Review

Microorganisms, the Ultimate Tool for Clean Label Foods?

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Abstract: Clean label is an important trend in the food industry. It aims at washing foods of chemicals perceived as unhealthy by consumers. Microorganisms are present in many foods (usually fermented), they exhibit a diversity of metabolism and some can bring probiotic properties. They are usually well considered by consumers and, with progresses in the knowledge of their physiology and behavior, they can become very precise tools to produce or degrade specific compounds. They are thus an interesting means to obtain clean label foods. In this review, we propose to discuss some current research to use microorganisms to produce clean label foods with examples improving sensorial, textural, health and nutritional properties.

Keywords: clean-label; technology additive; sensorial additive; anti-staling; bioremediation; biosurfactants; bio-preservation; antibiofilm; antinutrition; beneficial microorganism

1. Introduction

Clean label is a marketing concept aiming at giving confidence to consumers. Indeed, in the last few decades, consumers may have perceived the food industry as at risk of poisons in which all possibilities are used to do business at the expense of consumers, society and the environment. Applying the clean-label concept to food consists in washing the label from additives, especially those perceived as chemical and artificial, to go back to traditional foods reminding us of "Grandma's cooking".

Whereas in some fields, biotechnology is only limited by technical possibilities, in the food domain in which consumers are pushing the debate on ethical concepts, naturality and sustainability, biotechnology grows between many constraints that have arose to preserve people and the environment. As a result, the food biotechnologist is used to trying to bring about new technologies responding to technical issues as well as ethical and environmental ones. The use of microorganisms to wash the food label is one of these typical questions addressed by food biotechnologists. The food industry can produce high technical quality food, but this food is highly processed, using thorough cracking additives, many pesticides and crop preservation chemicals resulting in a high carbon cost, environmental pollution and food inducing metabolic syndrome and cancers in consumers. The concept of using microorganisms to achieve clean-label food is thus quite simple: microorganisms should, by their activity, produce active molecules from precursors naturally present in the food matrix. However, this concept should be usable also for non-fermented foods, meaning that, in this case, the microbial activity should not modify the sensorial properties of the product towards fermented notes.

From a regulatory point of view, this concept brings much discussion with business trying to occupy the field to obtain benefits while food and health agencies try to protect the consumers against this. In this review, we propose to present several examples of microbial applications in a clean-label strategy. These examples will deal with the use of microorganisms to replace technology, sensorial, biopreservation, bioremediation and health additives. We shall focus on recent work or current strategies and only refer briefly to already existing applications.

2. Technology Additives

Foods are usually very complex structures including all nutritional components, whatever their hydrophobicity, solubility, physicochemical status. Their textural organisation is thus prone to modification during shelf life and many chemical agents can be added to stabilise them. However, this domain of technology additives is very controversial as good quality products in terms of texture/structure and physico-chemical stability are often in the category of over-processed food, which results in bad marks in food score applications. In this context, microorganisms can bring a lot of functionalities without addition of chemicals. In this part, we will present some examples concerning how we can use microorganisms to avoid starch retrogradation in bread products and how microbial biosurfactants can bring interesting textural properties to food.

2.1. Staling

Starch retrogradation occurs in bread and starch products [1]. It is an issue in this field as it is responsible for stale bread, but it brings also desirable properties to other products like breakfast cereals or rice vermicelli. It is the result of a rearrangement of amylose and amylopectin molecules from gelatinised starch upon cooling [2]. During cooling, amylose forms a network around amylopectin granules. This network is reinforced by the rearrangement of amylose into double helices crystalline structures. Later during storage, amylopectin rearranges to form also crystalline structures, contributing to the hardness of the system. Several additives can interact with amylose, mobilising the molecules out of the network. For instance, monoglycerides, coded as E471 additives in the European system, can decrease amylose crystallisation. However, these E471 additives are typically a target of the clean-label strategy.

In the microorganism-induced clean-label strategy, microbial catalysts hydrolyse triglycerides present in natural plant oil into diglycerides, monoglycerides and free fatty acids. Contrasting with the use of enzymes, they can be labelled in the well-accepted "starter" category. One microorganism we have tested is the yeast *Yarrowia lipolytica*. This species is well-known and studied for its capacity to degrade hydrophobic compounds [3]. It possesses a wide family of lipases, including extracellular ones that are produced depending on the fatty substrate present in the medium [4]. From a technological point of view, mutants altered for the regulation of lipase synthesis or lipase production would be more attractive as they can be more efficient in the precision catalysis required. However, one of the constraints of microorganisms for foods is that, in almost all world markets, microorganisms for food usage cannot be genetically modified and only natural mutants are usable. This constraint is often not insurmountable even if no examples are available to produce specific lipases in *Yarrowia lipolytica*. Indeed, the difficulty is to find the right and easy-to-use screening procedure. Natural improvement of the tolerance of *Y. lipolytica* to toxic alcohols has already been made [5]. Another constraint is that *Y. lipolytica* must not exhibit any sensorial impact other than decreasing staling. This yeast species is well known for its ability to degrade lipids and proteins, producing thereby aroma compounds [3,6]. In the case of this aerobic yeast, this point can also be relatively easily overcome through a sequential utilisation of the yeast in the production process and inactivation after use. Eventually, the yeast must not pose any risks to consumers' health and this yeast, which is Generally Recognized As Safe (GRAS), has been studied for its applications as a starter showing high benefits [7].

Another family of additive popular to limit staling is composed of glucidic hydrocolloids. These compounds can have an impact on the plasticity of the amorphous regions of crumbs, where they can increase water retention or inhibit gluten-starch interactions [8]. Lactic acid bacteria can produce several products of this family under the form of exopolysaccharides [9]. Dextrane is one such bacterial compound which effect has been studied on starch retrogradation [10,11].

2.2. Microbial Biosurfactants

Emulsifiers are amphipathic compounds i.e., compounds possessing both hydrophobic and hydrophilic parts, exhibiting surface activity properties. They tend to accumulate at interfaces making them suitable to stabilise emulsions. These molecules can come from diverse origins, including petroleum industry and they can also exhibit many bioactivity properties. They could thus have a role to play in many modern food-related diseases [12]. Research has thus been oriented towards the development of new natural emulsifiers [13]. Biosurfactants are produced by living cells, especially microorganisms like bacteria, molds and yeasts. As emulsifiers, they are like chemical synthetic surfactants, amphiphilic compounds [14] consisting of hydrophilic and hydrophobic moieties and they can reduce surface and interfacial tensions [15]. In biosurfactants, hydrophilic moieties can be carbohydrates, carboxylic acids, phosphates, amino acids, cyclic peptides, and alcohols. However, the hydrophobic moieties of the biosurfactants are usually long–chain fatty acids, hydroxyl fatty acids and α-alkyl-ß-hydroxyl fatty acids [16]. Based on their chemical structures, the microbial biosurfactants are classified into four groups: glycolipids, phospholipids, and fatty acids, lipopeptides and polymeric biosurfactants [17,18] as shown in Table 1.

Table 1. Microbial biosurfactants.

Biosurfactants	Producing Microbes	References
Glycolipids		
Rhamnolipids	*Pseudomonas aeruginosa*	[19]
Sophorolipids	*Candida bombicola*	[20]
Trehalolipids	*Rhodococcus erythropolis*	[21]
	Mycobacterium sp.	[22]
Lipopeptides		
Putisolvins I and II	*Pseudomonas putida*	[23]
Surfactin	*Bacillus subtilis*	[24]
Pseudofactin II	*Pseudomonas fluorescens*	[25]
Serrawettin	*Serratia marcenscens*	[26]
Iturin A	*Bacillus amyloliquefaciens*	[27]
Fengycin	*Bacillus licheniformis*	[28]

Biosurfactant agents also show potential properties such as emulsification, functional additives, detergency, lubrication, phase dispersion, foaming, and solubilisation in many industries [29,30]. They show unique advantages including lower toxicity, better environmental compatibility, higher biodegradability, and specific activity when compared with chemical agents [31]. Mouafo et al. (2018) [32] reported that a glycolipid biosurfactant produced by *Lactobacillus* spp. could be used as an emulsifier in the food industry. Varvaresou and Iakovou (2015) [33] reviewed that sophorolipid ester was interesting as an ingredient in cosmetic products such as rouge, lip cream, and eye shadow. Furthermore, trehalose lipid produced by *Rhodococcus erythropolis* 3C-9 exhibited oil spill cleanup application [34]. In food, it can be noted that the bacteria themselves can exhibit surface active properties as shown on the use of *Lactococcus* strains to stabilise or destabilise emulsions [35–37].

Several studies are currently being carried out to develop the use of microbial biosurfactants instead of chemical ones in food. However, biosurfactants not only show the aforementioned properties, but they can also exhibit biological activities such as anti-microbial, anti-adhesion, and anti-biofilm formation activities. These properties can be of interest, but they require also a complete check before using a biosurfactant-producing microorganism.

3. Sensorial Additives

A major quality of food is to be attractive for consumers. This is true when a company wants consumers to buy back its products as well as to maintain a good nutritious state for patients losing their appetite. In the food transition towards a more sustainable system, sensorial properties are particularly important when new products are formulated with plants bringing off flavour or off colours. The traditional strategy in this case consists in using flavours or flavour-masking compounds that will lengthen the list of ingredients while the microorganism-based clean-label strategy proposes to select microorganisms able to produce flavour or colour and degrade off-flavours. Some examples concerning the bitterness of naringin and legumes off-colours are given in this section.

3.1. Naringin

Naringin (4′,5,7-trihydroxy flavanone 7-rhamnoglucoside) is a flavanone glycoside that is abundant in citrus fruits, mostly in the albedo and the peel [38]. With the limonin glycoside, naringin is considered as the molecule responsible of their bitterness, major off flavour when processing juice from citrus [39]. The naringin content is closely linked to the maturity of the fruit, its content being reduced with the maturity of the fruit [40]. Because of its high rate, the industrial processing of citrus generally uses immature fruits containing high contents of naringin. Thus, researchers have put efforts into finding ways to decrease the content of naringin in citrus. To do so, some physico-chemical methods have been developed, generally implying the use of resins, affinity polymers, cyclodextrin [41–43]. But these techniques involve the inclusion of additives and tend to impact the organoleptic characteristics of the processed juice [43,44]. Naringin can also be converted into naringenin by naringinase, an enzyme containing both α-L-rhamnosidase (E.C 3.2.1.40) and β-D-glucosidase (E.C 3.2.1.21) activities [43,45]. First, the enzyme breaks the bond between the rhamnose and glucose moieties of the naringin, producing pruning. Pruning is then hydrolysed, producing both D-glucose and naringenin, bitterless compound. This enzyme can directly be added to the juice—freely or immobilised [42,43] and can easily be produced by microorganisms, mostly filamentous fungi [43,46–48]. The enzyme production is generally induced by the addition of naringin, from 0.1 to 0.5% of the total medium nutrients [49]. The purified enzymes have a maximum activity temperature around 50 °C but are more thermically stable at 40 °C [50,51]. The range of pH stability is generally from 4 to 8 [45,50,51]. In 2016, Srikantha et al. [52] reached an activity as high as 449.58 U/g of dry matter in solid state fermentation for *Aspergillus flavus*. Some studies focused on the capacity of bacteria to produce naringinases, such as *Bacillus* spp. [53–55], *Lactiplantibacillus* (*L.*) *plantarum* [56], *Clostridium stercorarium* [57] or *Pseudomonas paucimobilis* [58]. Under optimum conditions for submerged culture, the production of naringinase reached 12.05 U/L for *Bacillus methylotrophicus* [54]. Similarly, Zhu et al. [55] characterized an enzyme produced by *Bacillus amyloliquefaciens*, which could reduce 97% of initial naringin in a pomelo juice. These results clearly indicate that both filamentous fungi and bacteria have the capacity of debittering citrus in juice processing industry. The goal now is to find a microorganism able to degrade multiple phenolic glycosides, which could be used for different applications. Indeed, most of enzymes have an activity highly specific for the nature of the bond between the glycosidic and aglyconic moiety (rutinoside-7-O-heperetin versus rutinoside-3-O-quercetin for example) and for the nature of the bond between the two sugars moieties (2 versus 6-O-α-L-rhamnosyl-D-glucose for example). Information about enzymes showing activities independent of the nature of the bond are scarce but are highly interesting for futures screening of glycosidases-producing microorganisms, which can possibly be used for a wide variety of applications.

3.2. Green-Notes in Legume Products

Legume-based products represent an interesting source of non-animal proteins due to their rich amount and diversity of essential and non-essential amino-acids [59]. In Europe, the main issue for the development of such products is the sensory acceptance by consumers. Indeed legume-based products are linked to "green", "grassy" or "leafy" descriptors [60,61]. Removing or masking undesirable tastes by means of biotechnology is a way of developing new alternative food products without using additives or heavy processes. The development of green-notes flavours is linked to the oxidative degradation of fatty-acid by enzymatic and non-enzymatic pathways during process and storage [62,63]. Green notes are related to many volatile compounds such as aldehydes, alcohols, esters, or ketones [64]. Hexanal and its derivatives have been wildly associated with green characteristics such as cut grass and leafy descriptors [65,66]. Nevertheless, green characteristics appear to depend not on the presence of isolate molecules but on the association of multiple compounds leading to various green description. Moreover, each modification on the aromatic mix leads to changes on the green perception balancing between green fruity and green grass/leafy [67]. Reducing the green characteristic of legume-based products might be complex according to multiple origins of it and its evolution during the making process. Fermentation appears to be a safe, cheap, and natural way to try to improve aromatics properties of legume-based products. This process has been wildly used since thousands of years in order to preserve and improve food quality. Fermentation by lactic acid bacteria (LAB) on legumes derivatives products such as protein extract, legume-based milk or raw legumes have been investigated among the literature. Fermentation of pea and lupin protein extracts by *L. plantarum* and *Pediococcus pentosaceus* separately, leads to a modification of green markers quantity, such as a diminution of hexanal content [68,69]. Fermentation of soy milk and peanut milk by *L. acidophilus*, *L.* (*Lacticaseibacillus*) *casei*, *L. delbruckii*, *Streptococcus thermophilus* also demonstrates the ability to decrease and eliminates hexanal from milk [70,71]. The elimination of hexanal is a good start for improving organoleptic quality of legume-based products, but not enough to completely eliminate green notes due to other compounds. Fermentation by co-cultures of *L. delbruckii* ssp. *bulgaricus* and *S. salivarius* ssp. *thermophilus* leads to a modification of the aromatic profile of peanut milk, by decreasing green flavour and enhancing creamy flavour and sourness [71]. The transformation by LAB allows us to modify the aromatic profile by decreasing green-related compounds and enhancing other flavour. Moreover, the anti-green note-effect provided by some microbial cultures can be sufficient in one food matrix but not in another. Investigations are still needed to apply this clean label mean of inactivation of off flavours in all conditions but reaching this goal might be possible by selecting strains exhibiting precise metabolic activities. Our recent results have shown that when screening LAB activities towards aldehydes, it was possible to discriminate between strains reducing all aldehydes and strains reducing preferably a class of aldehydes depending on carbon chain saturation or length [72].

4. Bio-Preservation and Bioremediation Agents

The use of microorganisms for bio-preservation purposes has already been the subject of several reviews papers and will not be developed in this section. Bacteria able to produce antifungal weak acids are already used in bread applications to avoid the use of chemical preservatives [73] and bacteria able to produce antimicrobial peptides such as bacteriocins are used as starter in several products [74]. In this section, we will review the use of biosurfactants-producing microorganisms in bio-preservation strategies.

4.1. Antibacterial Activity of Biosurfactants

Biosurfactants exhibiting antimicrobial activity to control the growth of food pathogens are the subject of many studies [75–83]. Biosurfactant often exhibit detergent properties causing cell membrane destructuration and permeabilisation [84]. These interactions are according to the theory of "like dissolves like". Therefore, their combinations cause the leakage of variety of substances [85].

Several microbial biosurfactants have shown antimicrobial activity against bacteria and fungi [86–88]. Rhamnolipid biosurfactant from *Pseudomonas aeruginosa* AT10 showed inhibitory activity against several microorganisms including, *Escherichia coli*, *Micrococcus luteus*, *Alcaligenes faecalis*, *Staphylococcus epidermidis*, *Penicillium crysogenum* and *Rhizoctonia solani* [89]. Rufino et al. (2011) [88] reported that a biosurfactant named after the author Rufisan, produced by *Candida lipolytica* UCP 0988 showed antibacterial activity against *Streptococcus* spp. with concentration of 12 mg/mL. Padmapriya et al. (2013) [90] reported that biosurfactant from *Candida tropicalis* also showed antimicrobial activities against *Bacillus* spp., *C. albicans*, *Citrobacter* spp., *E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella* spp. and *Staphylococcus aureus*. A potent anti-bacterial activity of *Brevibacterium casei* affected the growth reduction of *P. aeruginosa* and *E. coli* [91]. Lipopeptide from *B. licheniformis* strain M104 demonstrated high activity against *S. aureus* [92]. Falardeau et al. (2013) [93] also reported that cyclic lipopeptides produced by *B. subtilis* showed anti-microbial activity against plant pathogenic fungi. Biosurfactant from *B. pumilus* DSVP18 showed anti-microbial activity against *B. cereus*, *S. aureus*, *S. enteritidis*, *E. coli*, and *Paenibacillus larvae* with concentration of 30–35 µg/mL [94]. The lipopeptide derived from *B. cereus* NK1 showed anti-microbial properties against Gram-positive, Gram-negative bacteria, and fungi [95]. In addition, Das et al. (2008) [96] also reported that lipopeptide biosurfactant from marine *B. circulans* showed growth inhibition of *E. coli*, *S. typhimurium*, and *S. aureus*.

However, all these strains are hardly usable as clean label starters in food because of potential hazards or sensorial impact. Fortunately, lactic acid bacteria which are often Qualified Presumption of Safety species used in foods, are also microbes reputed to produce biosurfactants [97]. Biosurfactants derived from *Lactococcus lactis* showed microbial inhibition against multi-drug resistant pathogens including *E. coli* and methicillin resistant *S. aureus* [98]. *Lactocaseibacillus paracasei* biosurfactant presented an antibacterial activity against *E. coli*, *Streptococcus agalactiae* and *S. pyogene* with concentration of 25 mg/mL [87]. Sharma and Saharan (2014) [99] also reported that biosurfactants from *L. casei* MRTL3 showed antimicrobial activity against several pathogens, including *S. aureus* ATCC 6538P, *S. epidermidis* ATCC 12228, *B. cereus* ATCC 11770, *Listeria monocytogenes* MTCC 657, *L. innocua* ATCC 33090, *Shigella flexneri* ATCC 9199, *S. typhi* MTCC 733 and *P. aeruginosa* ATCC 15442. Biosurfactant produced by *L. plantarum* CFR 2194 also showed antimicrobial activity against *E. coli* ATCC31705, *E. coli* MTCC 108, *S. typhi*, *Yersinia enterocolitica* MTCC 859 and *S. aureus* F 722 by using well diffusion method [100]. Gudina et al. (2015) [101] reported that 5 mg/mL of biosurfactant from *L. agalis* CCUG31450 exhibited the growth inhibition of *S. aureus*, *P. aeruginosa* and *S. agalactiae*.

4.2. Antifungal Activity of Biosurfactants

Biosurfactants also represent antifungal activity against fungal mycelium and spore [102]. *Botrytis cinerea*, a fruit spoilage mold was inhibited by the lipopeptide biosurfactant from *B. amyliquefaciens* [103]. Kilani-Feki et al. (2016) [104] also reported that microbial biosurfactants showed a 79% decay inhibition of tomato colonisation by *B. cinerea*. The lipopeptide biosurfactant derived from *Bacillus marinus* B-9987 was published as a safe antifungal substance against grey mold of *B. cinerea* [105]. In addition, Torres et al. (2016) [106] reported that the biosurfactants from *Bacillus* spp. showed antifungal activity against soybean pathogenic fungus of *Macrophomina phaseolina*. An interesting antifungal activity was reported by Abalos et al. (2001) [89]. They showed that *Aspergillus niger*, *B. cinerea*, *Chaetonium globosum*, *P. chrysogenum*, and *Rhizoctonia solani* were inhibited by rhamnolipid produced by *P. aeruginosa* AT10.

This activity can also concern pathogenic molds. This is of course less related with food processing but can contribute to decrease the number of pesticides in food. For instance, *Phytophthora cryptogea*, causing rotting of fruits and flowers, was inhibited by lipopeptide produced by strains of *P. fluorescent* [107]. Mnif et al. (2015) [102] revealed that *Fusarium solani*, a potato pathogenic fungus was undergoing a 78% inhibition by *B. subtilis* SPB1 lipopeptide biosurfactant after 20 days of incubation. Moreover, the 0.02 and 3.3 mg/mL SPB1 lipopeptide biosurfactant also inhibited the seed-borne pathogenic fungus of *R. bataticola* and *R. solani*, respectively [83]. Furthermore, Joshi et al. (2008a) [81] studied the antifungal activity of *B. subtilis* 20B lipopeptide biosurfactant by using the disc diffusion method. The results of this study showed that *B. subtilis* 20B lipopeptide biosurfactant has antifungal activity against several natural contaminating fungi such as *Fusarium oxysporum*, *Alternaria burnsii*, *Crysosporum indicum* and *R. bataticola*. The antifungal activity of biosurfactant was explained by González-Jaramillo et al. (2017) [108]. They studied the effect of fengycin C, a lipopeptide biosurfactant from *B. subtilis* EA-CB0015 on *Mycosphaerella fijiensis* mycelium and spore morphology changes by using dipalmitoyphosphatidylcholine (DPPC), a fungal membrane model. The results revealed that fengycin C, the lipopeptide biosurfactant was able to change the fungal membrane model by dehydrating the polar head groups of cell membranes bilayer, causing the loss of its permeable properties. Moreover, repulsion of charges of amino acid and polar bilayer might also be involved in the destabilisation of cell structure [108].

Interestingly, Jadhav et al. (2011) [109] studied the biosurfactant produced by *Enterobacter* sp. MS16 on *A. niger* and *P. chrysogenum* spore germination. These fungal spore germinations were also inhibited by 12.5 mg/mL biosurfactant. Yoo et al. (2005) [110] also demonstrated that the rhamnolipid and sophorolipid biosurfactants showed the zoospore lysis activity against *Phylophthra* spp. and *Pythium* spp. Gond et al. (2015) [111] investigated for antifungal activity by iturin A from *B. amyloliquifaciens* against maize phytopathogenic fungus, *F. moniliforme*. They revealed that this lipopeptide biosurfactant with 500 µg/disk was strongly inhibited the mycelium elongation of *F. moniliforme* by interacting with fungal hyphae.

As a conclusion, many microbial biosurfactants are efficient against food spoilage or pathogenic strains. LAB biosurfactants can be used against food bacteria whereas bacilli bacteria produce often antifungal compounds. However, it is important to check whether these surface-active compounds can exhibit other properties that could limit their use in food.

4.3. Bioremediation

Apart from bio-preservation, numerous microorganism can also exhibit some ability to degrade toxic substances. This is referred to as a "bioremediation process" which is a bioprocess that can convert toxic substances (e.g., pesticides) or toxic contaminants (i.e., mycotoxins) or anti-nutrients such as phytates (which cause a decrease in iron availability) or biogenic amines. Nowadays, a worldwide serious agricultural threat is mycotoxin. It is recognized as an unavoidable risk. Many factors that influence the contamination level are environmental (such as weather and insect infestation) which are difficult or impossible to control. Therefore, this section attempts to review and discuss mainly on mycotoxin bioremediation.

Mycotoxins, a large group of toxic secondary metabolites, are produced primarily by a group of filamentous fungi mainly in the genera *Fusarium*, *Penicillium*, *Aspergillus* and *Alternaria*. They can contaminate food and feedstuffs at pre- and post-harvest stages. Currently, approximately of 60–80% all global agricultural commodities are contaminated with mycotoxins [112]. The most frequently found are aflatoxins, ochratoxins, zearalenone, deoxynivalenol, fumonisin B_1, T2 and HT-2. There are numerous strategies, either based on physical or chemical treatments, that can be applied to mitigate against this problem. However, the application of biological means of mycotoxin reduction using microorganisms is received increasing interest from scientists due to its low cost, the broad spectrum of mycotoxins that can be targeted, the minimal side effects regarding nutrient status of the food, minimal training requirements for those applying the microorganisms, and its suitability for a wide range of liquid and solid food types [113]. Mechanism of action will involve either adsorption by cell wall or degradation by enzyme depending on species and strains of microorganisms. Watanakij et al., 2020 [114] demonstrated the application of an extracellular fraction from *Bacillus subtilis* BCC42005 with water as a soaking agent for maize. The result revealed that aflatoxin B_1 was reduced after 120 min contact time without any changed appearance of the corn kernel. Table 2 summarises some microorganisms which exhibit the potential to reduce mycotoxin loads.

Table 2. Potential microorganism for mycotoxins bioremediation.

Mycotoxin	Microorganism	Reduction Capacity (%)	References
Adsorption			
Aflatoxins	*L. casei* *L. plantarum* *L. fermentum*	25–61	[115]
	L. casei	14–49	[116]
	L. rhamnosus GG *L. rhamnosus* LC-705	80	[117]
	Lactobacillus spp. *Bifidobacterium* *Lactococcus* strains	5.6–59.7	[118]
	Enterococcus faecium M74 and EF031	29.0–33.7	[119]
	L. plantarum	45–100	[120]
	B. bifidum 1900 *B. pseudolongum* 20,099 *B. infantis* 1912 *L. casei*	20–50	[121]
	Lactobacillus delbrueckii subsp. *bulgaricus* CH-2	18.7	[122]
	L. plantarum *Lactococcus lactis*	81	[123]
	S. thermophilus *L. bulgaricus* *L. plantarum*	11–34	[124]
	L. paracasei LOCK 0920 *L. brevis* LOCK 0944 *L. plantarum* LOCK 0945	39–55	[125]
	L. plantarum C88	60	[126]
Fumonisins	*L. paraplantarum* CNRZ1885	2–27	[127]
	LAB strains	32–100	[128]
Zearalenone	*L. rhamnosus* GG *L. rhamnosus* LC-705	47–52	[129]
	Lactobacillus spp.	26–69	[130]
	L. paracasei *L. lactis*	55	[131]
Deoxynivalenol	LAB strains	13–54	[128]
	L. plantarum GT III	56–66	[132]
Patulin	*Enterococcus faecium* M74 and EF031	41.6–45.3	[119]
	LAB strains	3–78	[133]
	L. brevis 20023	ND	[134]
Ochratoxins	LAB strains	2–96	[133]
	LAB strains	31–57	[135]
	Oenococcus oeni	26–33	[136]
	L. casei LOCK 0920 *L. brevis* LOCK 0944 *L. plantarum* LOCK 0945	50	[127]
	L. acidophilus VM20 *B. animalis* VM12	95	[133]
	Pediococcus parvulus	90	[137]
	L. rhamnosus CECT 278^T	97	[138]

Table 2. *Cont.*

Mycotoxin	Microorganism	Reduction Capacity (%)	References
Degradation			
Aflatoxins	*B. subtilis*	74	[139]
	B. subtilis BCC42005	45	[114]
Ochratoxins	*B. subtilis*	92.5	[139]
Zearalenone	*B. licheniformis*	100	[140]
	B. natto	75	[141]

5. Nutritional Additives and Properties

With the population becoming older, consumers are getting more interested in health issues and big industrial food groups transform their strategy and communication around health [142]. However, putting away compounds that are undesired by some consumers may be difficult and adding some healthy additives is still based on additives. In this section, some examples of use of microorganisms to selectively destroy antinutritional factors or to produce vitamins will be given.

5.1. Cleaning Food of Their Antinutritional Factors (ANF)

Antinutritional factors (ANF) are present in cultivated legumes, seeds and cereals [143]. ANF regroups multiple compounds which are lowering nutritional value of foods by inhibiting protein digestion and nutrient intakes, have deleterious effect on the digestive tract and health or cause gut disorders like flatulence [144,145]. Based on the previous literature, protease inhibitors, tannins, phytic acid are the main molecules responsible for the decreasing of proteolytic activity due to the inactivation of gut protease and denaturation of protein (protease inhibitors and tannins respectively) and the capture of positive-charged mineral ions (phytic acid). Lectins are glycoproteins characterised by their ability to interfering with intestinal epithelium leading to inflammatory state and a lack of nutrient absorption. Flatulence is linked to the digestion of α-galactosides like raffinose, stachyose and verbascose by the microbiota. The development of legume-based diet as protein source and the demands for healthy product poses the challenge for developing processes that keep nutritional benefits and clear products from ANF. First approach consisting in thermal processes as boiling, microwaving or pressurised cooking, such processes have shown great efficiency for decreasing trypsin inhibitors, phytic acid, hemagglutinins activity (lectins), saponins and some oligosaccharides of chickpeas [146]. The second approach is based on the supplementation of the cooking by germination or fermentation. The germination of seeds has shown significant results by eliminating flatulence-linked oligosaccharides [147] and decreasing the level of phytic acid, tannins and trypsin inhibitors [148]. The combination between germination and cooking allows us to significantly decrease or eliminate ANF in seeds and cereals. Nevertheless, few legume-based foods are produced following the germination process. Fermentation could appear as a safe way to tackles ANF from ungerminated legumes. Lactic acid fermentation by *L. plantarum* on bean flour shown multiple effects on ANF, such as the elimination of oligosaccharides and a significant diminution of lectins level [149]. The fermentation by *L. brevis* also shown great improvement on soybean digestibility due to the reduction of protease inhibitors and oligosaccharides [150]. Significant decrease of raffinose, stachyose, trypsin inhibitors and tannins have been reported for lactic acid fermentation of black bean by *L. casei* and *L. plantarum* [151]. Similar results have been reported for lactic acid fermentation of pearl millet [152]. Fungi fermentation can also eliminate ANF, and *Rhyzopus oligosporu* has shown significant activity against oligosaccharides and protease inhibitors [147]. But the fungi fermentation must be well characterised to avoid the production of any toxic compounds. As reported by the literature, fermentation could help to reduce or eliminate some ANF without using heavy processes or chemical treatments. It can be used on raw products or at further stage of transformation. More investigations are needed due to the variability of fermentation effects caused by strains and legumes' specificity. Indeed, lactic acid fermentation of plant-based product could lead to the production of biogenic amines [153], and this production is hugely dependent on the strains and the variety of legumes. The combination of thermic processes, germination and fermentation seems to be a great way for improving nutritional quality of plant-based product, but studies must be carried out to avoid any deleterious effects. Characterisation of plant cultivars composition and the activity of microorganism on it is the only way to develop clean and healthy plant-based products.

5.2. Vitamins Like Folate

Vitamins are organic compounds involved in several metabolic functions including energy production, red blood cell synthesis, etc. They are grouped into 2 main groups: lipid-soluble (vitamins A, D, E, K) and water-soluble (vitamin C and eight kinds of B vitamins) vitamins [154].

Vitamins of group A comprise retinoids, retinol, retinal, retinoic acid and retinyl esters. Pro-vitamin A is composed of various carotenoids (β-carotene, α-carotene, and β-cryptoxanthin), which are then converted in their active forms in the body [154].

Vitamin D derives from cholesterol and ergosterol. Cholesterol is converted into 7-dehydrocholesterol, which can be cleaved by ultraviolet (UV)-radiation to form cholecalciferol (vitamin D3), while ergosterol results in ergocalciferol (vitamin D2). Vitamins D2 and D3, used by humans, require further hydroxylations [154].

The vitamin E group is formed by different chemical forms: four tocopherol and four tocotrienol forms. Tocopherols are often used as dietary supplements for humans, food preservatives, and in manufacture of cosmetics and sunscreens. However, α-tocopherol is the most predominant and active form in most human and animal tissues [155].

Vitamin K can be divided into phylloquinone (vitamin K1) with a phytyl group obtained from plants and menaquinones (vitamin K2) [154]. Vitamin C or ascorbic acid is an essential dietary component that humans are unable to synthesize.

B vitamins contain thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), pantothenic acid (vitamin B5), vitamin B6, biotin (vitamin B7 or vitamin H), folic acid (vitamin B9), and cobalamin (vitamin B12) [154].

The absence of adequate amounts of these compounds in the diet can cause several health problems not only to humans but also to animals. Therefore, they are produced industrially and used widely not only as food and feed additives, but also as cosmetics, therapeutic agents and health and technical aids [154]. However, these processes require the use of solvents, which are undesirable pollutants harmful to the environment. To overcome this drawback several studies are focused on the selection of microorganisms able to produce vitamins (Table 3).

Table 3. Vitamins, their functions and microorganisms used for their production.

Vitamin	Physiological Functions	Microbial Producer	References
Vitamin A	Immune system regulation, vision, reproduction, cellular communication, cell growth and differentiation.	*Cyberlindnera jadinii* (teleomorph *Candida utilis*), *Saccharomyces cerevisiae*, *Pichia pastoris*, *Y. lipolytica*	[156–160]
Vitamin D	Calcium absorption and mineralization of bones, modulation of cell growth, neuromuscular, immune and inflammation functions	*S. cerevisiae*, *Saccharomyces uvarum* and *Cyberlindnera jadinii* (teleomorph *C. utilis*)	[161]
Vitamin E	Antioxidant activity, cellular membrane stabilizer	Microalgae: *Spirulina platensis*, *Dunaliella tertiolecta*, *Synechocystis* spp., *Nannochloropsis oculata*, *Tetraselmis suecica*, *Chlorella* spp., *Clamydomonas* spp., and *Ochromonas* spp., *Euglena gracilis*, *Dunaliella salina*, *Isochrysis galbana*, and *Diacronema vlkianum*	[155]

Table 3. *Cont.*

Vitamin	Physiological Functions	Microbial Producer	References
Vitamin K	Blood coagulation	*Flavobacterium* sp., *B subtilis*, and *Propionibacterium freudenreichii*	[162–164]
B vitamines	Energy production, red blood cell synthesis	*B. subtilis*, *Corynebacterium ammoniagenes*, *L. plantarum*, *Leuconostoc mesenteroides*, *Lactococcus lactis*, *Rhodococcus rhodochrous*, *Agrobacterium* sp., *Corynebacterium glutamicum*, *Flavobacterium* sp., *Sinorhizobium meliloti* (ex *Rhizobium meliloti*), *B. sphaericus*, *Serratia marcescens*, *Propionibacterium shermanii*, *Pseudomonas denitrificans*, *Bacillus megaterium*, *Methanobacterium ivanovii*, *Rhodobacter capsulatus*, *Ashbya gossypii*, *Candida parapsilosis*, *Candida flaeri* and *Candida famata* (teleomorph *Debaryomyces hansenii*).	[165]
Vitamin C	Antioxidant activity, biosynthesis of collagen, l-carnitine and certain neurotransmitters, protein metabolism	*Gluconobacter* spp., *Acetobacter* spp., *Ketogulonicigenium* spp., *Pseudomonas* spp., *Erwinia* spp., and *Corynebacterium* spp.	[166,167]

Presently, several studies are focusing on Vitamin B9 or folate since it plays very important functions in human health including amino acid metabolism and DNA replication and repair and is thus essential for cell division. In pregnant women daily intake of folic acid is recommended since it reduces the risk of low birth weight, maternal anemia and neural tube defects (NTD): spina bifida and anencephaly [168]. There are many forms of vitamin B9, called vitamers, which are more resistant to technological processes. Folic acid, the synthetic form of B9 vitamin, presents only a glutamate molecule, while naturally occurring forms are characterized by a polyglutamate chain. In addition, folic acid exhibits a fully oxidized pteridine ring, while the other vitamers are generally either partially reduced (at the 7,8-position) in the case of dihydrofolate forms, or fully reduced (at the 5,6,7,8-position) in the case of tetrahydrofolate compounds [169].

Humans do not synthesize folate de novo and folate deficiency represents a problem worldwide. In fact, several countries adopted mandatory fortification programs in foods of mass consumption such as flours and rice [169]. The main strategies used to address the problem of vitamin deficiencies are (i) supplementation, (ii) food fortification, and (iii) dietary diversification [170]. Unfortunately, folate-rich foods are not always available, depending on the season, and on the geographic, agro-ecological and socio-economic context, and the intake of folic acid could exert some adverse secondary effects, such as masking symptoms of vitamin B12 deficiency and possibly promoting colorectal cancer. These side effects are not observed when natural folates, such as those found in foods or produced by certain microorganisms, are consumed [169].

The main producers of folate are LAB and bifidobacteria (Table 4). Folate production is strain-dependent and is influenced by growth kinetics and medium composition. Several studies reviewed in [169] highlighted that folate bacterial production occurs during the exponential growth phase or at the beginning of the stationary phase and is then consumed.

Table 4. Main bacterial species producing folate.

Microorganism	Outcome Range (ng/mL)	References
Bifidobacteria		
Bifidobacterium (B.) adolescentis	50–150	[171]
B. dentium	0–25	
B. animalis	1–65	[172]
B. bifidum	26	
B. breve	1	
B. catenulatum	1–3	
B. longum	3	
B. pseudocatenatulum	29	
B. adolescentis	10–30	[173]
Lactic acid bacteria		
Lactobacillus acidophilus	0–38	[174]
Lb. amylovorus	75–87	
L. casei	0–2	
L. paracasei	0–40	
Levilactobacillus brevis (ex *Lb. brevis*)	0–150	[175]
Latilactobacillus curvatus (ex *Lb. curvatus*)	0–20	
Fructilactobacillus fructivorans (ex *Lb. fructivorans)*	0–20	
Lb. helveticus	2–89	
Limosilactobacillus reuteri (ex *Lb. reuteri*)	0–125	
Loigolactobacillus coryniformis (ex *Lb. coryniformis)*	80–100	[176]
L. pentosus	0–4	
Lb. sakei	101–107	
Pediococcus. parvulus	40–60	
Pediococcus pentosaceus	0–40	
Weissella confusa	0–20	
Lb. delbrueckii	50–200	[171]
L. plantarum	36–60	
L. fermentum	0–148	[177]
Lb. johnsonii	28	[178]
Lactococcus lactis	57–291	[179]
Leuconostoc lactis	45	
Leuconostoc paramesenteroides	44	
S. thermophilus	0–170	[180]

The majority of studies concerning folate production by eukaryotic microorganisms were carried out on *S. cerevisiae* and *A. gossypii* [173]. However, also other yeast genera are reported as folate producers such as *Candida*, *Debaryomyces*, *Kodamea*, *Metchnikowia*, *Wickerhamiella* [174]. *A. gossypii* can naturally synthesize 40 µg/L of folates and after metabolic engineering is able to reach 6595 µg/L. This result was obtained overexpressing 3 genes involved in folate production (*FOL1*, *FOL2*, *FOL3*) and deleting the gene *MET7* which encodes for a FPGS (folypolyglutamate synthetase) which catalyses the polyglutamylation of folates in their gamma-carboxyl residue [173]. The elimination of competing pathways, such as riboflavin and adenine favours folate production [173].

Despite the efforts undertaken so far, microbial folate production is still low and not competitive in terms of cost and final concentration with industrial processes. A possibility to increase folate production could be the development of co-cultures of folate producing strains or folate vitamers that are resistant to oxidation, acid pH, and heat treatments. Finally, the possibility to use probiotic strains could be an advantage since folate could be produced in the gut. Future research should also focus on the understanding the complex regulatory mechanisms governing the enzymatic activities involved in the folate pathway; the optimization of the fermentation conditions and further development of downstream processes for the recovery and purification of the product.

6. Use of Taste-Active Microbial Amino Acids, and Peptides in Food Fermentation

Eventually, we will see some examples concerning inactive microorganisms that can be used for some compounds active for food properties.

Salt is an irreplaceable additive, flavouring foods. Culinary salt is a chemical compound consisting of the elements sodium and chlorine. Salty taste is given mainly by Na^+. The ions of the alkaline metal group exhibit also a salty taste but causing less feeling than Na^+. The size of the ions Li^+ and K^+ is also close to that of Na^+, creating a salty taste that is almost similar. The salinity of substances is assessed in comparison to the sodium chloride standard [181,182]. KCl is the main ingredient used to replace salt with an index of 0.6 (when the salinity of NaCl is 1).

Monosodium glutamate (MSG) gives the taste of meat and umami, which is one of the five basic tastes with sourness, sweetness, saltiness, bitterness. In 1909, Kikunae Ikeda discovered MSG from seaweed. The taste strength of glutamate is quite strong. The sensory threshold of MSG is 1/3000 (one gram over three liters of water). This intensity is much stronger than salt and sugar. However, in addition, glutamate enhances also the perception of salty taste, and helps therefore to reduce the amount of salt added to food. Reducing salt is a goal in daily meals for humans to avoid certain diseases such as high blood pressure, kidney failure. But reducing salt will lead to food with poor taste. Using KCl as a substitute for culinary salt will create a bitter and metallic taste. Research results have shown that MSG combines culinary salt, significantly improving the sensory properties of foods. Yamaguchi [183,184] reported that the addition of MSG to broth could help to decrease the rate of sodium chloride for a similar sensorial result. Thus, MSG can replace culinary salt while ensuring the deliciousness of food.

MSG is present in different amounts in most natural food sources such as tomatoes, fish meat or oysters. It can be present as a free form or bind with other amino acids to create certain peptides and proteins. The content of MSG in nature has been determined [185,186]. The highest content of free glutamate in food (100 g) are found in Pamesano cheese, 1.680 mg; seaweed, 1.608 mg, oyster, 140 mg; tomatoes, 246 mg, or Japanese fish sauce, 1.323 mg.

In the human body, approximately 70% of body weight is water, 20% is protein and of which glutamate accounts for about 2%. MSG is a natural part of metabolism and about 50 g per day is formed by the human body. The average person consumes 10–20 g of bound glutamate per day and about 1 g of free food glutamate. Daily intake of glutamate is the main source of intestinal energy.

Saccharomyces yeast is a rich-in-protein source (protein content accounts for 48–50% dry matter) and yeast hydrolysed products are considered as rich sources of amino acids and peptides. They have many applications in food such as salad dressings, ice creams, crackers or meat products. They are used as additives, enhancing the flavour of the food products. Beer production can be a source of yeast. For instance, in a country like Vietnam with beer consumption of about 4.6 billion litters in 2019 according to data from the World Bank and Euromonitor, the production can generate around 7000 tons of spent yeast that can be used for either food consumption and feed. Utilising a large source of protein from brewer's yeast to produce hydrolysed products for application in food and food additives has a high real-life benefit. The composition of some amino acids in the brewer's yeast hydrolysates (BYH) varies depending on hydrolysis techniques. Continuous circulation hydrolysis method with heat shock and processed by autolysis gives the highest total amino acid content. The glutamate content accounts for 3.14 g/ 100 g BYH (55% dry matter) when the total amino acid composition achieved 32.3 g/100 g BYH.

However, bitterness in hydrolysates is one of the major undesirable aspects for various applications in food processing. It has been reported that the bitterness of brewer's yeast hydrolysate obtained by using flavourzyme is the lowest and that this product keeps a good umami taste [187].

The second limitation in the use of yeast and hydrolysate is the high content of nucleic acid in the yeast. There are many methods for reducing or separating nucleic acids in hydrolysed products such as extracellular ribonuclease enzymes, chemical agents, thermal shock and autolysis. Using extracellular ribonuclease enzyme for hydrolysis of nucleic acid gives good efficiency but suffers high production cost. Chemical agents negatively affect the quality of the hydrolysed products used in the food industry. It has been reported that a method using combination of heat shock treatment, autolysis and continuous circulation hydrolysis techniques gave the smallest content of nucleic acid in the brewer's yeast hydrolysate in comparison with using the batch and continuous overflow process [188].

In addition to the contribution of inactivated yeast to the taste of products, this popular microorganism can also bring health-active compounds. One of the most economically important components of yeast biomass is ergosterol, which, as already discussed in the previous paragraph, could be used as a precursor of vitamin D2 and another sterol drug [189]. Thanks to advanced technology in biotechnology, modified strains of yeast have been developed to enhance the production of ergosterol or the co-production of ergosterol with other products [190–192]. In Vietnam, the National Institute of Nutrition has conducted investigation on the production of ergosterol from *S. cerevisiae* and its application in functional food production. From 50 yeast samples of bakerhouses and 50 samples of fresh grapefruits from markets in Hanoi, two yeast strains, namely MB14.2.2 and N42.2.2, were found with the highest concentration of ergosterol in comparison with dry biomass (3.7% and 3.5%, respectively). Furthermore, optimized conditions and apparatus system for ergosterol production from these strains were established. For the applications in function foods, cookies (for children) and soya milk powder (for adults) were supplemented with vitamin D2 (1600 IU/100 g and 2261 UI/100 g in cookies and soya milk powder, respectively), that was transformed from ergosterol using radiation method. After using the products, the group of children had better transformation of the z-score index height/age and body mass index (BMI). The adult group improved bone health and improved blood biochemical indicators. Concentrations of 25- (OH) D of both groups with vitamin D2 were significantly higher than that of the control group ($p < 0.001$). The percentage of vitamin D deficiency noticeably decreased in both intervention groups.

Furthermore, brewing yeast is a great source for β-glucan. When yeasts are grown for seasoning purposes, molasses from sugar production is used as raw material for yeast fermentation. Presently, there are three products: spray-dried whole cell yeasts, yeast extract in paste form and spray dried yeast extract. The yeast cell wall separated after centrifuge goes to wastewater and causes complications and costs in wastewater treatment. Therefore, there would be a great opportunity to add value to yeast by using the cell wall as a source for production of β-glucan, a functional food.

7. Conclusions

With the growing concern of consumers towards the food that they eat, the clean label strategy has been generalised in many companies. From the first efforts which could often been assimilated to green washing, some companies have now developed a systematic struggle against additives. In this cleaning effort, microorganisms can be an efficient tool. This review illustrates what microorganisms can bring to the clean label concept through examples of recent strategies. In fact, besides the use of microorganisms producing antifungal weak acids in bread products, exopolysaccharides or of strains able to consume lipids or sugars to decrease the caloric properties of foods, or compounds with a positive effect on human effects, the efficacy of microbial strains to obtain good foods without additives is always subject to evaluation. The use of microrganisms could be useful to reduce the employment of additives since some strains are able to transform food components, degrade off-flavors, antinutritional factors, toxins, and chemical pollutants, or bring new molecules that are active for taste or health. Further studies are necessary to improve this "clean label" approach to reduce the list of ingredients used in food products.

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