Novel strategies of addressing increasing complexity in flavour research

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Abstract

Foods and beverages are highly complex systems in terms of composition and chemical and material changes during processing. The expected quality and benefits represent a delicate balance between sensory properties (aroma, taste, mouthfeel, texture), nutrition, health, and safety. This calls for a holistic and system-type approach to obtain the best product quality. Therefore, it is important to consider chemical and physical interactions, to study the formation kinetics of both desired and undesired compounds, and to know more about the release of bioactive compounds from the food and beverage matrix including the consumption event as well as during digestion and resorption. This requires sophisticated experimental setups, the use of non-targeted ("omics"-type) analytical methods and advanced data processing, working at the interface of scientific disciplines and establishing correlations between product quality and consumer benefits.

Introduction

Flavour research has been a key activity in academia as well as in flavour and food industry. Many key odorants and taste compounds have been identified, their sensory characteristics described, their formation mechanisms studied using thermal and/or bio-assisted approaches, and ways for their formulation and controlled release developed. For a long time, the discovery of new molecules has been the primary focus, using targeted analytical methods as well as synthetic chemistry. More recently, high-throughput receptor-based assays have been designed for the screening of taste-active components. While identifying new sensorially relevant molecules will remain an active area of interest, generating and delivering the desired, complex, and well-balanced flavour profile by natural means and mild processing has become a major focus. This paper briefly describes new approaches of dealing with increasing complexity in flavour research and options to transform challenges into opportunities.

Results and discussion

Flavour Chemistry. Our understanding of complex phenomena in food chemistry is largely based on the attempt to simplify intricate systems and to study individual phenomena in model systems, i.e. typically lipid oxidation and Maillard-type reactions. This approach has led to major breakthroughs highlighting the reaction mechanisms and relevant parameters of control. However, in food matrices these reactions cannot be seen in isolation, as food is composed of many different chemical entities such as lipids, carbohydrates, amino acids, peptides and proteins, but also polyphenols, alkaloids, vitamins, terpenoids, minerals, etc. They interact at various stages of different reaction cascades and influence and shape food properties and quality attributes such as aroma, taste, colour, texture and the nutritional profile. In this respect, chemical transformations taking place in foods can be regarded as a subset of "systems chemistry". In the following, complex food chemistry will be depicted from different perspectives, such as *i)* chemical

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interactions, *ii)* food as a complex system, *iii)* formation of defined molecules from various precursors, and *iv)* flavour generation in self-assembly systems.

As examples, amino acid degradation products characteristic for Maillard-type reactions (e.g. Strecker aldehydes, thermogenic amines, vinylogous compounds) can be produced in the presence of lipid oxidation products such as α,β-unsaturated aldehydes [1]. Lipid-derived reactive aldehydes can also be replaced by polyphenols as shown for the Strecker degradation of phenylalanine in the presence of *o*- and *p*-diphenols [2]. Epicatechin reactions have been shown to influence the mechanism of Maillard product formation in low moisture systems [3]. Hydroxyhydroquinone, a degradation product of chlorogenic acids, is trapping 2-furfurylthiol (FFT), a character-impact odorant of coffee aroma, in the presence of transition metals, thus changing the overall coffee aroma from fresh to stale [4]. The triple role of polyphenols has recently been discussed, resulting in a multitude of chemical interactions based on their chelating, free radical-scavenging, and carbonyl-trapping regions [5].

Looking at food as a complex system, it is mandatory to perform studies not only in simplified model systems but in real food matrices. Coffee constitutes one of those examples. The coffee bean can be seen as a mini-reactor. Consequently, the most appropriate approach studying chemical transformations upon roasting is using the coffee bean itself as a reaction system. Therefore, it is not surprising that the formation of FFT in coffee is different from what we learned from the respective model systems. It has been shown that FFT is generated in arabinose/cysteine model systems *via* 3-deoxypentosone and furfural maintaining the intact carbon chain [6]. However, 'in-bean' experiments using fully ^{13}C-labelled arabinose resulted in only 1% fully labelled FFT upon coffee roasting while almost 90% of the FFT formed was not labelled at all [7]. This strongly suggests alternative formation pathways of FFT in coffee, which are still not well understood.

A specific molecule can derive from one individual source material or, on the other side, from many different precursors. As an example and depending on the food composition, furan might be formed from various sugars, amino acids, polyunsaturated fatty acids (PUFAs), carotenoids, and ascorbic acid [8, 9]. Therefore, it is mandatory to screen for all potential sources to mitigate the formation of this undesirable compound during food preparation. Contrastingly, acrylamide is primarily formed from asparagine as a well-defined precursor [10] while 2,4-decadienal is known as a lipid degradation product of PUFAs. As recently shown [11], the choice of the oil in combination with heat treatment has a strong impact on the level of acrylamide and flavour active components (2,4-decadienals) exhibiting deep fried notes (Table 1). Therefore, it is recommended to study the formation of undesirable and desirable compounds in parallel in order to enable mitigation while delivering desired sensory properties.

Table 1: Concentrations (µg/kg) of acrylamide and 2,4-decadienal (sum of the (*E*,*E*)- and (*E*,*Z*)-isomers) in potato chips after deep-frying at 180 °C and 140 °C for 2.5 min

Odorant	*Safflower oil 180 °C*	*Safflower oil 140 °C*	*Linseed oil 180 °C*	*Linseed oil 140 °C*
Acrylamide	160	94	1690	1240
2,4-Decadienal	4697	468	321	46

A characteristic feature of systems chemistry is the formation of self-assembled structures, also referred to as mesophases, which can be observed in many food products. Molecular organisation of flavour precursors can play an important role in food systems

containing ingredients that tend to form self-assembly structures, as for example in reversed microemulsions. This may lead to increased yields in flavour formation due to favouring certain formation pathways and increased flavour stability by protecting labile flavour compounds in compartments of the structured medium. As shown in Figure 1, the yield of FFT generated from xylose (Xyl) in the presence of cysteine (Cys) increased continuously during the entire heating period in both reaction media. However, highest FFT yields were obtained in the mesophasic system as compared to phosphate buffer [12].

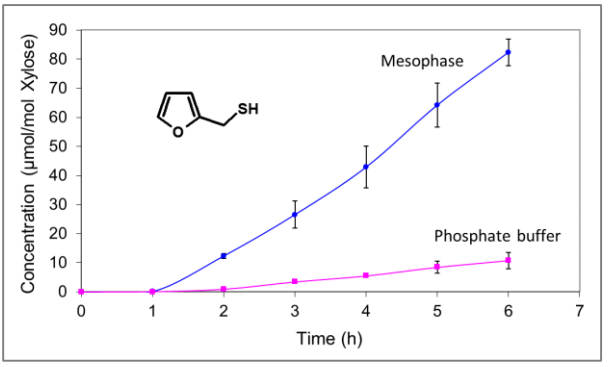

Figure 1: Formation of 2-furfurylthiol (FFT) from Xyl/Cys in phosphate buffer and in self-assembled structures (mesophase: reversed microemulsion) at 95 °C and as a function of time.

In such structures, three domains of submicrometre size are present, i.e. aqueous, amphiphilic, and lipophilic. A given molecule is preferably dissolved in one particular domain and may display a specific spatial orientation. When two molecules are located in the same domain (e.g. amphiphilic domain), their concentration is higher, thus increasing the probability of reaction. On the other hand, a molecule in the aqueous domain is unlikely to react with a molecule in the lipid domain. As a result, this domain fragmentation (compartmentalization) may favour certain reactions while inhibiting others. For Maillard-type reactions involving cysteine and xylose, both reactants are hydrophilic whereas the reaction products are more lipophilic, such as FFT for example. Thus, this type of reactions in mesophases may lead to high yields considering that the product concentration in water will remain low, as formed FFT will migrate into the lipophilic domain once generated. Furthermore, isolation of FFT in the lipid domain may protect it against reactants present in the aqueous media.

Advanced Analytics. Novel insights and the data quality obtained usually correlate with the advancement in analytical techniques applied. New key odorants and tastants have been identified thanks to sensory-guided chemical analyses, i.e. GC-Olfactometry [13, 14] and LC-Taste [15]. Quantitative results can be obtained using the Stable Isotope Dilution Assay (SIDA) method [16]. Reaction mechanisms can be elucidated using labelling experiments and the relative importance of concurrent pathways estimated by the carbon module labelling (CAMOLA) technique [17]. These techniques, e.g. primarily targeted methods, have contributed to major new discoveries and our current understanding of flavours.

We have applied the CAMOLA technique in kinetic studies to study the formation of 2,3-butanedione (diacetyl) from various precursors [18]. Figure 2 shows the formation of diacetyl from sucrose and other sources, e.g. bound carbohydrates. While the total amount of diacetyl is constantly increasing over time and with roasting degree (up to seven minutes and in particular after three minutes), sucrose is progressively losing

importance as a source of diacetyl in favour of other precursors (e.g. bound carbohydrates). Furthermore, the contribution of the intact carbohydrate skeleton decreases with increased roasting level (data not shown) due to fragmentation favoured at higher temperatures. These data give a new insight into the relative role of various formation pathways, which are the base for adapting process conditions and selecting raw materials. Understanding the relative importance of various alternative reaction pathways helps to single out the relevant formation patterns and to identify how they could potentially be influenced *via* adapted processing conditions.

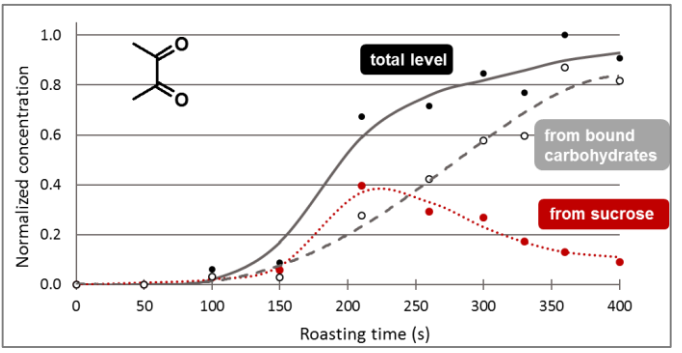

Figure 2: Formation of 2,3-butanedione (diacetyl) upon coffee roasting obtained in a CAMOLA study using ^{13}C-labelled and unlabelled sucrose in a 1:1 ratio.

The techniques mentioned above are suitable to study known compounds and relationships in a targeted manner. However, they show some limitation when it comes to unknown molecules and intricate formation pathways. Data independent acquisition (DIA) of mass spectrometry (MS) data has been proven a very effective tool in Life Sciences to unravel complex correlations and, thus, identify new molecular targets and mechanistic relationships. In the food context, they may correlate with phenomena of interest such as aroma, taste, and health benefits. The sequential window acquisition of total high-resolution mass spectra (SWATH-MS) method is measuring all signals in one run. SWATH-MS is a DIA method that generates, in a single measurement, a complete recording of the fragment ion spectra of all analytes in a sample for which the precursor ions are within a predetermined *m/z* versus a retention time window [19]. SWATH-MS results in a digital fingerprint of the sample (digital twin) allowing retrospective data interpretation. It can be used as a new method in food and flavour research to compare differences between samples and changes upon processing. Targeted analysis can subsequently be performed with a focus on significant chemical differences. This untargeted method is considerably gaining importance in food research as a complementary approach to targeted molecular characterization. It is frequently associated with the term “foodomics” as shown at the recent RAFA symposium [20].

Release Phenomena. Aroma and taste components present in foods and beverages need to reach the respective receptors in order to elicit the desired aroma note or taste response. One critical step is the release of those aroma- or taste-active molecules during mastication in the mouth and their transport in the saliva. In-mouth release phenomena are studied with the aim of maximizing the inherent flavour potential of sensory-active components before they are being swallowed [21, 22]. During this in-mouth process, flavour compounds are progressively released from the food matrix. This phenomenon is mainly dependent on food texture, composition, in-mouth breakdown, and on

impregnation with saliva. The saliva composition and its activity may represent another opportunity of influencing flavour perception. As all these factors will affect release kinetics, this could potentially be an option to reduce the amount of ingested sodium and sugar while maintaining the desired taste characteristics.

Tailored design of materials in the solid state, for instance as a co-crystal, constitutes a novel concept to modulate taste perception. Co-crystals are little known in the food industry [23], however, co-crystallization as a concept has been broadly applied in the pharmaceutical industry to improve solubility and bioavailability of the respective active compound. In a food context, modulating dissolution kinetics could be of interest for delivering salt and sweet taste through the use of co-crystalline salt and carbohydrate materials. Co-crystals of glucose and NaCl are well known in the literature and easy to obtain *via* direct crystallization from aqueous solution [23]. Synthetic protocols to obtain co-crystals of sucrose and NaCl have not been described previously. This material is preferably accessible *via* isomorphous seeding with the co-crystalline NaBr heterologue: their synthesis and physico-chemical characterisation have recently been reported [24].

The dissolution kinetics in saliva are key for the sensory perception of water-soluble tastants consumed in the solid state, e.g. salt and sugar. This concept has been explored in the past *via* micronization, e.g. using powdered sugar or dusted salt. Interestingly, co-crystalline formulations can display faster dissolution properties, possibly giving rise to a stronger taste impact. Figure 3 shows the dissolution kinetics of pure NaCl, anhydrous glucose, glucose monohydrate and the respective co-crystal $(Glucose)_2 \cdot NaCl \cdot H_2O$, the structure of which is presented in the bottom right of Figure 3.

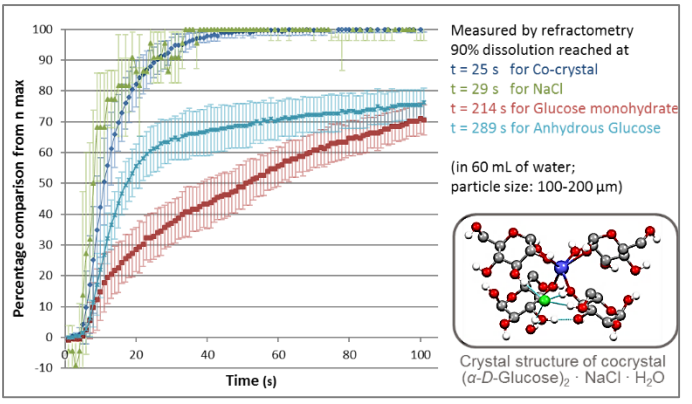

Figure 3: Dissolution kinetics of co-crystalline glucose sodium chloride *vs.* its individual pure ingredients indicating that the co-crystal dissolves faster than glucose or glucose monohydrate alone.

This co-crystalline material dissolves faster compared to pure glucose or pure glucose monohydrate alone, taking into account parameters like crystal size, crystal size distribution, concentration and molar composition. It dissolves comparably to NaCl with respect to kinetics. From a sensory perspective, the salt taste perception is much stronger than the simultaneously perceived faint sweetness of glucose. However, the co-crystal also dissolves faster than a simple dry-mix of glucose with NaCl. Therefore, such co-crystalline forms of NaCl could potentially offer a boost of saltiness, as carbohydrates are omnipresent in food products [25].

In conclusion, flavour research is facing an increasing complexity. Product quality is not only depending on one individual attribute, e.g. aroma, but on a multitude of

features (e.g. taste, mouthfeel, texture) which need to be well balanced. In addition, it is equally important to ensure nutrition and to maintain or develop health benefits (e.g. appropriate amount of carbohydrates, minerals and lipids), as well as mitigation of process contaminants. While the concurrent study of all these phenomena represented a clear challenge in the past, we have got emerging analytical techniques from Life Sciences using extensively non-targeted methods ("omics"). Their transfer and application to food science ("foodomics") has become a trend and it is an excellent opportunity to connect flavour research with other disciplines delivering additional benefits. Another recent development of equal importance stems from Material Sciences allowing the use of tailored solid-state structures to better master flavour formation and release.

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