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Product-oriented flavor research: Learnings from the past, visions for the future

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In the past flavor research and the development of new flavorings were constantly driven by the interaction of flavor analysis, structure elucidation, and chemical synthesis accompanied by sensory. Highly potent flavor compounds were identified in numerous food products and helped to establish a powerful toolbox for flavorists. Nowadays we experience the merging of various scientific disciplines, for example medicine, biology, chemistry, and various technologies in the field of flavor research, which shows direct impact on our understanding of flavors. At the same time modern life has profoundly changed our eating habits. This situation generates new challenges for product development teams, which represent all facets of technologies. This paper will illustrate different examples for the evolution of product-oriented flavor research and future trends.

Keywords: Chiral analysis / MDGC/MS / Isotope Ratio Mass Spectrometry / Flavor / LC/MNR / Raman Spectroscopy

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1 Introduction

One of the first ever reported formulas is cited in the Holy Bible [1]. The use of combinations of essential oils, extracts, roots, and barks has a long history in the enjoyment of life, and in the middle ages many perfumers were driving the world of creative development. In the industrial age, the curiosity of chemists revealed the chemical nature of numerous flavoring substances. The so-called great cycle of the chemical industry, identification, laboratory synthesis, large-scale synthesis, and commercialization, introduced important aroma chemicals for example cinnamic aldehyde, benzaldehyde, methyl salicylate, coumarin, phenyl acetaldehyde, and vanillin between 1830 and 1890.

In 1874, Wilhelm Haarmann tried to overcome the limited availability of vanillin *via* a synthetic route starting with the cambial sap of fir trees, isolation of the glucoside of coniferyl alcohol, cleavage, and a chemical oxidation process [2]. The application of single aroma chemicals in multiple food flavorings showed immediate benefits with regard to impact and total intensity of flavored foodstuffs. For many years trained flavorists created products with prominent flavor top notes such as green apple liqueur or ice tea with peach flavor.

2 Materials and methods

For chiral analysis of 4- and 5-alkanolides the condensate water phases were analyzed and obtained after lyophilization of strawberries and raspberries from Germany and Poland.

2.1 Instrumental analysis

The enantio-GC/MS analyses of 4- and 5-alkanolides in strawberry and raspberry water phase were performed on a fused-silica column (25 m × 0.25 mm, film thickness 0.25 μm) from MEGA capillary columns laboratory (Legnano, Italy). The commercially available column was coated with heptakis (2,3-*O*-acetyl-6-*O*-*tert*-butylsilyl)-beta-cyclodextrin 30% in OV 1701.

For detection in SIM mode a GC/MS system (Agilent MSD 5973 equipped with GC HP 6890) was operated under positive chemical ionization conditions (PCI-SIM) with 25%

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Abbreviations: IRMS, isotope ratio MS methane as a reagent gas, temperature program 80–200°C, flow 1.0 mL He/min. The following SIM ions were selected.

SIM-Mass 4- and 5-C8: 83, 97, 125, 143; SIM-Mass 4- and 5-C9: 139, 157, 185, 197; SIM-Mass 4- and 5-C10: 153, 171, 199, 211; SIM-Mass 4- and 5-C11: 167, 185, 213, 225; and SIM Mass 4- and 5-C12: 181, 199, 227, 239.

^{1}H-NMR spectra were recorded on a Varian $^{\text{Unity}}$INOVA with 400 MHz in $CDCl_3$ at 25°C using TMS as internal standard. GC/MS experiments were performed using a MAT 8200 from Thermo Finnigan (70 eV ionization energy, EI mode) equipped with a DB-1 capillary column (30 m, film 0.25 µm, id 0.25 mm). The column temperature was programmed from 80 to 280°C at 4°C/min and helium was used as carrier gas. GC/FTIR was recorded on a BioRad Tracer System, consisting of a Hewlett Packard 5890 Series II gas chromatograph (DB-1, 60 m × 0.25 mm, film thickness 0.25 µm; 60–240°C at 4°C/min, carrier gas: helium), a 575C FTIR spectrometer, and a tracer unit. The Raman spectra were obtained from a FT Raman spectrometer Bruker RFS 100 (Bruker Optics, Ettlingen, Germany) with 500 mW excitation intensity at 1064 nm. Spectral resolution was 2/cm, and 50 scans were accumulated *per* Raman spectrum. Raman spectra were recorded from neat material in a sealed glass tube.

2.1.1 1,4 Diethyl tetrathiane

^{1}H-NMR (isomeric mixture, $CDCl_3$): $\delta = 1.07/1.08$ (*t*, $J = 7.2$ Hz, 6H), 1.78 (bs, 4H), 4.22 (bs, 2H); GC/MS (70 eV) *m/z* (%): 41(33), 45(43), 64(19), 73(100), 106(9), 138(29), 147(40), 170(1), 212(40); GC/FTIR $\tilde{\nu}$ (RI = 2137, /cm^{-1}) = 2962, 2927, 2868, 2844, 1454, 1374, 1331, 1281, 1219, 1162, 1081, 905, 804, 735; $\tilde{\nu}$ (RI = 2157, /cm^{-1}) = 2962, 2928, 2869, 2844, 1453, 1373, 1328, 1281, 1220, 1163, 1081, 904, 804, 712; Raman $\tilde{\nu}$ (isomeric mixture, /cm^{-1}) = 2967, 2929, 2897, 2851, 1436, 1022, 902, 805, 632, 606, 533, 513, 477, 379, 367, 341.

HPLC/NMR experiments were performed on a Varian $^{\text{Unity}}$I-NOVA system (400 MHz) using a $^1H\{^{13}C/^{15}N\}$PFG triple resonance indirect detection microflow LC/NMR probe (IFC probe) with a detection volume of 60 µL at 20°C. The HPLC system consisted of a ternary Varian ProStar 230 pump, a Varian ProStar 330 Photodiode Array Detector, and a Varian ProStar 510 column oven (50°C). Chromatographic separation was carried out on an OmniSpher C18 column (250 mm × 4.6 mm, particle size 5 µm) running gradients with ACN and D_2O (containing 0.01% TFA v/v). The chromatogram was acquired at 280 nm and UV-Vis spectra were measured in the range of 200–550 nm. Solvent suppression was employed by executing a WET pulse sequence [3]. Chemical shifts were referenced to ACN (1.93 ppm). LC/MS experiments were performed on a system consisting of a ThermoQuest LCQ mass spectrometer with an APCI interface and a Hewlett Packard HP 1100 HPLC system. APCI experiments were carried out in the positive mode. Nitrogen was used as sheath blanket gas. Gradients with H_2O (containing 0.01% formic acid) and ACN were run with a flow rate of 0.2 mL/min, and a Varian C18 column (125 mm × 2 mm, particle size 3 µm) was used for chromatographic separations.

2.1.2 Quercetin diglucoside

^{1}H-NMR (CH_3CN/D_2O) $\delta = 7.59$ (s, 1H), 7.57 (dd, $J = 8.3$, 1.7 Hz, 1H), 7.17 (d, $J = 8.3$ Hz, 1H), 6.49 (s, 1H), 6.26 (s, 1H), 5.05 (d, $J = 7.2$ Hz, 1H), 4.9 (d, $J = 7.7$ Hz, 1H), 3.78 (d, $J = 12.1$ Hz, 1H), 3.64 (dd, $J = 12.4$, 5.5 Hz, 1H), 3.36–3.56 (m, 7H), 3.23 (dd, $J = 9.0$, 9.0 Hz, 1H), 3.27 (dd, $J = 9.2$, 9.2 Hz, 1H), 3.08–3.14 (m, 1H); ^{13}C-NMR (CH_3CN/D_2O)*$\delta = 119.8$ (CH), 115.1 (CH), 113.7 (CH), 100.2 (CH), 98.3 (CH), 97.8 (CH), 93.1 (CH), 74.4 (CH), 72.5 (CH), 71.1 (CH), 67.8 (CH), 66.5 (CH), 59.9 (CH_2), 59.4 (CH_2); LC/MS (APCI pos.) = 627 $[M+H]^+$, 465 $[M+H-162]^+$, 305 $[M+H-2\times162]^+$. *Signals which could be clearly identified by a gHSQC experiment.

2.2 Isotope ratio MS (IRMS)

Traditionally, the determination of $^{13}C/^{12}C$, $^{18}O/^{16}O$, and $^2H/^1H$ ratios of organic molecules is based on the coupling of an IRMS with an elemental analyzer (EA) operating in the combustion (C) and the pyrolysis (P) mode, respectively. Nowadays, the on-line coupling of GC (HRGC) with IRMS through specific interfaces (for combustion and pyrolysis mode, respectively) enables the direct analysis of individual constituents of complex flavorings. The analysis of $^{13}C/^{12}C$ ratio is based on the conversion of carbon atoms in the molecules to CO_2 gas (combustion mode). After removal of water the carbon isotopic ratio is determined by simultaneous measuring *m/z* 44, 45, and 46. For measurements of $^{18}O/^{16}O$ and $^2H/^1H$ ratios, organic molecules are transformed to CO and H_2, besides nitrogen (pyrolysis mode). Isotopic ratio determination is performed by simultaneous monitoring *m/z* 28, 29, and 30 and *m/z* 2 and 3, respectively. Based on calibration of the system against standardized reference material, the isotope ratios of $^{13}C/^{12}C$, $^{18}O/^{16}O$, and $^2H/^1H$ are represented in *per* mil ($^0/_{00}$) deviation relative to the PDB and V-SMOW international standard as $\delta^{13}C_{PDB}$, $\delta^{18}O_{VSMOW}$, and δ^2H_{VSMOW} values. For details about sample preparation and IRMS setup, see literature [4–6].

3 Results and discussion

In order to avoid fraud in the trade of flavored and nonflavored food products, various techniques have been devel-

Table 1. Enantiomeric distribution of 4- and 5-alkanolides in concentrated strawberry water phase, samples A–F

(a)

	5-C8		4-C8		4-C9		5-C10	
	S(−)	*R*(+)	*R*(+)	*S*(−)	*R*(+)	*S*(−)	*S*(−)	*R*(+)
A	>60%	<40%	65.1	34.9	67.5	32.5	53.7	46.3
B	>60%	<40%	67.7	32.3	55.7	44.3	62.0	38.0
C	74.2	25.8	67.5	32.5	71.0	29.0	72.5	27.5
D	>60%	<40%	67.5	32.5	71.4	28.6	60.6	39.4
E	60.2	39.8	64.6	35.4	57.0	43.0	52.5	47.5
F	61.2	38.8	64.7	35.3	56.4	43.6	49.3	50.7

(b)

	4-C10		5-C12		4-C12	
	R(+)	*S*(−)	*S*(−)	*R*(+)	*R*(+)	*S*(−)
A	98.2	1.8	33.0	67.0	99.4	0.6
B	98.3	1.7	35.8	64.2	99.4	0.6
C	98.4	1.6	34.1	65.9	99.5	0.5
D	98.4	1.6	35.0	65.0	99.5	0.5
E	98.1	1.9	34.0	66.0	99.5	0.5
F	98.4	1.6	34.6	65.4	99.5	0.5

oped for the authenticity control of flavor compounds, essential oils, extracts, spices, and complete food systems. Besides GC/MS analysis, chiral separation of enantiomers and IRMS are receiving increasing attention [7]. The lactone chemistry of strawberries has been the subject of numerous studies [8].

Starting with derivatization and nonchiral column techniques, multidimensional GC equipped with chiral and especially cyclodextrin GC columns enabled a rapid determination of enantiomeric excess data. Lehmann *et al.* [9] identified trace amounts of 4-hexanolide and 4-octanolide in strawberries of the Elsanta variety. 5-Hexanolide was found to occur as a racemic mixture. In 2001, Kreck *et al.* [10] applied stir bar sorptive extraction (SBSE)-enantio-MDGC/MS for the analysis of fresh Andalusian strawberries and illustrated the dominance of the corresponding *R*-enantiomer of 4-octanolide, 4-decanolide, and 4-dodecanolide in pulp and jam. Recent studies on concentrated water phase obtained from strawberries and raspberries revealed the presence of 4-nonanolide, 4-octanolide, 5-octanolide, 5-dodecanolide in strawberry in trace amounts and with slight enantiomeric excess values. Kreck *et al.* [10] reported a purity of *R*-(+)-4-octanolide >99%, which however was not confirmed in this study. Table 1a shows an average ratio of approximately 66.2:33.8 (*R*:*S*).

The situation looks similar for the trace analysis of alkanolides in raspberry water phase (Table 2).

Table 2. Enantiomeric distribution of 4- and 5-alkanolides and α-ionone in raspberry waterphase

(a)

	5-C8		4-C8		5-C9		4-C9	
	S(−)	*R*(+)	*R*(+)	*S*(−)	*S*(−)	*R*(+)	*R*(+)	*S*(−)
G	99.4	0.6	49.4	50.6	nd	nd	38.1	61.9
H	>99.5	<0.5	49.6	50.4	nd	nd	38.3	61.7
I	>99.6	<0.6	50.9	49.1	nd	nd	33.4	66.6
J	>99.5	<0.5	49.1	50.9	nd	nd	34.0	66.0
K	>99.5	<0.5	49.7	50.3	Trace	nd	35.4	64.6

(b)

	5-C10		4-C10		4-C12		α-Ionone	
	S(−)	*R*(+)	*R*(+)	*S*(−)	*R*(+)	*S*(−)	*R*(+)	*S*(−)
G	99.0	1.0	>90	<10	>90	<10	99.4	0.6
H	99.1	0.9	Trace	nd	92.5	7.5	98.9	1.1
I	98.9	1.1	>90	<10	95.7	4.3	99.4	0.6
J	99.0	1.0	>90	<10	96.3	3.7	99.4	0.6
K	99.1	0.9	>90	<10	93.9	6.1	>99.5	<0.5

The occurrence and the enantiomeric distribution of 4- and 5-alkanolides in concentrated strawberry and raspberry water phase samples were confirmed by enantioselective MDGC/MS (full scan mode).

The data for alkanolides in strawberries and raspberries are in good agreement with the data published in the thesis of Bernreuther [11].

Analysis of caffeine (1,3,7-trimethylxanthine) using multielement IRMS techniques was successfully applied for the provenance assignment of green coffee (*Coffea arabica*) [4] as well as for authenticity control of Guarana (*Paullinia cupana*), tea (*Camellia sinensis*), and maté (*Ilex paraguariensis*) [5, 6]. In conclusion, and especially based on the $\delta^{18}O_{VSMOW}$ values of caffeine, highly significant discrimination between caffeine of natural sources (green coffee, maté, green tea, black tea, Guarana seeds) and synthetic origin was obtained. Using statistical evaluation tools like LDA and CART on the basis of the $\delta^{18}O_{VSMOW}$ and $\delta^{2}H_{VSMOW}$ values of caffeine, geographical origin assessment of green coffee beans (samples from North and South America, Africa, Indonesia, Jamaica and Hawaii) could be performed at very low error rates. Latest research for strategies authenticating coffee of different botanical origins resulted in a methodology for chemical discrimination of arabica and robusta coffee by FT Raman spectroscopy [12].

In parallel flavor research also followed multiple pathways. The ongoing search for new powerful aroma chemicals relies continuously on improved analytical tools like GC/MS. Prominent examples are the analysis of trace compounds in buchu leaf oil [13] and sulfur compounds in Scheurebe wine [14].

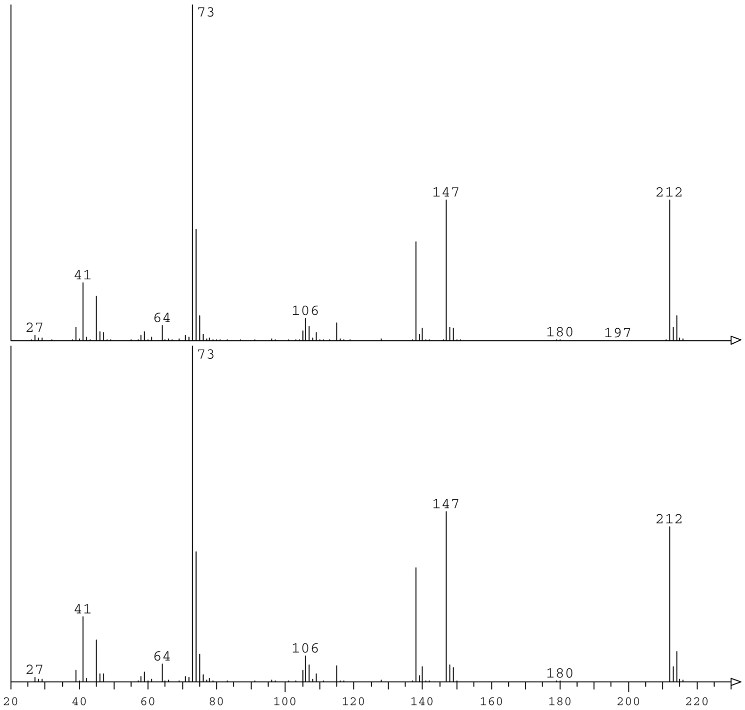

Figure 1. Mass spectra of *cis*/*trans* isomeric 1,4-diethyl tetrathianes, retention index 2137 and 2157, 60 m DB-Wax.

Modern mint research revealed numerous powerful aroma compounds, which provide together with l-menthol, both fresh breath and a pleasant mouthfeel in oral care, chewing gum, and sweet applications [15].

A continuous completion of the jig saw puzzle of compound details for many fruits and vegetables enabled a better understanding of the biochemical pathways leading to the formation of flavor compounds and revealed the relevance of precursor molecules such as the furaneol glucoside in tomatoes [16]. At the same time degradation processes and flavor stability are of high interest for modern product development and flavor research as well. The thermal treatment of paprika powder leads to the breakdown of colored carotenoids and gives rise to powerful flavor compounds [17].

Process flavors based on the reaction of amino acids and sugars in the so-called Maillard reaction stimulate the development of sweet flavor profiles as well as savory notes. Besides the formation of pyrazines and Strecker aldehydes, numerous heterocyclic compounds such as thiazolines and thiazolidines were studied [18].

Powerful tools in flavor research such as solvent-assisted flavor evaporation (SAFE) [19] and, in particular, methodologies like the aroma extract dilution analysis (AEDA) were established as valuable tools for the investigation of complex systems like culinary preparations. Recent studies on shallots, fried in palm oil at 120 and 160°C, showed major differences in the pattern of volatile flavor compounds (Table 3), based on the selection of compounds with a flavor dilution factor of 512 [15].

In this context Raman spectroscopy was applied for the structure elucidation of diethyl tetrathianes, highly symmetrical sulfur compounds, which were found in cooked onions as well as in shallots fried at 160°C in palm oil by GC/MS. Figure 1 shows the mass spectra of the *cis*/*trans* isomers of 1,4-diethyl tetrathiane.

The Raman spectrum in Fig. 2 shows bands due to *S*-*S* vibrations at 533 and 514/cm and due to *C*-*S* vibrations at 632 and 606/cm [20]. Raman spectra of the isomers purified by preparative GC of 1,4-diethyl tetrathianes and the related 1,4-dimethyl tetrathianes revealed that the Raman band at 533/cm is assigned to the *trans*-isomer whereas the Raman band at 514/cm is assigned to the *cis*-isomer. The absence of a Raman band near 490/cm which is a marker for *S*-*S*-*S* vibrations is consistent with the absence of 1,3-diethyl tetrathianes in significant amounts. Details will be reported separately [21].

In this study both the volatile and the nonvolatile flavor chemistries of culinary Allium preparations were included in order to achieve a comprehensive understanding of the aroma and taste chemistry. LC Taste®, a newly introduced tool based on high-temperature LC for the ortho and retro nasal evaluation of aroma and taste compounds showed the presence of several bitter compounds.

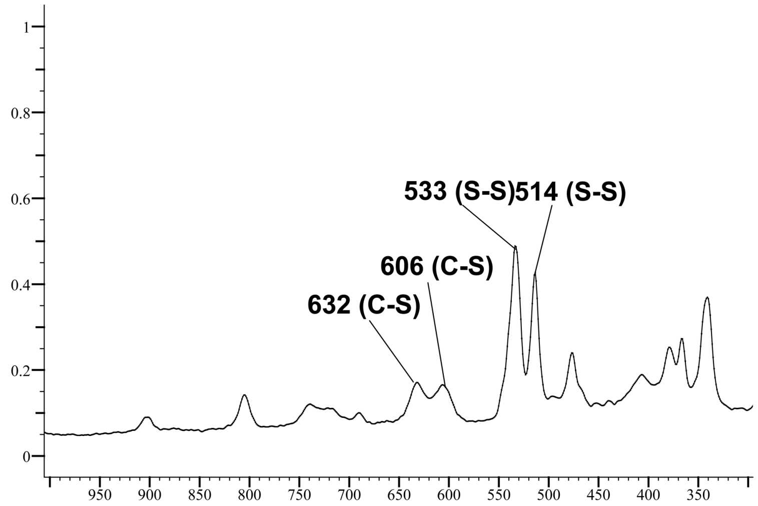

Figure 2. Raman spectrum from 1000 to 300/cm of a mixture of *cis*/*trans* 1,4-diethyl tetrathianes. Raman bands discussed in the text are labeled.

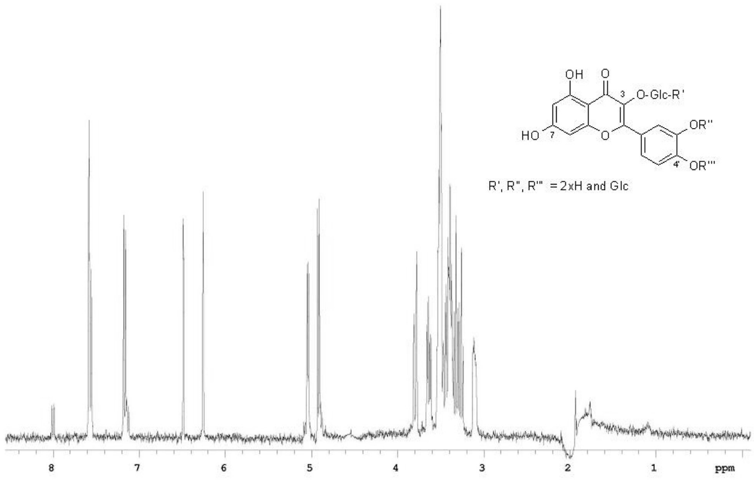

Figure 3. ^{1}H-LC/NMR spectrum from a fried shallot LC fraction.

Table 3. Volatiles detected upon frying shallots in palm oil at 120 and at 160°C

	Compound selected *via* AEDA FD 512	120°C/ppm	160°C/ppm
1	methyl 1-propenyl sulfide	<0.01	0.03
2	methyl (1*E*)-propenyl disulfide	0.05	2.02
3	6*D*-dimethyl trisulfide (standard)	1.00	1.00
4	dimethyl trisulfide	<0.01 (FD 128)	2.93
5	(1*E*)-propenyl propyl disulfide	0.32 (FD 128)	1.28
6	2-ethyl-3,6-dimethyl-pyrazine	n.d.	6.5
7	2–ethyl-3,5–dimethyl-pyrazine	n.d.	0.74
8	Unknown: *m/z* 87, 116, 45, 39, 53, contains *S*	n.d.	0.49
9	Tentatively: 2-ethyl-3,5,6-trimethyl pyrazine	<0.01	0.66
10	methyl propyl trisulfide	0.09	2.05
11	2-nonanol (standard for quantification)	1.00	1.00
12	2-isobutyl-3,5–dimethyl-pyrazine	n.d.	<0.3
13	Tentatively: methyl 1-propenyl trisulfide	<0.02	Approximately 1.5
14	5-methyl-6,7-dihydro-5H-cyclopentapyrazine	<0.01	0.44
15	(2*E*,4*E*)-decadienal	<0.01	0.6
16	4-hydroxy-2,5-dimethyl-(2*H*)-furan-3-one	0.01	1.28

LC/NMR coupling becomes an increasingly valuable tool in the investigation of natural products [22] allowing a reliable characterization of the main components in extracts or enriched fractions. LC/NMR and LC/MS studies on a preparative LC fraction of Allium species, showing a slightly bitter and astringent organoleptic profile, revealed a quercetin diglycoside, which has been tentatively assigned (Fig. 3).

Using higher magnetic fields and cold probe technology as well as applying the broad range of 1D- and 2D-NMR experiments full structure elucidation of the compounds of interest will be more accessible.

Bitter compounds play an ambivalent role in many foodstuffs. In numerous alcoholic beverages bitter notes are highly appreciated. In sweet applications bitter notes originating from ingredients like caffeine are subject to masking with higher sweetness levels or taste modifiers. The evaluation of bitter masking flavanones from Herba Santa (*Eriodictyon californicum* (H.&A.) Torr.), Hydrophyllaceae showed that the flavanones homoeriodictyol, its sodium salt and eriodictyol have remarkable bitter masking effects without exhibiting any additional strong taste or flavor [23]. This finding underlines the importance of flavor modifiers for tailor-made flavor and product development.

Functional ingredients, vitamins, and nutrients are of steadily increasing importance for consumer acceptance. Healthy raw materials such as fish oils, phytosterols, isoflavone-enriched soy products create a strong need for intelligent flavor solutions, smart formulation technologies like encapsulation, and a strong interaction between flavor research, nutrition sciences, physiology, and food technology.

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