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Analytical Chemistry in Food Science

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Abstract. Analytical chemistry presents a traditional and important tool in food science. Its development grew out of governmental food control to verify food composition and wholesomeness. In modern food chemistry, analytical methods are applied to study food constituents, additives and contaminants and their interactions and reactions during processing and storage. Research activities of the Food Chemistry group at ETH comprise investigations on carbohydrates and on chiral flavour compounds. Experimental work in food technology relies on simple analytical methods that are suitable for large series of processing trials. Such methods are used by the Food Technology group to optimize lipid stability of heat sterilized meat and hot-air roasted nuts. Analytical chemistry is taught at ETH in lectures and laboratory courses to all food science majors.

Introduction

The production and distribution of wholesome and palatable food in sufficient amounts belongs to the prerequisites of human existence. While in the old days food preparation and preservation was mainly a task for the individual household and the small trade, industrialisation has caused a continuous shift of these activities to the industrial scale. Industrialisation also brought about changes in lifestyle which in turn stimulated developments such as the marketing of convenience foods in ready-to-cook or ready-to-eat form, or the setup of an increasing number of catering operations to meet the demand for out-of-home eating of a large proportion of the population. Furthermore, industrialisation has lead to a dense and efficient network of worldwide trade and exchange of food produce, without having been able, however, to solve the never-ending problem of an unbalanced world food market. Therefore, food shortage for an increasing portion of the world population has become one of the most important demographic, economic and political issues in many areas of the world.

There is no question that the complexity of the many problems of present and future food supply asks for an increasing effort in many areas of food science. Food science as an academic field started to emerge after the Second World War and today comprises disciplines such as food chemistry, engineering, technology, microbiology and biotechnology. However, activities in some disciplines related to foods are much older. Analytical chemistry belongs to these traditional fields, mostly in conjunction with food control from the legislative point of view. It is, therefore, not surprising that one of the oldest Swiss journals in chemistry, the '*Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene*', published investigations on analytical chemistry and its application to food ever since its foundation. The 'Schweizerische Gesellschaft für Angewandte und Analytische Chemie', now 'Schweizerische Gesellschaft für Lebensmittel- und Umweltchemie', presents the oldest professional chemical society in Switzerland and has indeed always played a key role in representing and promoting analytical chemistry as applied to food.

As will be shown elsewhere in this series, analytical chemistry is still the most important basis in the activities of governmental and non-governmental food control. Likewise, analytical chemistry is indispensable in food science research and education. Therefore, it also belongs to the core topics of food chemistry and technology on the university levels. The description of selected current research projects may illustrate the developments and application of analytical chemistry in food chemistry and in food technology at ETH-Zürich. In addition, some information about teaching analytical chemistry for food science majors is given.

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Analytical Chemistry in Food Chemistry

Food chemistry comprises not only the knowledge of chemical structures of food constituents, food additives and contaminants, but the understanding of interactions between the different compounds present in food and of the chemical reactions that occur during processing and storage of food as well. The use of analytical tools is, therefore, of great importance in food chemistry. This is of course also true for all research projects carried out by the Food Chemistry group of the Institute of Food Science at ETHZ. Out of the broad range of topics covered, some considerations related to projects in carbohydrate and in flavour chemistry, respectively, will be presented.

Carbohydrate Analysis

Carbohydrates represent a qualitatively and quantitatively important group of food constituents. On the one hand, carbohydrates are responsible for several sensory properties of food. Low molecular weight carbohydrates (mono- and disaccharides, the so-called simple sugars) such as glucose, fructose and sucrose taste sweet, whereas intermediate (oligosaccharides) and high molecular weight carbohydrates (polysaccharides) such as dextrins, starch, plant cell wall polysaccharides or gums contribute to the texture or consistency of food. On the other hand, digestible carbohydrates represent the most important energy source for all living organisms (microorganisms, plants and animals) and, therefore, are of outstanding nutritional importance. The overall carbohydrate content of food is generally determined spectrometrically after acid treatment of polysaccharides (*e.g.*, in conc. sulphuric acid) and coupling of the liberated and degraded monosaccharides (*e.g.*, furfural, hydroxymethyl furfural) to, *e.g.*, anthrone, orcinol or phenol. Monosaccharides are routinely quantified either by gas-liquid chromatography (GC) after derivatisation to alditol acetates, aldononitrile acetates or trimethylsilyl (TMS) derivatives, respectively, or by high performance liquid chromatography (HPLC). In addition, capillary electrophoretic methods for the qualitative and quantitative determination of mono-, di- and oligosaccharides have been developed recently. The most common mono- and disaccharides (glucose, fructose, sucrose, *etc.*) can also be quantified by enzymatic methods (*e.g.*, by using the hexokinase/glucose-6-phosphate dehydrogenase or the glucose oxidase method for glucose determina-

tion). The Food Chemistry group of the Institute of Food Science has been involved in the development of carbohydrate analysis at different levels and stages. A reinvestigation of the derivatisation of monosaccharides as aldononitrile peracetates resulted in the detection of by-products which necessitated a comment on the scope and limitation of this reaction for the purpose of quantification [1]. A high performance anion exchange chromatography method with pulsed amperometric detection (HPAEC-PAD) was adapted for quantification of monomeric constituents of polysaccharides obtained by acid hydrolysis from insoluble and soluble dietary fibre [2]. In collaboration with the research group of the late Prof. *H.M. Widmer* (*Ciba*, Corporate Analytical Research, Basel), methods for the determination of mono-, di- and oligosaccharides by capillary electrophoresis were developed that were mainly based on derivatisation of reducing end groups with 8-amino naphthalene-1,3,6-trisulfonic acid (ANTS) and laser-induced fluorescence detection [3][4]. The main efforts in carbohydrate research in our group is focused on the analysis of plant cell wall components, especially pectic substances. This group of polysaccharides is known to be responsible for the texture of fruits and vegetable products together with cellulose and hemicelluloses. In order to understand the changes that occur during ripening and senescence of plant tissues, *e.g.*, tissue softening and maceration, it is important to investigate the structures of the cell wall polysaccharides at different stages of maturity and ripeness of fruits and vegetables [5–7]. Structural investigations on isolated pectic substances from apples of different degrees of ripeness are carried out by combining biochemical methods like specific partial enzymatic degradation of pectic substances, followed by classical methods used in carbohydrate analysis, like determination of the monomeric constituents by GC and/or HPLC and methylation analysis (GC/MS) to determine the linkage positions of the monomers in the polysaccharide. Finally, specific enzymes are used to establish the configuration of the glycosidic linkages. The building up of a library for all partially methylated alditol acetates (PMAA) of the monomers potentially present in cell walls was of primordial importance for the correct assignment of the linkage positions. As an example, in *Fig. 1* the gas chromatogram of all PMAA of galactose and in *Table 1* the corresponding retention times and linkage types in polysaccharides are shown. The resolution of double-peaks such as 3/

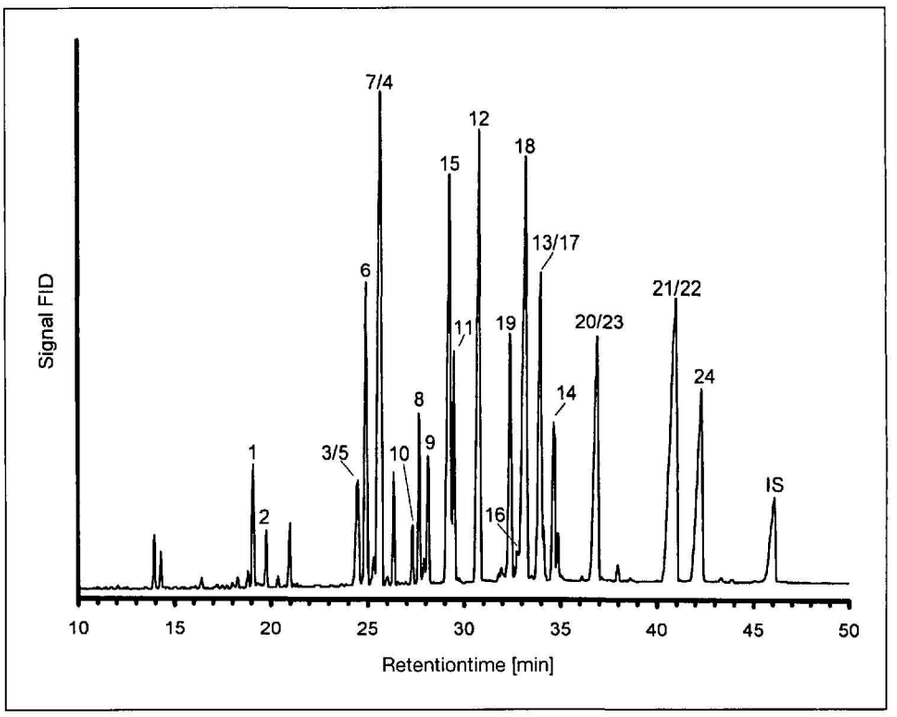

Fig. 1. *Gas chromatogram of the permethylated galactitol acetates as an example for the library of partially methylated alditol acetates used in plant cell wall analysis.* Peak numbering corresponds to the numbers given in *Table 1*. IS = Internal standard (inositol hexaacetate).

Table 1. *Relative Retention Times and Linkage Types of Partially Methylated Galactitol Acetates.* The numbering corresponds to the one shown in *Fig. 1*.

No.	PMAA-Derivative	t_R	Linkage type	
1	1,4-Di-*O*-acetyl-2,3,5,6-tetra-*O*-methylgalactitol	0.412	Gal*p*-T	
2	1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol	0.427		Gal*f*-T
3	1,2,4-Tri-*O*-acetyl-3,5,6-tri-*O*-methylgalactitol	0.527		1,2-Gal*f*
4	1,2,5-Tri-*O*-acetyl-3,4,6-tri-*O*-methylgalactitol	0.554	1,2-Gal*p*	
5	1,3,4-Tri-*O*-acetyl-2,5,6-tri-*O*-methylgalactitol	0.530		1,3-Gal*f*
6	1,3,5-Tri-*O*-acetyl-2,4,6-tri-*O*-methylgalactitol	0.537	1,3-Gal*p*	
7	1,4,5-Tri-*O*-acetyl-2,3,6-tri-*O*-methylgalactitol	0.552	1,4-Gal*p*	1,5-Gal*f*
8	1,4,6-Tri-*O*-acetyl-2,3,5-tri-*O*-methylgalactitol	0.598		1,6-Gal*f*
9	1,5,6-Tri-*O*-acetyl-2,3,4-tri-*O*-methylgalactitol	0.608	1,6-Gal*p*	
10	1,2,3,4-Tetra-*O*-acetyl-5,6-di-*O*-methylgalactitol	0.590		1,2,3-Gal*f*
11	1,2,3,5-Tetra-*O*-acetyl-4,6-di-*O*-methylgalactitol	0.636	1,2,3-Gal*p*	
12	1,2,4,5-Tetra-*O*-acetyl-3,6-di-*O*-methylgalactitol	0.662	1,2,4-Gal*p*	1,2,5-Gal*f*
13	1,2,4,6-Tetra-*O*-acetyl-3,5-di-*O*-methylgalactitol	0.731		1,2,6-Gal*f*
14	1,2,5,6-Tetra-*O*-acetyl-3,4-di-*O*-methylgalactitol	0.749	1,2,6-Gal*p*	
15	1,3,4,5-Tetra-*O*-acetyl-2,6-di-*O*-methylgalactitol	0.629	1,3,4-Gal*p*	1,3,5-Gal*f*
16	1,3,4,6-Tetra-*O*-acetyl-2,5-di-*O*-methylgalactitol	0.710		1,3,6-Gal*f*
17	1,3,5,6-Tetra-*O*-acetyl-2,4-di-*O*-methylgalactitol	0.731	1,3,6-Gal*p*	
18	1,4,5,6-Tetra-*O*-acetyl-2,3-di-*O*-methylgalactitol	0.714	1,4,6-Gal*p*	1,5,6-Gal*f*
19	1,2,3,4,5-Penta-*O*-acetyl-6-*O*-methylgalactitol	0.699	1,2,3,4-Gal*p*	1,2,3,5-Gal*f*
20	1,2,3,4,6-Penta-*O*-acetyl-5-*O*-methylgalactitol	0.794		1,2,3,6-Gal*f*
21	1,2,3,5,6-Penta-*O*-acetyl-4-*O*-methylgalactitol	0.879	1,2,3,6-Gal*p*	
22	1,2,4,5,6-Penta-*O*-acetyl-3-*O*-methylgalactitol	0.879	1,2,4,6-Gal*p*	1,2,5,6-Gal*f*
23	1,3,4,5,6-Penta-*O*-acetyl-2-*O*-methylgalactitol	0.794	1,3,4,6-Gal*p*	1,3,5,6-Gal*f*
24	1,2,3,4,5,6-Hexa-*O*-acetylgalactitol	0.911	1,2,3,4,6-Gal*p*	1,2,3,5,6-Gal*f*

The retention time (t_R) of the derivatives is given in relation to the internal standard (IS) used (inositol hexaacetate = 1.000). Gal*p*: Galactopyranose; Gal*f*: Galactofuranose.

5 or 13/17 in *Fig. 1* was achieved by using different chromatographic conditions. Three sets of gas chromatographic conditions has allowed to assign all peaks obtained after permethylation, hydrolysis, reduction and acetylation of pectic polysaccharides to the corresponding monomers and to a linkage type. The experimental details on the methodology for the determination of PMAA are given in [7].

Chiral Flavour Compounds

'Chiral recognition' is not only an important principle in pharmacology but also in flavour chemistry. The influence of the chirality of a flavour compound on its qualitative and quantitative characteristics (different odour/taste and different odour/taste thresholds, respectively) is well established.

Research on chiral flavour compounds can be connected with the following targets:

- knowledge of sensory properties of the pure enantiomers
- evaluation of the enantiomeric ratio of naturally occurring chiral flavour compounds, *e.g.*, to evaluate their authenticity.

These studies, therefore, emphasise synthesis and structure evaluation by NMR, IR, MS, and CD, and the development of separation methods (analytical and preparative HPLC with chiral and achiral phases, HR-GC and HR-GC/MS with chiral and achiral phases). They are also relevant to organic chemistry as the complete characterisation of a chiral compound includes the knowledge of the chiroptical properties of the enantiomers and the absolute configuration.

Phthalides are the key compounds of celery flavour and very important components in various food products, such as instant soups and vegetable juices. Several representatives of this class of compounds which are also of pharmacological interest, are chiral. Examples of phthalides studied are butylphthalide [8], one of the most prominent chiral phthalide, and 3-butylhexahydrophthalide [9].

The enantiomeric separation of racemic butylphthalide was carried out through formation and separation of diastereoisomeric derivatives. Sensory evaluation revealed differences in the quantitative characteristics (odour threshold) of the two enantiomers. Whereas in celery (*Apium graveolens* L.) and celery seed oil the (–)-(*S*)-enantiomer was predominant, in lovage seed (*Levisticum officinale* K.) only the (+)-(*R*)-enantiomer was found.

The relative configuration of the two diastereoisomers of 3-butyl-*cisoid*-3a,7a-hexahydrophthalide was assigned by NOE difference spectroscopy. All four stereoisomers of this phthalide were found to be present in celeriac (*Apium graveolens* L. var. *rapaceum*).

Analytical Chemistry in Food Technology

Food technology describes the processes which are necessary to produce, preserve, store and distribute food at the quality level requested and accepted by the consumer. Research on food technology follows the food chain from the raw material to the product on the plate and tries to optimise food quality by introducing new processing systems and improving existing methods of production and preservation. Traditionally, most developments in food technology have been empirical and based on a wealth of experience which was accumulated over the years. The aim of research in food technology is to replace empirism by first recording the changes occurring in a food system in relation to the parameters applied in a particular process. The changes may be investigated in different levels depending on the most relevant quality aspect.

Analytical chemistry is applied in almost any research project on food technology. It may involve as little as the determination of moisture or the assay of a particular vitamin, or it may be go as far as characterising the profile of a volatile flavour of a product. Needless to say that this is one reason why the Food Chemistry group and the Food Technology group form one administrative unit within the Institute of Food Science at ETH sharing the infrastructure of analytical chemistry, physical measurements, sensory evaluation and pilot plant operations.

As food technology research is usually based on large series of bench or pilot plant scale experiments of food processing, analytical methods for following up critical changes in food products should be as reliable as possible, but at the same time simple and not time consuming. The pilot plant experiment itself with its necessity to record process parameters such as temperature, residence times, humidity, pressure, *etc.*, does not allow the additional introduction of demanding analytical methods. This means that a compromise has to be found between the desire to apply the most sophisticated method giving a large amount of detailed information and the need to reduce the time, labour and equipment input by using a simplified method with reduced information.

Over the last few years, investigations on oxidative changes during processing of foods and on means to minimise or prevent oxidative reactions have been of particular interest. Oxidative changes primarily influence the lipid fraction of many food products and impair sensory quality by development of off-flavour and discolouration. Oxidative changes are studied in heat-sterilised menu components of vegetable or meat in cans, trays and pouches. They are monitored in a project on shelf-life designed packaging of dehydrated food products such as snacks or breakfast cereals. And they present the critical factor in the design of a new system for roasting oil containing nuts with improved storage stability. In the latter project, emphasis was placed on roasted hazelnuts which is an important intermediate product for the chocolate and confectionary industry.

In view of the importance of lipid oxidation in food processing, numerous simplified analytical methods have been developed and introduced to the food industry. In the present research projects, short chain alkanes in the headspace of heat sterilised products and of dehydrated foods packed in different packaging materials are determined by GC to monitor oxidative changes. Methane, ethane and pentane are formed as end product of the breakdown of fatty acids and accumulate over the progressing lipid oxidation during processing and storage. The headspace of heat-processed canned foods is fully saturated with water vapour and stays under a vacuum so that a special device for retrieving gas samples had to be developed [10]. The system was applied to monitor oxidative changes in vegetables and potatoes packed conventionally with brine and vacuum packed, respectively [11]. The analysis of short chain alkanes was also used to compare different packaging material and filling techniques for heat-sterilised meat preparation in trays [12]. These latter products have become quite popular as shelf-stable menu components, but are quite susceptible to oxidative changes and off-flavour development.

The short chain alkanes are mere indicator substances and do not contribute to the off-flavour itself. Nevertheless, their analysis proved to be advantageous in comparison with the analysis of, *e.g.*, hexanal which is known to be a component of rancid off-flavour, but is not always stable and, therefore, may not accumulate over a longer period of storage. Likewise, the application of the thiobarbituric acid test, which is frequently used in the dairy and meat industry, proved to be less reliable than the alkane analysis when the meat

samples surpassed a certain oxidation level.

In the case of hazelnuts, the oil fraction is recovered by mechanical pressing of nut samples, and the relative concentration of conjugated diens of the oil samples is measured by UV spectroscopy. Freshly pressed crude oil of raw hazelnuts is used as reference sample [13]. With this method, oxidative changes during different roasting processes were judged. Then, oxidation rates during storage of these nut samples were characterised by the rate of formation of conjugated diens, expressed as increase of UV absorption per day. This procedure made it possible to classify various traditional roasting machines according to the susceptibility of roasted nuts to lipid oxidation. Experiments on a laboratory fluidized bed roaster showed that dien formation during storage of roasted nuts was much more controlled by the roasting temperature than the roasting time (*Fig. 2*). In addition, dien formation rates during storage could be lowered when a two-step roasting process was introduced and the relative humidity of the air was increased in the first step (*Table 2*). The more favourable roasting conditions seem to minimize structural changes of the nut tissue so that oxygen diffusion into the tissue remains limited. Based on these results, a new roasting principle was developed which is now available for commercial application [14]. In all the numerous scale-up experiments, the determination of conjugated diens proved to be a valuable screening method for product behaviour during roasting and storage. It is clear that for the final evaluation of stability and flavour quality, complementary analytical investigations on selected nut samples will be necessary.

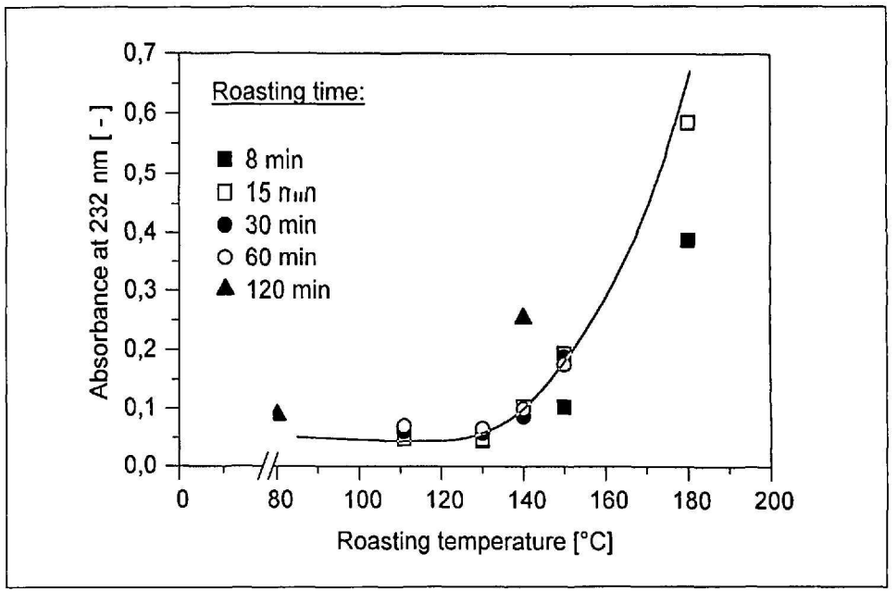

Fig. 2. *Influence of air temperature and roasting time on the oxidative stability of roasted hazelnuts after a storage period of 31 d at 37°.* Formation of diens expressed as absorbance at 232 nm.

Table 2. *Comparison of Oxidative Stability During Storage of Hazelnuts Roasted at Different Conditions.* Rate of dien formation expressed as increase of absorbance at 232 nm per day during storage at 37° for 40 d.

Roasting conditions Step 1 T [°]	p_v [kPa]	t [min]	Step 2 T [°]	t [min]	Rate of dien formation during storage [1/d·10^{-3}]
Reference process					
–	–	–	150	15	2.7 ± 0.1
Two-step roasting process					
130	22	8	150	12	1.7 ± 0.1
130	68	5	150	12	0.6 ± 0.3
130	68	8	150	12	1.1 ± 0.4
130	68	12	150	12	0.7 ± 0.4

T = Air temperature during roasting; p_v = water vapor pressure of air; t = roasting time.

Analytical Chemistry in the Curriculum in Food Science

As has been discussed in the previous sections, in food science and technology, a large number of analytical methods are used in research, food control, process control and trade. That is why analytical chemistry is an essential part of the education of our students and is taught in lectures as well as in practical courses. The goal is not to train students as analytical chemists but to provide an understanding of the fundamental principles of analytical chemistry and to show how these principles are applied in food chemistry. The lecture topics concentrate on routine instrumental methods and comprise optical spectroscopy (UV/VIS, MIR, NIR, AAS), chromatography (GC, HPLC), mass spectrometry, electrophoresis, refractometry and polarimetry. The purpose of the food chemistry laboratory course is to complete and extend the areas of the lectures in food chemistry and instrumental food analysis. Students will learn the techniques needed for determining constituents of foods and raw materials with emphasis on the main components (carbohydrates, lipids, proteins).

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