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Review

Bio-mediated generation of food flavors – Towards sustainable flavor production inspired by nature

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ABSTRACT

Background: The consumers' trend toward naturalness and "clean-label" products advocates the development of "bio-mediated" tools including new processes for the generation of flavors. Today, many fundamental studies demonstrate the feasibility of producing individual flavor compounds or more complex flavoring preparations by fermentation or by enzymatic reactions. However, to turn research into industrial applications, the processes have to be simplified and optimized by combining chemistry, biology and process engineering know-how.
Scope and approach: This review summarises recent basic research and development on cell and enzyme based formation of volatile flavors with focus on smart combinations of biocatalytic and thermal steps to enrich the natural flavor profile of foods. Ideally, targeted bioconversion of specific raw materials and ingredients will release flavor precursors required to generate the desired flavor profile by appropriate thermal processing.
Key findings and conclusions: The combination of fermentation or enzymatic treatment of raw materials with heat-induced food processes (e.g. drying, extrusion, roasting) represent an elegant approach in industrial food processing to generate flavors under mild conditions. This requires a good control of fermentation or enzymatic reaction steps to produce suitable substrates at the optimal concentrations adapted to the thermal processes. Using traditional cooking and minimal processing conditions (nature-inspired strategies) has become an attractive approach to generate authentic flavor profiles resonating with consumers' demand for more naturalness.

1. Introduction

While climate change, soil erosion and human intervention steadily decrease the agricultural areas, the growth of the human population calls for an increased agro-industrial productivity. The natural genetic diversity will have to be more intensively exploited, and transgenic plants surviving on poor soils and at warmer conditions will contribute remedies, but an all-encompassing solution is not in sight. Recognizing this situation, the European Commission and other institutions worldwide have launched programs, for example FP6, FP7 and Horizon 2020 (EC), 'The Bioeconomy to 2030' (OECD), worldwildlife.org/initiatives/food (WWF), to establish and develop novel biotechnologies for the sustainable generation of food and feed. These include the use of insects, algal and other marine sources as well as the introduction of biocatalysis in different industrial areas.

This trend is coinciding with consumers' demand for more naturalness when it comes to food and food flavors (Román, Sànchez-Siles, & Siegrist, 2017). They have reported how naturalness can be classified in three categories. First, the way the food has been grown, e.g. food origin (organic, local). Second, how the food has been produced, e.g. what technology and ingredients have been used (free from undesirable compounds such as additives, artificial flavors, preservatives, etc.), presence of natural ingredients, minimally processed, and use of traditional methods. Third, the properties of the final product play a role, e.g. healthy, eco-friendly, tasty and fresh. There are several options and opportunities in food industry to build on this insight and move towards natural flavor solutions.

Recent years have seen a rapid pace of the advances of food biotechnology including new processes for the generation of volatile flavors. In low application concentrations, these chemicals not only confer attractive sensory properties to food, but unfold other desirable bioactivities as preservatives, antioxidants and else (Ayseli & Ayseli, 2016). According to recent estimations, a set of 230 key compounds out of the 10^4 known volatile food flavors determines the characteristic sensory properties of foods (Dunkel et al., 2014, 2015). A group of "generalist" flavor compounds occurs in more than 20% of the > 200 food items investigated (Table 1). Compounds of the "individualist" group are derived from rare and partly unknown biogenetic precursors

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Table 1
Key food odorants[a]: Precursors and preferred occurrence indicated by +.

Precursor	Key flavor compound	Cold formation	Thermally treated/ Dried /Stored Foods
Various			
	Acetic acid	+	+
	Acetaldehyde	+	+
	Ethyl 2-/3-methylbutanoate	+	
	2-Acetyl-1-pyrroline	+[b]	+
	Butanoic acid	+	+
Amino acid			
	Methional	+	+
	2-/3-Methylbutanal	+	+
	2-/3-Methylbutanoic acid	+	+
	Sotolon (3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone)	+	+
	2-Methylpropanal	+	+
	2-Phenylethanol	+	
	Phenylacetaldehyde	+	+
	Ethyl 2-methylpropanoate	+	
Carbohydrate			
	Butane-2,3-dione	+	+
	Ethyl butanoate	+	+
	Furaneol® (4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone)	+	+
Lipids			
	(*E,E*)-2,4-Decadienal[c]	+	+
	Hexanal	+	+
	1-Octen-3-one	+	+
	(*E*)-2-Nonenal	+	+
	(*Z*)-3-Hexenal	+	+
	trans-4,5-Epoxy-(*E*)-2-decenal		+
Phenols			
	Vanillin	+	+
	2-Methoxyphenol	+	+
Terpenes			
	(*R,S*)-Linalool	+	
	(*E*)-*β*-Damascenone	+	

[a] Abundance in > 20% of 227 foods analysed referred to as "generalist" flavor compounds (based on Dunkel et al., 2015).
[b] Biosynthesis of 2-Acetyl-1-pyrroline (Poonlaphdecha et al., 2016).
[c] (Raffo, Carcea, Castagna, & Magri, 2015).

and may occur in a few or a single food item only. Both generalist and individualist flavors are nature's preferred agonists of the 270 functioning olfactory receptors in the human *Regio olfactoria*. These chemical signals of the sense of smell are converted to electrical signal patterns, compared with the internal odor pattern library and interpreted by deep regions of the brain resulting in often unconscious behavioral responses of a human being.

"Generalist" key flavor compounds apparently constitute prime targets of biotechnological approaches. Their widespread occurrence suggests origins from universal food constituents, such as fatty acids, amino acids and saccharides (Table 1). However, these flavor precursors exist in most foods in bound and buried forms, such as triacylglycerols and phospholipids, proteins and peptides, and homo- and heteropolysaccharides, which lower their availability. Traditionally, the release of these flavor precursors is performed by fermentation. This process is mainly carried out by the incidental or deliberate inoculation with bacteria or yeasts, and is characterized by multifarious concurrent hydrolytic reactions (Swain, Anandharaj, Ray, & Rani, 2014). Smaller molecules are liberated and accumulated resulting in increased nutritional value and taste, degradation of anti-nutrients, and, eventually, in the accumulation of potential flavor precursors. It cannot be mere incidence that cold origins were made likely for all of the "generalist" key food odorants mentioned in Table 1.

This review will present examples of cells and isolated enzymes based formation of flavors and investigate how combinations of biocatalytic and thermal steps can enrich the natural flavor profile of foods.

2. Flavor generation based on whole cell systems

2.1. Flavor generation based on fermentation

The roots of modern flavor biotechnology reach back to the empirical food fermentations. Although never strictly anaerobic, the term "fermentation" is still widely used for these traditional processes. Starter cultures were selected for technologically important traits, such as rapid formation of acid or ethanol, insensitivity against their own metabolites and competitiveness in the complex food matrix. Microbial formation of flavors was taken for granted, and little effort was made to understand, control or even improve what happened seemingly inevitably. Today, sophisticated instrumentation, such as Proton Transfer Reaction-Mass Spectrometry (PTR-MS) differentiates the flavor profiles of, for example, *Lactobacilli* (Benozzi et al., 2015). Among the discriminating compounds in yogurt cultures were acetaldehyde, methanethiol, butanoic acid, 2-butanone, butane-2,3-dione, acetoin, 2-hydroxy-3-pentanone and benzaldehyde, some of which occur on the list of "generalists" (Table 1).

Strains of *Bacillus* are common in Asian fermented foods, such as Natto (Beaumont, 2002). Their strong hydrolytic activities create a pool of flavor precursors, which becomes observable during incubation in the volatile flavor fraction, but also in the non-volatile fraction upon heat treatment. The resulting taste is often described as "meaty" or "savory", although the processes completely rely on soy, cereals or other plant sources. Many consumers are aware of the huge carbon footprint of animal mass propagation, reduce their meat intake accordingly, but still do not want to miss the occasional experience of meat flavor. The return to fermentation flavors offers a convenient option.

Several fermented foods are nowadays produced on industrial scales like cheese, yogurt, kefir, seasonings, soups and sauces. Efforts are still underway in Asia and Africa to master and improve the fermentation of beans, cereals, vegetables, and spices with a view to enhance their nutritive value, shelf-life and sensory quality. In Africa, fermented cereals (e.g. millet, sorghum, cassava, and maize) are particularly important as weaning foods for infants and as dietary staples for adults (Franz et al., 2014; Gabaza, Muchuweti, Vandamme, & Raes, 2016; Galati, Oguntoyinho, Moschetti, Crescimanno, & Settanni, 2014). However, to guarantee industrial scale fermentation of these cereals, a number of parameters must be met:

- Develop commercial and cheap starters with high performance
- Improve and master food safety through inhibition of pathogens and removal of toxic compounds
- Improve nutritional value (e.g. fortification with micronutrients, remove antinutrients)
- Improve sensory quality (e.g. mastering metabolic pathways)
- Process should be simple and product's price affordable

The use of genetically engineered yeasts in foods still meets a negative consumer perception. Thus, instead of genetically manipulating *Saccharomyces*, a "Yeast Flavor Diversity Screening" was proposed to improve the flavor of alcoholic beverages (Carrau, Gaggero, & Aguilar, 2015). Another recent approach is to assist or substitute *Saccharomyces* by adding wild yeast species, such as *Brettanomyces* (Steensels et al., 2015). Formerly classified as "spoilage" microorganisms, it was recognized that the gene pool of such wild yeasts may be exploited to add new sensory attributes to fermented beverages. As soon as some history of safe use was built up, the transfer of the responsible genetic trait back into a *Saccharomyces* would be a toxicologically preferable option.

Kluyveromyces marxianus, a rapidly growing food-grade wild yeast, was shown to produce a number of flavor compounds, among them 2-phenylethanol, a member of the "generalist" compounds (Morrissey,