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Production of plant metabolites with applications in the food industry using engineered microorganisms

Nicolai Kallscheuer[1], Thomas Classen[2], Thomas Drepper[3] and Jan Marienhagen[1]

Secondary plant metabolites are extensively used in today's food industries, for example, as coloring-agents, flavouring-agents or texturizing agents. In particular, metabolites with antioxidative properties find applications as preservatives or anti-browning agents. Today, extraction from plant material represents the major source of these metabolites, but progress in the field of metabolic engineering also enabled the microbial production of these valuable compounds as a more economic and ecological alternative. This review article presents the current state of metabolic engineering of microorganisms for production of plant metabolites with applications in the food industries. We focus on compounds, which are already used in food applications, discuss current limitations of microbial plant metabolite production, and outline strategies on how these challenges can be addressed in the future.

Addresses
[1] Institute of Bio- and Geosciences, IBG-1: Biotechnology, Forschungszentrum Jülich, D-52425 Jülich, Germany
[2] Institute of Bio- and Geosciences, IBG-1: Bioorganic Chemistry, D-52425 Jülich, Germany
[3] Institute of Molecular Enzyme Technology, Heinrich Heine University Düsseldorf Located at Forschungszentrum Jülich, D-52425 Jülich, Germany

Corresponding author: Marienhagen, Jan (j.marienhagen@fz-juelich.de)

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Introduction

Food additives typically improve the food quality, modify the food texture or structure, or increase the food's shelf life. Quality as well as texture and structure are related to the visual impression or taste, whereas an increased shelf life results from protection against spoilage or contamination with microorganisms such as fungi and bacteria. Most phytochemicals with applications in the food industries are secondary metabolites, which are not essential for plant growth and propagation, but enable interaction of the plant with its biotic and abiotic environment [1]. Natural functions of these compounds include protection against UV radiation, scavenging of radicals, defense against phytopathogenic bacteria, fungi or viruses, or attraction of pollinators [2]. Characteristics such as anti-oxidative and antimicrobial activities render plant metabolites interesting for food applications. Secondary plant metabolites can be grouped into three major classes, namely phenols, terpenoids and alkaloids. Compounds of the same secondary metabolite class are typically synthesized from the same set of precursors originating from the primary carbon metabolism. Plant phenols (including the large class of polyphenols) are derived from aromatic amino acids, whereas terpenoids are produced from intermediates of glycolysis (either acetyl-CoA or pyruvate/glyceraldehyde-3-phosphate) [3]. Alkaloids are a structurally more diverse class of *N*-heterocycles, which are either derived from the three aromatic amino acids or from glutamate, aspartate or glycine [4]. Since most of the plant-derived compounds relevant for food applications are either phenols (*e.g.* phenylpropanoids, hydroxybenzoic acids, flavonoids, coumarins, and curcuminoids) or terpenoids (*e.g.* monoterpenes, sesquiterpenes, or diterpenes), we focus on these and do not discuss alkaloids further. A small number of relevant compounds, which cannot be assigned to phenols or terpenoids are glycosides, amino acids, proteins, or vitamins.

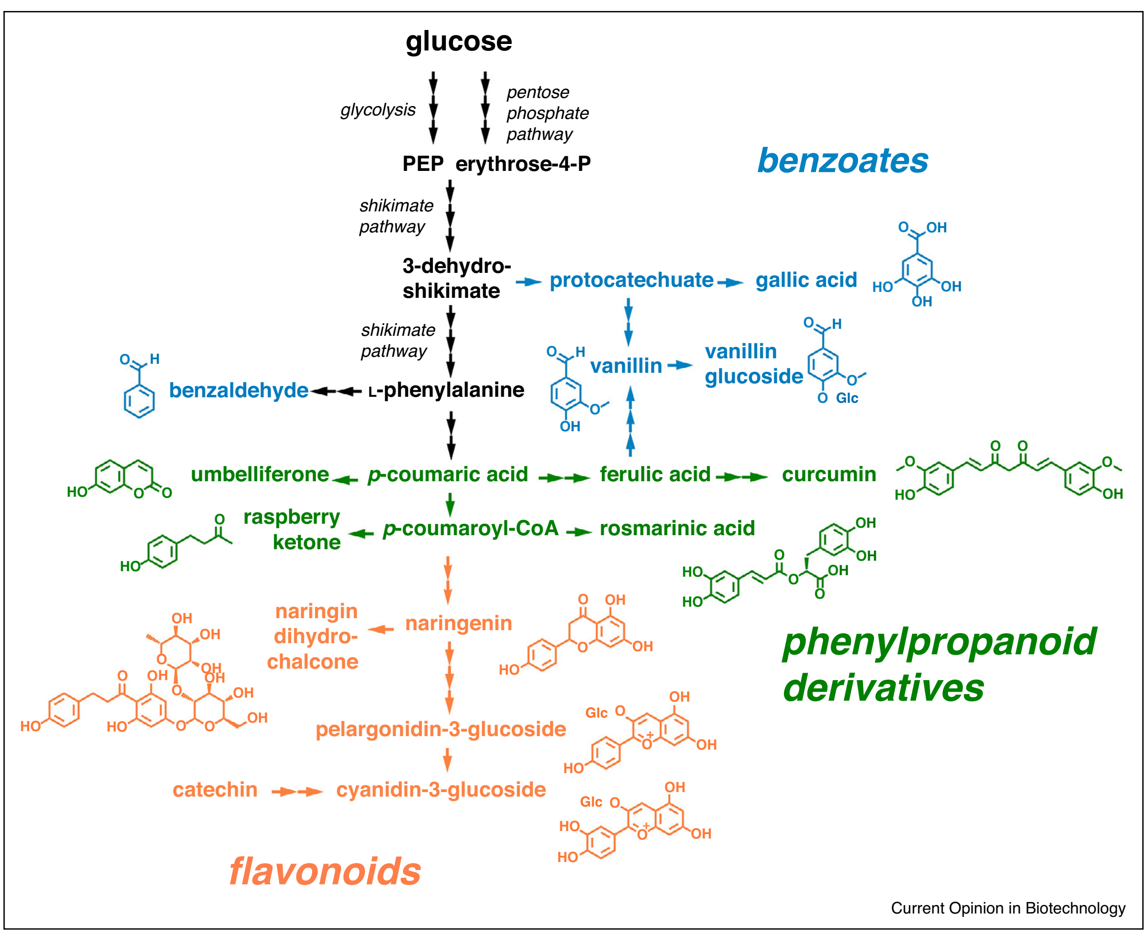
Today, most plant secondary metabolites are obtained by direct extraction from plant material. This strategy is only economically feasible for a very small number of plant-derived compounds. Extraction from plant material as a general strategy for getting access to these compounds is typically challenging because plants often contain complex mixtures of chemically closely related secondary metabolites. I addition, not every desired compound is produced at all times and in all plant tissues, and product concentrations are subject to seasonal and geographical variations. In contrast, microorganisms engineered for plant metabolite production represent a promising alternative as they reach high growth rates and can be easily cultivated in cheap cultivation media yielding high biomass concentrations.

In this review article we describe the current state of metabolic engineering of microorganisms for production of plant metabolites with applications in the food industries. We focus on compounds, which are already used in food applications, discuss current limitations of microbial plant metabolite production, and outline strategies on how these challenges can be addressed in the future. In the context of food applications, plant secondary metabolites can be classified in two ways: first, based on the same area of application or second, according to the natural compound class they belong to. Since the metabolic engineering strategy during microbial strain development largely depends on the respective precursor molecules, which need to be provided by the endogenous microbial metabolism, we organized the text according to the latter classification.

Phenolic compounds

Plant-derived phenols comprise a large family of aromatic compounds ranging from hydroxylated monocyclic benzoic acids to more complex polycyclic compounds such as stilbenes and flavonoids [5] (Figure 1). Natural monocyclic aromatics, which are used as flavoring agents, include, for example, vanillin (4-hydroxy-3-methoxy-benzaldehyde), benzaldehyde and raspberry ketone [4-(4-Hydroxyphenyl)-2-butanone]. Vanillin, the most important flavoring agent used world-wide, can in principle be extracted from beans of the vanilla orchid [6]. However, the low vanillin concentration in the natural producer renders large-scale vanillin extraction expensive. In fact, less than 1% of globally produced vanillin is obtained from the vanilla orchid (*Vanilla planifolia)* today, whereas most vanillin is produced with engineered microorganisms [6]. Usually, vanillin is produced by biotransformation of ferulic acid using *Escherichia coli*, *Pseudomonas fluorescens* or *Streptomyces sannanensis* as production hosts. To this end, microbial catabolic pathways for ferulic acid leading to vanillin are often exploited [7,8]. Alternatively, a combined cultivation process involving *Aspergillus niger* and *Pycnoporus cinnabarinus* [9–12] can be used. Noteworthy, the highest reported conversion yield of ferulic acid to vanillin was 75% in *Amycolatopsis* sp. [13,14]. In contrast, vanillin production from cheap glucose could be successfully established in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* [15•]. In this study, the natural aromatic amino acid-forming shikimate pathway was recruited for the production of the aromatic precursor protocatechuate, which was then converted to vanillin by

Figure 1



Biosynthetic pathways for plant-derived phenols relevant for food applications.