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Am Schluss der Arbeit ist ein *Literaturverzeichnis* anzugeben. Die Literaturzitate erscheinen in der Reihenfolge ihrer im Text festgelegten Nummern – im Text (1), (2) usw., im Verzeichnis 1, 2 usw. Sie setzen sich zusammen aus: bei Zeitschriften Namen und abgekürzte Vornamen der Autoren, Titel der Arbeit, Zeitschrift (in der Abkürzung nach Analytical Abstracts), Band (unterstrichen), Seite des Beginns und des Endes der Arbeit sowie Erscheinungsjahr in runden Klammern, bei Büchern Namen und abgekürzte Vornamen der Autoren, Titel, Auflage, Band, Seite, Verlag, Erscheinungsort und Erscheinungsjahr.
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Canned Food Consumption – Swiss Survey 2001

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Introduction

Knowledge of food consumption is essential to manage two areas of public health: nutrition and food safety. One of the objective of food safety is to assess the risk raised by the consumption of contaminated food. For this, it is necessary to evaluate the exposure of the population to the contaminants. Level of contamination (i.e. concentration in the food), amount and frequency at which the contaminated food is eaten, are key variables to measure the exposure. By comparing the exposure with toxicological data it is possible to determine a contamination level below which consumer's health is protected. Such a value is set afterwards as a limit in the legislation.

In Switzerland, the part of principal foods in the diet is deduced from domestic production, importation and exportation data (1). This information is often insufficient in order to establish an accurate risk assessment of contamination. Effectively, diet obtained from economic data only reports an averaged estimate of the consumption but does not reveal the real, complete image of food intake. Besides standard consumers, there are individuals who significantly eat less and others who eat more than the average. Yet, the legislation is supposed to protect everyone. Therefore it is fundamental to measure not only the *per capita* consumption, but also the extremes of dietary intake.

Contamination related to packaging represents a special case in risk assessment of contaminated food. Different packaging materials are used to pack a given foodstuff and a definite contaminant is not specific to a particular food. New developments in packaging technology as well as marketing trends induce rapid changes in the packaging systems in use. Therefore, it is often difficult to follow the pathway of a particular migrant in the diet and consequently to assess the real exposure. To manage this difficulty the European legislation utilises a worst-case estimate of the exposure. The legislation postulates that, in the case of a migration from a package

to the food, the consumer eats daily one kilogram of this possibly adulterated food during his lifetime. This assumption is used to define the *specific migration limit* (SML is the maximum concentration of the contaminant tolerated in the food) that is set in the legislation. It is evident that in most cases this assumption overestimates the real consumption value (e.g. for fatty foods). Canned food is one example where a 1 kg daily intake is recognised to be too high. European experts are evaluating presently the possibility to introduce correction or reduction factors (2, 3) for given foods and specific packaging systems in order to correct the inaccuracy of the regulation concept.

The primary goal of this survey was to determine the distribution of the consumption of canned food in Switzerland in order to check the new correction factors under discussion for an improved food packaging legislation. A secondary objective was to gain experience on the possibilities and difficulties of a survey carried out entirely by electronic means. This point will not be discussed in this paper.

Methodology

Collective

The participants were recruited in the federal administration. They were free to register and no registration was rejected. The study also included the family of each registered participant, the family was defined as all persons living in the household (regardless of relationship) and sharing the meals. Infants too young to eat canned food and family members away from the household for more than three days during the observation period were excluded from the survey. Information on the objectives of the study as well as detailed documentation on the procedure of the survey were provided to the subjects prior to their registration.

Study protocol

The survey was carried out in September 2001. Participants had to report the overall number of food cans used by the family during seven consecutive days. Only metallic food cans were recorded. Glass jars and canned beverages were excluded from the study. Canned food was classified in eight different categories: *vegetables, fruits, meat, fish, soups, ready-to-eat meals, milk products* and *other foods.* Only cans used at home or taken from home for lunch were considered for the survey.

The following information was also requested: age and gender of each participant in the family, the number of inhabitants in the municipality of residence. In addition to the family intake, participants were asked to estimate their individual consumption compared to the family average. Participants could declare themselves as *"normal consumers"*, *"low consumers"* or *"high consumers"*. They reported the intake frequency (9 choices) and the category of canned food most frequently eaten (for *"high consumers"*).

Recording of results

The survey was carried out exclusively by electronic means (E-mail and Internet) and data transfer was fully automated. The School of Engineering of Fribourg (Switzerland), Department of Telecommunication, developed the computer application and programs used in this study.

Each federal employee having an E-mail address was contacted (ca. 20000 addresses) with a brief invitation to participate. This message contained the address of an Internet site accessible in French, Italian and German. Information on the study objectives and documentation of the survey protocol could also be downloaded. The site was divided in two sections: *Registration* and *Results*. The two sections were not opened simultaneously to avoid possible mistakes. Registration was only possible between 4 and 12 of September and transmission of the collected information between 14 and the 28 of September. An E-mail address was accessible to the participants during the survey allowing them to report their problems and to ask questions. After registration, each participant received, per E-mail, a confirmation of her/his enrolment together with a *U*ser *ID*entification-code (UID). This UID was necessary to enter the *Results* section and to transmit the observed data. The UID could only serve for one data transmission. This process guaranteed the complete anonymity of the participants and protected the study against multiple, false entries. The figures of each participant were automatically added to a data file (CSV format), which was used for the statistical analysis.

Results and Discussion

Introductory remarks

This survey intended to determine an initial profile of canned food consumption in Switzerland. The participants were not selected according to specific criteria usual in statistical surveys. Therefore, results should not be interpreted as typical for the Swiss population without caution. Moreover, these reported data reflect only the mathematical analysis of the numbers reported to us. They do not include any positive or negative judgement on the diet patterns of the participants.

Participants

A total of 1477 people registered and received an UID. Out of this number, 562 result-forms were sent back. With all members of the families, the number of participants was 1466 persons (712 women and 754 men). The pyramid of ages depicted in figure 1 enables a better visualisation of the participants. It appears remarkably symmetrical; up to 65 years (retirement age) all ages are represented more or less equally with a small deficit between the 20–30 years. Figure 2 depicts the distribution of the family size. With ca. 40 %, homes with two members represent the majority of participants. Homes with more than five members represent

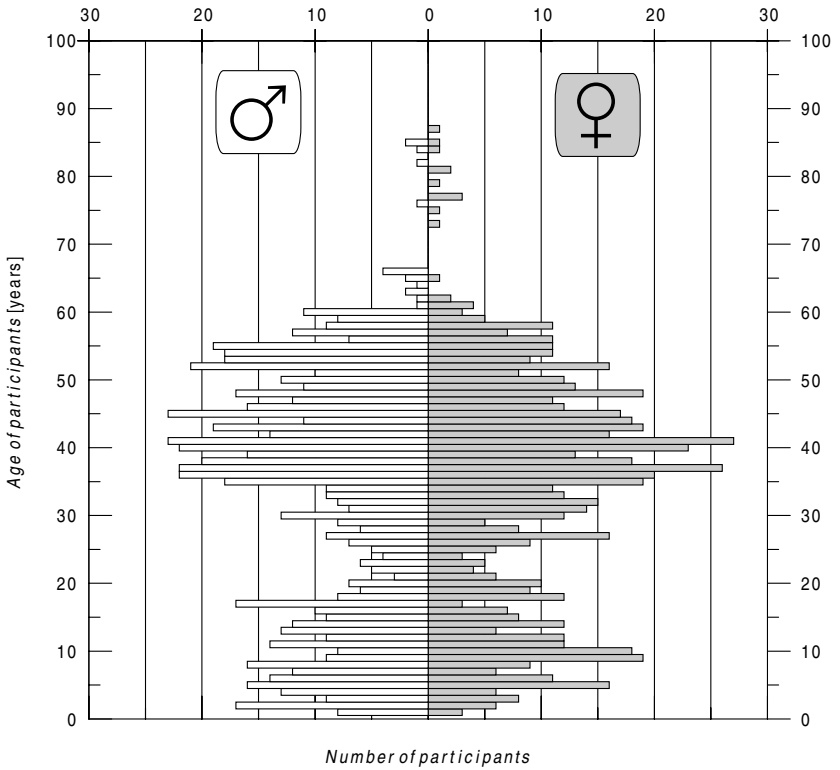

Figure 1 **Number of male and female participants by class of age**

only 2% of the answers. This fact is not surprising as it reflects the situation of the family size in Switzerland.

Consumption: average and extremes

Altogether, the 1466 participants ate 1126 food cans during the one-week survey. This corresponds to a per capita consumption of 0.77 can per person and per week. This number matches quite well with the average consumption in Europe (1 can per person and per week) reported by the industry based on market data (4). Each of the 562 participating families reported the total number of food cans used during a week by its members. With this data it is not possible to get an average consumption for each individual, but only an average for the family. By grouping the averages in class intervals of 0.5 (can/person/week) and drawing them in a histogram, one obtains the distribution curve of the consumption (fig. 3). The distribution is

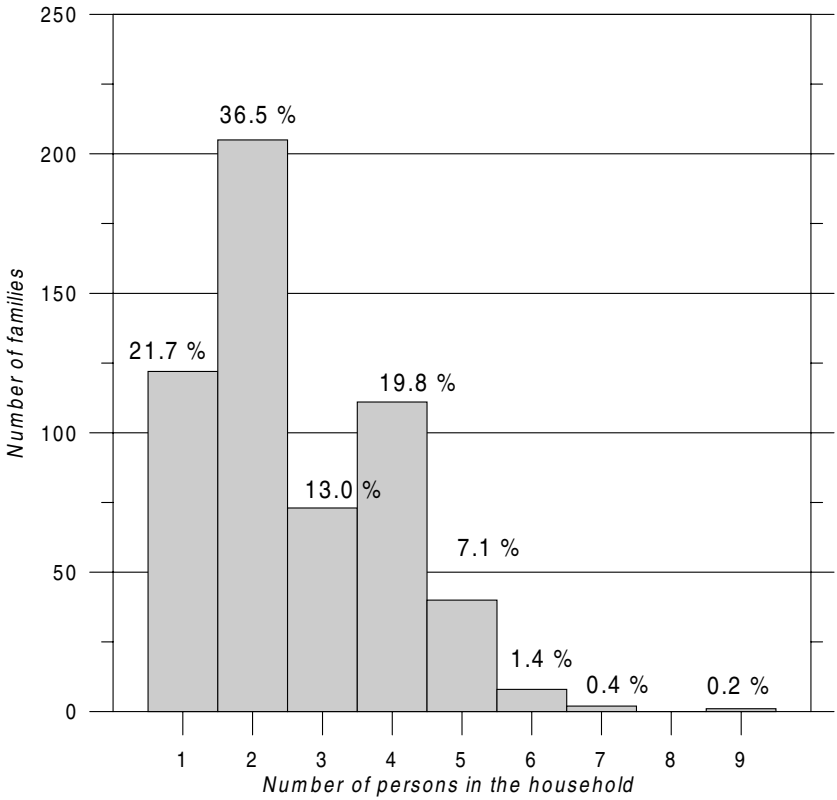

Figure 2 **Distribution of families by size**

strongly left skewed with the median (50% percentile) at 0.47 can/person/week. The average home consumption is 0.85 can/person/week with a standard deviation of 1.1. This home consumption should not be confused with the per capita consumption. It is the arithmetic mean of the average consumptions reported by the 562 families. The histogram is balanced (at equilibrium) at that point. This value gives a more exact image of the real average consumption because a can is not necessarily a portion but shared by the family members during the meals.

The histogram was plotted with 18 equal class intervals in order to make it more informative. Table 1 reports the complete set of the measured consumptions. From these data the following additional information can be obtained:

– 26% of the families did not eat canned food
– the highest measured consumption is nine cans per person and per week
– the 97.5 percentile of the histogram is located at four cans per person and per week.

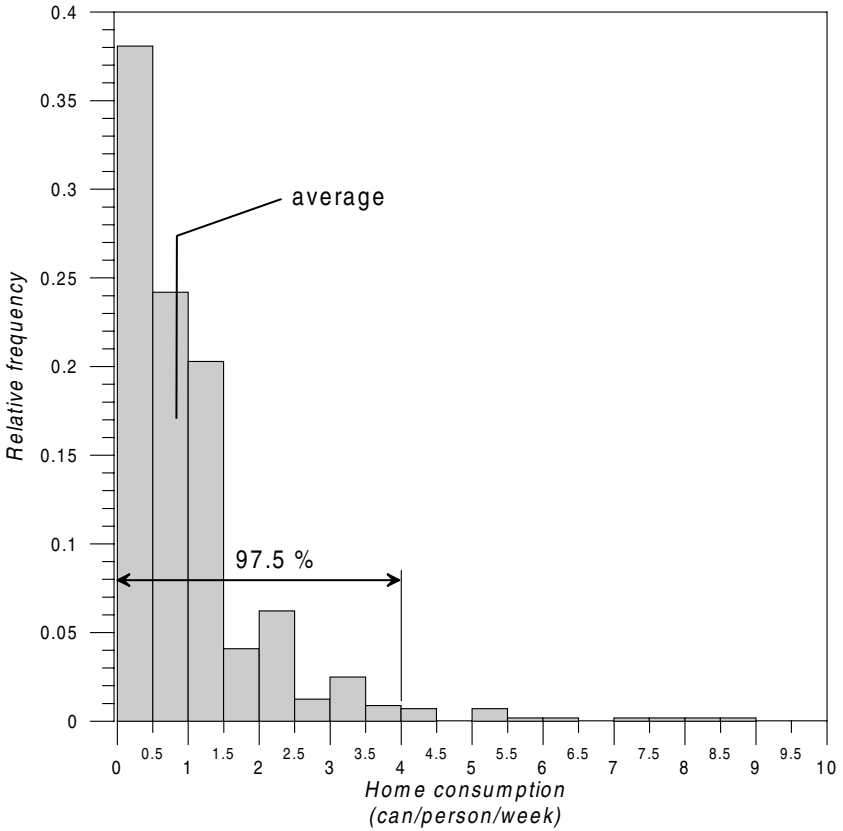

Figure 3 **Distribution of can consumption in family (histogram built up with left endpoint convention)**

It is generally accepted as a rule that, for staple foods, the 97.5 percentile deviates from the mean consumption by a factor of 3. The histogram indicates clearly that for canned food the distribution curve is more stretched with a factor 5 and 10! for the 97.5 and 99 percentile respectively.

The average consumptions given in table 1 show distinct peaks for values of 1, 1.5, 2, 2.5 and 3 can/person/week. This is purely incidental and due to the fact that both, the family size and the number of cans reported in the survey are discrete variables. Both variables increase by fixed amount of 1, 2 and so on. Although the average consumption is a continuous variable, some values are not possible whereas others are favoured: e.g. a family of two persons could eat food from 1, 2, 3, or 4 cans,

Table 1
Distribution of families and persons by reported, average consumption of canned food. Columns "histogram": percentage of families in class interval of 0.5 can/person/week (class intervals include the left endpoint, but not the right endpoint)

average consumption	*family*		*person*	*histogram*	
(can/person/week)	*(number)*	*(%)*	*(number)*	*class interval*	*% in that interval*
0.00	146	25.98	297		
0.20	13	2.31	65		
0.25	24	4.27	96		
0.33	23	4.09	75		
0.40	8	1.42	40	0–0.5	38.08
0.50	93	16.55	248		
0.60	10	1.78	50		
0.67	14	2.49	48		
0.75	16	2.85	64		
0.80	1	0.18	5		
0.83	2	0.36	12	0.5–1.0	24.20
1.00	92	16.37	190		
1.14	1	0.18	7		
1.20	2	0.36	10		
1.22	1	0.18	9		
1.25	6	1.07	24		
1.33	11	1.96	33		
1.40	1	0.18	5	1.0–1.5	20.28
1.50	19	3.38	42		
1.67	2	0.36	6		
1.75	2	0.36	8	1.5–2.0	4.09
2.00	34	6.05	55		
2.25	1	0.18	4	2.0–2.5	6.23
2.50	6	1.07	14		
2.75	1	0.18	4	2.5–3.0	1.25
3.00	14	2.49	20	3.0–3.5	2.49
3.50	5	0.89	10	3.5–4.0	0.89
4.00	4	0.71	5	4.0–4.5	
				4.5–5.0	
5.00	4	0.71	5	5.0–5.5	
5.75	1	0.18	4	5.5–6.0	
6.00	1	0.18	1	6.0–6.5	2.49
				6.5–7.0	
7.33	1	0.18	3	7.0–7.5	
7.50	1	0.18	4	7.5–8.0	
8.00	1	0.18	2	8.0–8.5	
9.00	1	0.18	1	8.5–over	
total	562	100	1466		100

generating a consumption of 0.5, 1, 1.5 and 2 respectively. The use of class intervals of 0.5 can/person/week masks this effect in the histogram.

Food cans on the market are available in many different sizes but this information was not considered during the survey to keep it simple. The size factor could possibly bias the reported results because a meal could be prepared with a foodstuff from several small cans or from one big "family-can". However, the amount of canned food used in the meal preparation is supposed to be similar in both examples. To estimate the intake of canned food by weight and not by number of cans, the average can size needs to be guessed. A can of 400 g (dimensions: ∅ 75 mm, h 110 mm) can be considered as a large can and consequently as a worst case for the calculation. This leads to the result that 97.5 % of the consumers eat less than 230 g of canned food per day (4 cans of 400 g per week). It follows that an intake of 1 kg exaggerates the real consumption by a factor of 5 for the majority of consumers. For the very few, extreme consumers (9 cans/week), the factor is still 2.

The participants were asked to give an estimate of their individual canned food intake. The reason for this was to reveal individuals with extreme consumption habits whose records could have been hidden in the average consumption of the family. 323 families declared "non average" consumers: 686 low consumers (disseminated in 293 families) and 57 high consumers (in 30 families). Analysis of data suggests that the canned food consumption is rather homogeneous within the family, i.e. all persons in a family have the same consumption habits. Effectively, 7 % of these 323 families include both "normal" and "non-average" consumers in the same family (only 1.2 % with both "low" and "high consumers"). The majority of the families consists of persons with a similar eating habit. Table 2 gives the number of "extreme" consumers by the frequency of intake. It is not possible to directly compare these figures with average data reported for the family (table 1). The average values are real, counted data whereas the individuals answers are the result of an estimate. However, some limited information can be drawn. It is evident that the 32 persons, who declare to never eat canned food, do match the 297 persons (26 % of participants) with an intake of 0 can/week during the survey. This indicates that

Table 2
Repartition of individual consumers by periodicity of intake

Periodicity	*Low consumers*	*Average consumers*	*high consumers*
never	32		
<1× per 3 months	50		
<1× month	158		
<1× week	446		
1× a week			4
2× week			12
3× week			24
1× day			17
>1× day			0
total	686	723	57

real "non-consumers" are a minority in the group of participants. The distribution of the consumption (histogram) misses precision in that range of intake probably because the time of survey is too short compared to the intake frequency of the consumers. For high range, it is interesting to see that the highest value reported is one can/day. This appreciation corresponds well with the highest measured intake of nine cans/week and consequently can be taken with confidence as the most extreme intake for canned food.

Types of food eaten

The participants reported the total number of cans eaten classified in eight different categories of food. Table 3 shows that vegetables, accounting for 41 % of all cans, represent the largest fraction. Fish (17 %) and fruits (15 %) come in second and third rank, respectively. Besides this trio, the consumption of other food types appears to be limited. This information is important to understand the exposure to migrants. Effectively, vegetables and fruits are aqueous and acidic type of foods and fish can be considered as fatty food. The migrants, which could possibly diffuse from can coatings into foodstuff, change accordingly with the solubility characteristics of the food. However, these results should take into account the fact that the survey was carried out in September. It is likely that the intake of vegetables and fruits are governed by a seasonal variation.

Table 3
Repartition of canned food eaten by category. (a) from ref. 4 (1997 data)

food category	*this survey (%)*	*Europe (a) (%)*
vegetables	40.9	29.2
fruits	15.5	6.7
meat	3.6	8.0
fish	16.9	24.2
soups	2.0	4.1
ready-to-eat meals	10.3	12.0
milk products	3.8	9.5
other foods	7.0	6.4
total	100	100

The pattern of consumption in Europe appears to be quite different from this survey. In average, the Europeans eat more fish (+7 %) and less vegetables (–12 %) and fruits (–9 %) cans. The divergence is likely to be even higher if individual countries are considered as regional diets between countries, even developed European countries, are known to be different (5). The results in table 3 show the difficulty of estimating the exposure from global production data because the specificity of regional consumption is not reflected.

Food types preferred by high-consumers are slightly different from the family average. Vegetables with 49 % of the answers come first and are followed by fish (19 %) and ready-to-eat meals (16 %). All other food categories make up less than 10 % of the answers.

Influence of family size

The family size has a direct influence on the consumption of canned food as shown in figure 4: a larger family definitively eats less canned food than a smaller one. There are various reasons to explain this trend, but they were outside the scope of the study and were not further investigated. The tendency is particularly obvious for vegetable and fish cans, which together represent 55 % of all cans. The pattern of

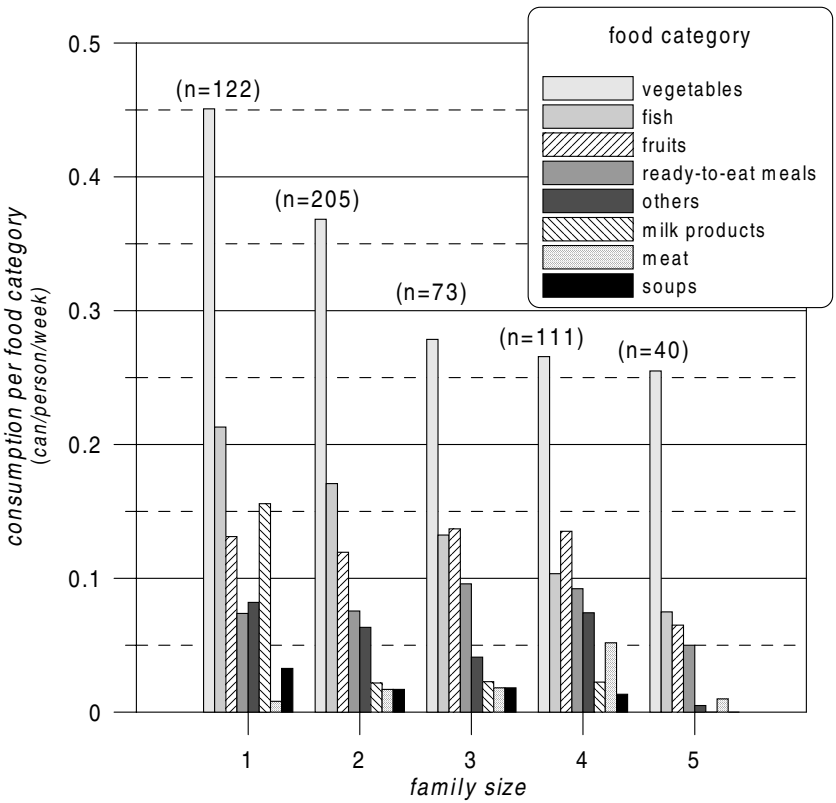

Figure 4 **Consumption of the different food categories by family size. Number in bracket is the number of families in this class**

consumption for the other food categories appears to remain unchanged with the number of persons in the family. The category "milk products" makes exception to the rule: persons living alone consume at least seven times more canned milk products than families with two or more persons.

Other observations

A selection of families composed only by adults (age>16 years) of the same sex allows to check the influence of the gender on canned food consumption. It appears that women eat less canned food (0.8 can/person/week) than men (1.4 can/person/week). This result should be considered with caution as the size of the subpopulation is small (women: $n=77$, men: $n=82$) and other factors may also play a role.

The influence of the place of residence was also investigated. It was not possible to show a difference in the can consumption among families living in an urban or a country community.

Conclusions

For the first time a food consumption survey related to packaging was carried out in Switzerland. The measured average intake is close to the *per capita* consumption reported by the European industry. Although the group of participants was not defined, this fact denotes the quality of estimates and supports the idea that the distribution curve is also representative of the real diet in Switzerland. It is clear that the survey needs to be repeated to increase the confidence in the results and to check the importance of factors such as seasonal or regional variations. However, these first data of the intake of canned food serve as a basis for a better appreciation of exposure to migrants from can coatings.

Summary

The consumption of canned food in Swiss households was recorded in a seven-day survey. The average and the 97.5 percentile intake were measured at 0.85 and four cans per person and week respectively. Vegetables, fish and fruits are the principal food categories and represent 74 % of canned food consumed in this study. The results show that the risk assessment in Europe overestimates canned food intake by approximately a factor 5.

Zusammenfassung

Der schweizerische Verbrauch von Lebensmitteln aus Konservendosen wurde in einer Befragung über sieben Tage ermittelt. Der durchschnittliche Verbrauch und die 97,5-Perzentile betrug 0,85 bzw. vier Dosen pro Person und Woche. Gemüse, Fisch und Früchte sind die wichtigsten Nahrungsmittelkategorien und machen 74 % des betroffenen Gesamtverzehrs aus. Die Resultate zeigen, dass die in der EU

gebräuchliche Modellrechnung das von Konservendosen ausgehende Risiko ca. um einen Faktor 5 überschätzt.

Résumé

La consommation d'aliments en conserve dans les familles suisses a été mesurée dans une enquête de sept jours. La consommation moyenne ainsi que la valeur à 97,5 % de la courbe de consommation ont été mesurées à 0,85 et quatre conserve/personne et semaine. Les légumes, le poisson et les fruits sont les aliments principaux et représentent 74 % des conserves consommées dans cette enquête. Les résultats indiquent que, dans l'évaluation du risque en Europe, la consommation des conserves est surestimée d'un facteur 5 environ.

Key words

Food consumption, Food can, Diet, Exposure

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5 The sixth World food survey FAO Rome, 1996.

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Determination of Phthalates in Toys and Childcare Articles Made of PVC with HPLC and HPTLC

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Introduction

Phthalates are used as plasticizers to improve the flexibility or processability of plastic utensils, rubber items and other technical products such as paints. They are also used as fixatives for fragrances, as insect repellents and as solvents for pesticides. Bis-(2-ethylhexyl) phthalate (DEHP) is the most commonly used plasticizing agent for polyvinylchoride (PVC) with contents ranging between 10 and 40 % in this plastic. It is therefore found everywhere in the environment. Despite its low acute toxicity, it is known to cause tumours in rats and mice exposed to high doses over a long period (1). Some phthalates also seem to influence the reproductivity of mammals (2). In 1998 the SCTEE (Scientific Committee for Toxicity, Ecotoxicity and the Environment) first established migration limits for six phthalates in soft PVC toys (3). In 1999 the European Commission banned childcare articles and toys made of soft PVC containing phthalates if these were intended to be put in the mouth by "under three year olds" (4). The initial approach based on migration limits was abandoned due to analytical problems and followed by a limit of 0.1 % for the total content. Switzerland on the other hand has banned DEHP for these items since 1986 (5). A provisional limit referring to the total content of 10 mg/kg DEHP was later set to 0.1 % for six phthalates in accordance with EC regulations. Reported methods concerning the determination of total content either used supercritical fluid, Soxhlet or Sonication extraction followed by identification and quantitation with gas chromatography (6–8). In this paper we would like to present our method for the determination of seven phthalates using tetrahydrofuran as an extractant and HPLC or HPTLC for identification and quantitation.

Method

Materials and instruments

Lab-shaker, Adolf Kühner AG, Switzerland; magnetic stirrer; rotary evaporator, Büchi R114 Switzerland; fluted filter, Schleicher & Schuell 602 EH; polyethylene syringes 1 ml; 0.45 µm Nylon filter, Gelman Acrodisc; HPLC system: quaternary low pressure mixing pump, Waters 600 MS; column oven, Waters Model 600; degasser, ERMA Tokyo ERC 3811; photodiode-array detector, Waters 996; Millennium chromatography software; column: Nucleosil C18,3 µm 120 Å, 250×4.0 mm, Macherey-Nagel; HPTLC system: CAMAG Reprostar 3 with Videoscan software; CAMAG Linomat IV sample applicator; CAMAG horizontal developing chamber for 10×10 cm plates; CAMAG TLC plate heater; HPTLC plates 10×10 cm RP-8_{W254S}, Merck.

Reagents

Tetrahydrofuran p.a., e.g. Merck 9731; methanol gradient grade, e.g. SDS 09337G16 for HPLC; acetonitrile gradient grade for HPLC, e.g. Lichrosolv, Merck 00030; demin. water Nanopur.

Reference materials

di-(2-ethylhexyl)-phthalate, *DEHP,* ≥97% (Fluka 80032) CAS-Nr. 117-81-7,
di-butyl-phthalate, *DBP,* 99% (Merck 800919) CAS-Nr. 84-74-2,
benzyl-butyl-phthalate, *BBP,* 98% (Aldrich 30,850-1) CAS-Nr. 85-68-7,
di-n-octyl-phthalate, *DOP,* ≥98% (Fluka 80153) CAS-Nr. 117-84-0,
di-iso-nonyl-phthalate, *DINP,* >99% (Aldrich 37666-3) CAS-Nr. 28553-12-0,
di-iso-decyl-phthalate, *DIDP,* 99.8% (Fluka 80135) CAS-Nr. 26761-40-0,
di-cyclohexyl-phthalate, *DCHP,* 96% (Merck 800920) CAS-Nr. 84-61-7

Procedures

Calibration solutions for HPLC

Stock solutions

Prepare 100 ml solutions of 100 mg of each reference compound in acetonitrile (1 mg/ml). These solutions are stable for at least four weeks if stored at 4°C in the dark.

Calibration solutions

Pipette 5 ml of each stock solution into the same flask and dilute to 50 ml with acetonitrile (dilution 1). Pipette 25 ml of dilution 1 into a flask and dilute to 50 ml (dilution 2). Pipette 10 ml of dilution 1 into a flask and dilute to 50 ml (dilution 3).

Pipette 5 ml of dilution 1 into a flask and dilute to 50 ml (dilution 4). Pipette 1 ml of dilution 1 into a flask and dilute to 50 ml (dilution 5).

Calibration

Inject 10 µl of dilution 1 to 5. The corresponding amounts (depending on weighed-in quantity) are: dilution 1 (1 µg), dilution 2 (0.5 µg), dilution 3 (0.2 µg), dilution 4 (0.1 µg), dilution 5 (0.02 µg).

HPLC parameters and eluant

Temperature: 40° C, flow rate: 0.80 ml/min, detection wavelength: 225 nm, run time: 26 min, injection volume: 10 µl, DAD: Wavelength range: 200–320 nm, measuring time: 26 min, rate: 2.0 Hz, resolution: 1.2 nm (table 1).

Table 1
Peak retention time of phthalates

compound	*retention time in minutes*
DBP	4.5
BBP	5
DCHP	7.5
DEHP	14.1
DOP	15.1
DINP	highest peak at 18.6 (6 maxima)
DIDP	highest peak at 21.0 (3 maxima)

Calibration solutions for HPTLC

Prepare 100 ml solutions of 100 mg of DINP respectively DIDP in acetonitrile.

Solvent for HPTLC

Mix 25 ml of acetonitrile, 17.5 ml of tetrahydrofuran and 7.5 ml of water in a glass stoppered Erlenmeyer flask.

Setting of linomat IV

Plate width: 100 mm, start position: 8 mm, band: 5 mm, space: 4 mm, sec/µl: 15, volume: variable

Apply bands 7 mm from lower plate edge.

Application scheme for HPTLC

track 1: 1 µl each of DINP and DIDP solution (corresponds to 1 µg of each analyte)
track 2: 10 µl of sample extract
track 3: 2 µl each of DINP and DIDP solution
track 4: 10 µl of sample extract and 1 µl solution of the presumed phthalate
track 5: 3 µl each of DINP and DIDP solution

track 6: 10 µl of sample extract
track 7: 4 µl each of DINP and DIDP solution
track 8: 10 µl of sample extract and 1 µl solution of the presumed phthalate
track 9: 5 µl each of DINP and DIDP solution

Development of HPTLC plates

Fill developing chamber with solvent and condition plate for 15 min. Let solvent migrate for 50 mm. Dry for 5 min at 85° C with plate heater.

Documentation and quantitation

Record and quantitate HPTLC plates at 254 nm.

Sample preparation for HPLC and HPTLC

Weigh 1 g of sample in a 300 ml stoppered Erlenmeyer flask. Add 50 ml of tetrahydrofuran and stir about 15 min until the sample is dissolved. If the sample hasn't dissolved completely, place for another 15 min in an ultrasonic bath. Slowly add 150 ml of methanol while stirring. Depending on sample composition a precipitate might form. Store flask in an (explosion proof) refrigerator for 2 h. Filter suspension over a fluted filter and rinse filter with 50 ml methanol. Evaporate filtrate with a rotary evaporator at 40° C and 200 mbar and dry for 3 min at 70 mbar. For HPLC: Redissolve the oily residue with 50 ml acetonitrile. (For HPTLC: Redissolve the oily residue with 5 ml acetonitrile.) Store the suspension for 2 h in a refrigerator. Filter an aliquot through a 0.45 µm filter with a syringe. The filtrate is then ready for HPTLC or HPLC.

Evaluation of chromatograms (HPLC)

Calculate peak areas at 225 nm. Peaks are assigned according to retention time and UV spectrum (the UV spectra of the different phthalates are very similar) (fig. 1).

DINP and DIDP are isomere mixtures and give broad humps instead of discrete peaks with definite retention times. They therefore are easier to quantify with HPTLC.

Evaluation of chromatograms (HPTLC)

DINP and DIDP give dark bands at 254 nm. The rf-values are 0.24 (DINP) and 0.20 (DIDP).

Quality control

Samples should be stored at 4° C because phthalates tend to decompose. In case of positive samples, identification should be verified and recovery rates determined by repeating procedure with a sample aliquot spiked with the presumed phthalate. Even though phthalates are ubiquitously distributed in the environment and are

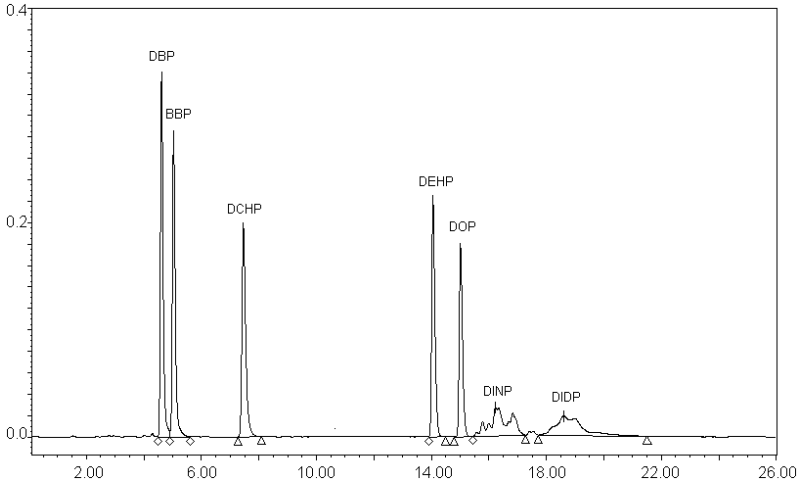

Figure 1 **HPLC run of phthalate standards**

known as laboratory contaminants, blank values are not a problem when supervising the limit of 0.1 %.

Results and discussion

With the HPLC procedure, phthalates can be directly determined in concentrations ranging from 0.01 % to 0.5 %. Quantitation with HPTLC can be directly applied between 0.05 % and 0.25 %. Samples with higher contents must be diluted prior to quantitation. Detection limits for DBP, BBP, DEHP and DOP are 5 mg/kg and for DINP and DIDP 0.025 %. The HPLC method was used in an interlaboratory test to verify, if it was adequate for supervising the current Swiss limit of 0.1 % phthalate. Four laboratories participated using the HPLC method and one laboratory used its own GC/MS method. Three samples were analysed of which sample 1 had no detectable amounts of phthalate, sample 2 contained about 27 % DEHP and sample 3 had about 24 % DINP. Only one laboratory used HPTLC to quantify DINP. All in all the results of the five laboratories were in good agreement. None of the laboratories detected any phthalates in sample 1. Interlaboratory relative standard deviation for sample 2 containing DEHP was 5 % and for sample 3 containing DINP was 4 %. Even though HPLC was not intended for quantifying DINP, results did not differ from those obtained with HPTLC. Recovery rates determined in our laboratory on a sample spiked with all seven phthalates lay between 88 % for DIDP and DINP (HPTLC method) and 97 to 101 % for DBP, BBP, DCHP, DEHP and DOP (HPLC method). When used as a plasticizer in PVC, phthalate concen-

trations are at least hundred times higher than the limit of 0.1 %. In such high concentrations the presence of phthalates can be detected already by the telltale odor of the decomposition products (alkyl alcohols). Supervision of the limit, however, can be tricky if phthalates are present only as contaminants in the plastic or were only used as additives for the application of colors on the toys. In both cases, concentrations can be near the limit and in the second case distribution in the sample varies considerably. Cryogenic homogenization of samples as described in (8) or analyzing multiple aliquots should then be taken into account in order to obtain representative results.

Our survey in 2000 showed that no teethers or nipples made of PVC were found on the Swiss market. This could be a consequence of the Swiss DEHP ban for these products which has been in effect already since 1986.

Acknowledgements

We would like to thank the following laboratories who, by participating in the round robin test helped us establish the ruggedness of our method: Clariant (Schweiz) AG, Qualitätskontrolle/Chromatographie, Muttenz; Kantonales Laboratorium Zürich; Laboratoire cantonal vaudois, Epalinges; Laboratorium des Bundesamtes für Gesundheit, Bern.

Summary

A HPLC and HPTLC method for the determination of seven phthalates in childcare articles and toys made of PVC is presented. Extraction is performed by dissolving the PVC in tetrahydrofuran. Identification and quantitation is either performed by HPLC for phthalates giving discrete peaks or by HPTLC for DINP and DIDP. The method was used for an interlaboratory test and for a market survey and proved to be suitable for supervising the legal limit of 0.1 % phthalate.

Zusammenfassung

Es wird eine HPLC und eine HPTLC Methode beschrieben, mit der Phthalate in Spielzeugen, Beissringen und Saugern aus PVC bestimmt werden können. Die Extraktion wird durch Auflösen des PVC in Tetrahydrofuran erreicht. Die Identifikation und Quantifizierung wurde für Phthalate, welche diskrete Peaks ergaben, mit HPLC und für DINP und DIDP mit HPTLC durchgeführt. Die Methode wurde in einem Ringversuch getestet und für eine Marktkontrolle verwendet. Sie erwies sich als geeignet für die Überwachung der Limite von 0,1 % Phthalat.

Résumé

La méthode présentée permet de déterminer les phthalates dans les jouets et les sucettes en PVC. L'extraction se fait par dissolution du PVC dans du tétra-hydrofuranne. L'identification et la quantification sont faits par HPLC pour les phthalates donnant des pics discrets ou par HPTLC pour le DINP et le DIDP. La méthode a

été contrôlée par un test interlaboratoire et a été utilisée pour un contrôle du marché. Elle s'est avérée être une méthode praticable pour surveiller la teneur limite de 0,1 % phthalates.

Key words
Phthalates, HPLC, HPTLC, Toys, Teethers, Childcare articles

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Koffein als Störfaktor bei der Ochratoxin-A-Analyse

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Einleitung

Ochratoxin A (OTA) ist ein Sekundärmetabolit von Schimmelpilzen der Gattung *Penicillium* und *Aspergillus*. Dieses Mykotoxin wird auch in kalten oder gemässigten Klimazonen auf Lebens- und Futtermitteln gebildet. Die Bildung wird besonders durch die Temperatur sowie den Feuchtigkeitsgehalt während der Ernte und der Lagerzeit beeinflusst und ist durch geeignete Trocknungs-, Transport- und Lagerbedingungen weitgehend vermeidbar (1). OTA wurde in Getreide, Nüssen, Gewürzen, getrockneten Früchten, Fruchtsäften, Kaffee, Soja, Kakao, Mais, fermentierten Getränken (Wein, Bier), Fleisch, tierischen Organen sowie auch in Humanproben nachgewiesen (2) und hat karzinogene, nephrotoxische, teratogene sowie immunotoxische Eigenschaften (3, 4). Zum Nachweis von OTA wurden verschiedene Methoden publiziert, so z.B. konventionelle Säulenchromatographie mit HPLC-Detektion (5) oder immunochemische Methoden wie ELISA (6, 7). Die meisten dieser Methoden haben den Nachteil, dass sie nur für eine beschränkte Anzahl von Matrizes verwendbar sind. So zeigte die von uns verwendete Methode, die auf Arbeiten von *Pittet et al.* (8) basiert, in Kaffee wesentlich schlechtere Wiederfindungsraten als z.B. in Hafer, sobald die Beladung der Immunoaffinitätssäule einen bestimmten Wert überschreitet (OTA-haltiger Kaffee: ca. 0,6 g bei einer Konzentration von 6,5 mg Kaffee/ml Extrakt). Zudem ist mit der von Pittet beschriebenen Methode die OTA-Belastung von Gewürzen, wie z.B. Pfeffer, nicht messbar, da bei der HPLC-Analyse Störpeaks auftreten.

In dieser Studie wird eine modifizierte Methode für die OTA-Analyse in Kaffee[1] beschrieben. Diese Methode scheint auch für Gewürze verwendbar zu sein. Wie in Abbildung 4 ersichtlich, kann sie z.B. für weissen Pfeffer verwendet werden.

[1] Diese Studie wurde mit löslichem, gefriergetrocknetem Kaffee durchgeführt.

Experimentelles

Alle in dieser Arbeit dargestellten Ergebnisse sind Mittelwerte aus Dreifach- oder Vierfachbestimmungen.

Material

Geräte

Schüttelgerät: SM25 (E. Bühler Laborgeräte); Vakuumsystem: Visiprep (Supelco); Stickstoff-Abdampfapparatur: Vapostat Berkely (Hugi); Vortexgerät: Vibrofix VF1 Elektronik (IKA-Labortechnik); HPLC-System HP 1050 bestehend aus: Autosampler HP 1050, Gradientenpumpe HP 1050, On-Line-Entgaser und Fluoreszenzdetektor HP 1046 AX, HP Chemstation.

Chemikalien

Alle verwendeten Lösungsmittel wiesen analytische Qualität auf. Als HPLC-Laufmittel wurden Lösungsmittel zur Chromatographie eingesetzt.

Bortrifluorid in Methanol (Fluka 15716); Koffein (Fluka 27602); Natriumacetattrihydrat (Merck 1.06267); Natriumhydrogencarbonat (Fluka 71630); Ochratest Immunoaffinitätssäulen (Vicam); Ochratoxin A (Sigma); Phosphate-buffered saline tablets (Oxoid); Stickstoff N50 (Carba Gas).

Proben

Für die Aufarbeitung der Proben wurde destilliertes Wasser verwendet. Für die Experimente wurden gefriergetrocknete, lösliche Kaffees verwendet: Blue Dream, koffeinfrei (Voncafe, Migros), Koffeingehalt 0,08 %[2]; Columban (Voncafe, Migros), Koffeingehalt 2,5 %[1].

Methoden

25,0 g feingemahlene und homogenisierte Probe wurden mit 200 ml Methanol/3 % $NaHCO_3$ (1:1 v/v) versetzt. Zur Extraktion wurde die Mischung 1 h auf dem Schüttelgerät geschüttelt und anschliessend filtriert. Zur Abtrennung von störender Matrix wurden 10,0 ml des Rohextraktes zweimal mit je 10 ml Dichloromethan extrahiert. Nach sorgfältiger Phasentrennung (bei Emulsionsbildung zentrifugieren bei 4000 rpm) wurden 5,0 ml der oberen Phase (entkoffeinierte wässerig-methanolische Lösung) in einen 100-ml-Messzylinder pipettiert und mit gesättigter phosphatgepufferter Salzlösung (PBS) auf 100 ml verdünnt. Die verdünnte Extraktlösung wurde mit einem Fluss von ca. 1 Tropfen/s durch die Ochratestsäule eluiert. Anschliessend wurde die Säule mit 10 ml dest. Wasser (Fluss von ca. 2 Tropfen/s) gespült und mittels Vakuum kurz trocken gesaugt. Noch vorhandene Wasserspuren (Säule, Säulenausgang) wurden mittels Kleenexpapier und Wattestäbchen vorsichtig

[2] Koffeinbestimmung nach SLMB, Kapitel 36 C.

entfernt. Das gebundene Ochratoxin A wurde mit 4 ml Methanol in ein 10-ml-Braunglasröhrchen eluiert (Fluss ca. 1 Tropfen/2 s). Das Eluat wurde bei 50° C am N_2-Strom gerade zur Trockne eingeengt. Der erhaltene Rückstand wurde mit 250 µl mobiler Phase versetzt und durch 30 s schütteln (Vibrofix) gelöst. Vor dem Einspritzen in das HPLC-System wurde der gelöste Rückstand filtriert (Spartan-Einmalfilter 13/20) (9). Endkonzentration der Probenextrakte: 2,50 g Probe/ml Eluent. Die Extrakte bleiben bei Raumtemperatur ca. 12 h stabil.

Chromatographie

Säule:	Spherisorb ODS II, 5 mm, 250 × 3 mm
Vorsäule:	LiChroCART, 4-4 Kartusche mit LiChrospher 100 RP-18 (5 µm)
Mobile Phase:	40 % Acetonitril und 60 % eines Gemisches (19+1 v/v) bestehend aus einer wässeriger 4 mM Natriumacetatlösung sowie Essigsäure
Flussrate:	0,6 ml/min
Trenntemperatur:	50° C
Injektionsvolumen:	80 µl
Detektion:	Fluoreszenz; Anregung bei 330 nm, Emission bei 470 nm, Kantenfilter: 370 nm (FLD HP 1046 AX)

Nachweisgrenzen und Kalibrierung

Die Nachweisgrenze (S/N ca. 2) lag bei ca. 0,2 µg/kg Ochratoxin A in der Probe. Die Kalibrationen waren im untersuchten Bereich linear und verliefen durch den Nullpunkt.

Resultate und Diskussion

Diese Untersuchung wurde durchgeführt, weil bei Analysen von OTA in gefriergetrocknetem, löslichem Kaffee deutlich tiefere Wiederfindungen erhalten wurden als z.B. in Hafer (Abb. 1). So lagen die Wiederfindungsraten bei OTA-Gehalten in gefriergetrocknetem, löslichem Kaffee von über 5 µg/kg teilweise sogar unter 60 %, wenn die auf Arbeiten von *Pittet* basierende Methode (8) (ohne Extraktion mit Dichloromethan) verwendet wurde.

Aus Abbildung 1 ist ersichtlich, dass die Wiederfindung von OTA in Hafer auch bei steigender OTA-Konzentration relativ konstant bleibt (ca. 80 %), während jene von gefriergetrocknetem, löslichem Kaffee bei steigender OTA-Konzentration deutlich sinkt (von ca. 80 % bei einer OTA-Konzentration von 1 µg/kg auf ca. 60 % bei 8,5 µg/kg).

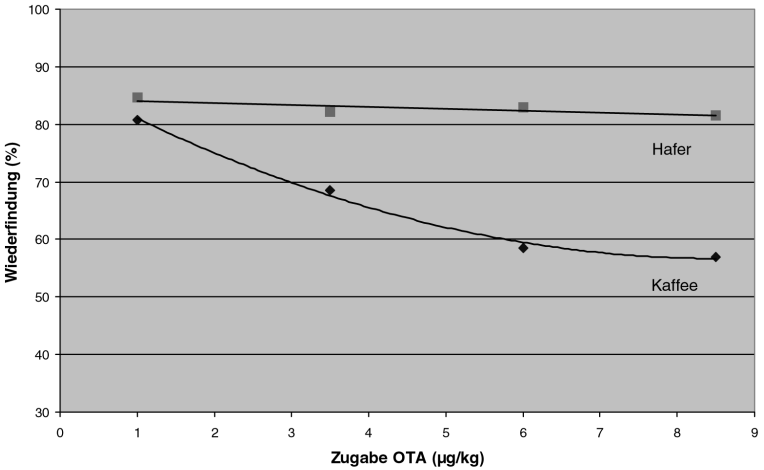

Abbildung 1 **Wiederfindung von OTA in gefriergetrocknetem, löslichem Kaffee und in Hafer**

Tabelle 1
Wiederfindung von OTA in verschiedenen Matrizes

Matrix	*OTA-Zusatz* (µg/kg)*	*Koffein-zusatz (%)*	*Wieder-findung (%)*	*(%)*	*(%)*	*(%)*	*Durchschnitt WF (%)*
Kaffeepulver	1		83	87	73		81
(gefriergetrocknet,	3,5		64	69	73		69
Koffeingehalt 2,5%)	6		57	56	62		58
	8,5		56	59	55		57
Hafer	1		89	70	95		85
	3,5		83	84	80		82
	6		82	83	83		83
	8,5		84	76	84		81
Wasser**	5	0	100	90	93	89	93
	5	2	83	71	83	81	80
	5	4	55	68	59	59	60
	5	6	43	43	47	45	45
	5	8	38	34	38	31	35
Kaffeepulver	5	0	83	80	83		82
(gefriergetrocknet,	5	2	68	64	61		64
Koffein-Gehalt 0,08 %)	5	4	57	52	50		53
	5	6	46	47	42		45
	5	8	46	47	44		46

* Bezogen auf Gehalt in Matrix; ** Zugabe gleicher Menge wie bei Kaffeepulver

Wie aus Abbildung 2 ersichtlich, ist der Matrixeffekt der Kaffeeproben auf Koffein zurückzuführen. Sowohl eine Lösung von OTA in Wasser als auch koffeinfreier löslicher Kaffee (Koffeingehalt 0,08 %) wurden mit verschiedenen Mengen Koffein versetzt (Koffeinzusatz ausgedrückt als Gehalt im Kaffeepulver) und auf die entsprechenden Wiederfindungen geprüft. Die Wiederfindungen nehmen mit steigender Koffeinkonzentration drastisch ab.

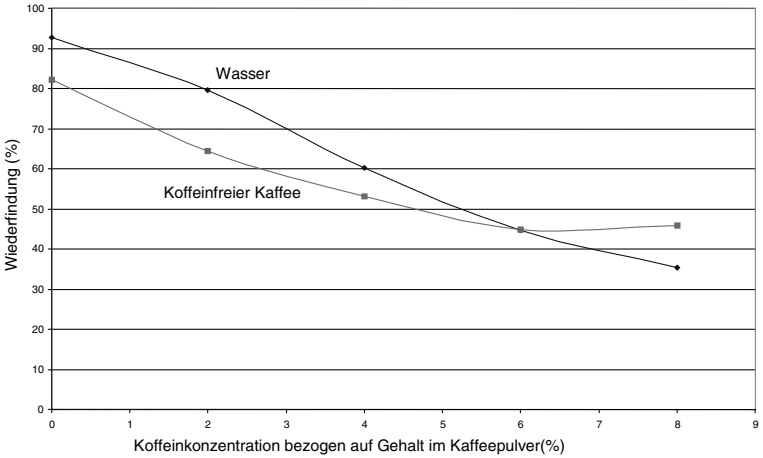

Abbildung 2 **Wiederfindung von OTA bei steigender Koffeinkonzentration in Wasser und in koffeinfreiem, löslichem Kaffee**

Kaffee kann bis zu 3 % Koffein enthalten, dies macht eine Koffeinentfernung bei der OTA-Analyse unabdingbar. Industriell bekannte Verfahren zum Entkoffeinieren von Kaffee sind: Extraktion mit Dichloromethan, kontinuierliche Entkoffeinierung mit überkritischem Kohlendioxid, Verwendung von Quellkohlensäure/Wasser bzw. von Wasser mit Kohlefiltern.

Da Dichloromethan ein problematisches Lösungsmittel ist (Toxikologie/Ökologie), wurde in der Folge untersucht, ob Alternativlösungsmittel verwendet werden können.

Ethylacetat, Diethylether, t-Butylmethylether und i-Butylmethylketon ergaben aber, auch teilweise unter Aussalzen, eine schlechte Phasentrennung. Butanol, Aceton sowie Ethylmethylketon brachten, auch mit Aussalzen, keine Phasentrennung. Eine gute Phasentrennung von Kaffee-Extrakt wurde hingegen mit chlorierten Lösungsmitteln wie Dichloromethan, Chloroform und 1.1.1-Trichloroethan sowie mit Toluen erreicht.

Wie aus Tabelle 2 ersichtlich ist, ergibt nur die Verwendung der chlorierten Lösungsmittel sowie jene von Ethylacetat eine effiziente Extraktion von Koffein. Deutlich schlechter schneidet i-Butylmethylketon ab, und als unbrauchbar betrachtet werden können t-Butylmethylether und Toluen. Die Wiederfindung von OTA nach der Extraktion betrug mit Dichloromethan 82–92 %, mit i-Butylmethylketon 43–58 % und mit Ethylacetat 9–10 %.

Tabelle 2
Koffein- bzw. OTA-Gehalt nach Extraktion mit verschiedenen Lösungsmitteln

Lösungsmittel	*Koffeingehalt (zweimalige Extraktion) (%) des Anfangsgehaltes*	*Wiederfindung OTA (%)*
t-Butylmethylether	38	
Toluen	52	
Dichloromethan	3	82–92
i-Butylmethylketon	27	43–58
Ethylacetat	10	9–10
Chloroform	1	

Diese Experimente zeigen deutlich, dass Dichloromethan mit den untersuchten Lösungsmitteln nicht ersetzt werden kann.

Dass die Wiederfindung mittels der neu entwickelten Methode konstant in einem Bereich von 80–90 % liegt, zeigte die Untersuchung von mehr als dreissig verschiedenen Kaffeesorten. Sowohl löslicher als auch gerösteter Kaffee wurde mit jeweils 5 µg/kg OTA versetzt und die Wiederfindung bestimmt.

Unter Verwendung der verbesserten Methode bei der Extraktion von gefriergetrocknetem, löslichem Kaffee steigen nicht nur die Wiederfindungsraten, auch die Chromatogramme sind wesentlich besser auswert- und interpretierbar. Dies ist in Abbildung 3 ersichtlich, wo ein Chromatogramm eines Kaffee-Extraktes, der nach der Pittet-Methode (8) hergestellt wurde (links) mit einem Chromatogramm eines Extraktes mit zusätzlicher Dichloromethan-Extraktion (rechts) verglichen wird.

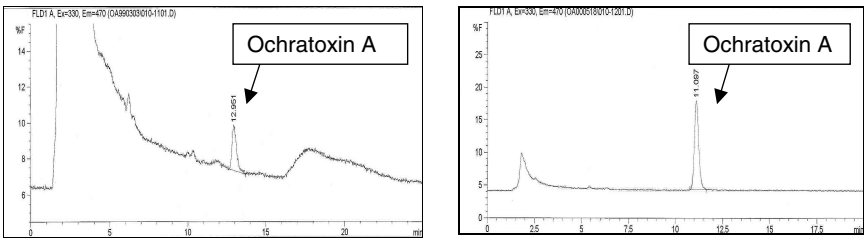

Abbildung 3 **Vergleich der Chromatogramme von löslichem Kaffee ohne/mit zusätzlicher Dichloromethan-Extraktion**

Die Nachweis- und Bestimmungsgrenze konnte mit diesem zusätzlichen Extraktionsschritt um den Faktor drei gesenkt werden.

Ein noch drastischeres Bild wird erhalten, wenn Gewürze untersucht werden. Auch bei Gewürzen werden Substanzen, die die Chromatographie erheblich stören, durch die zusätzliche Extraktion entfernt. In Abbildung 4 werden Chromatogramme von weissem Pfefferextrakt gezeigt. Auch hier wird der Vorteil der Dichloromethan-Extraktion mehr als deutlich (rechtes Chromatogramm: weisser Pfefferextrakt nach Dichloromethan-Extraktion).

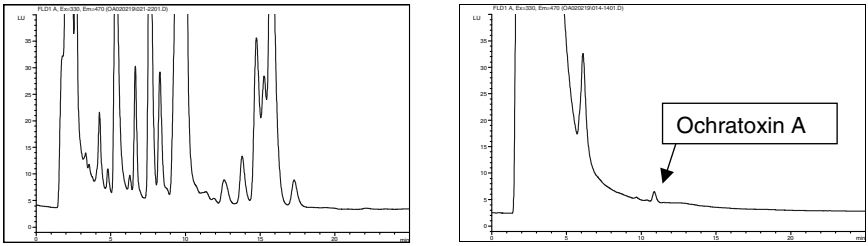

Abbildung 4 **Vergleich der Chromatogramme von weissem Pfeffer ohne/mit zusätzlicher Dichloromethan-Extraktion**

Zusammenfassung

Es wird eine modifizierte auf IAC-HPLC mit Fluoreszenzdetektion basierende Methode zur Bestimmung von Ochratoxin A in Kaffee beschrieben, mit der bereits mehrere Hundert Proben untersucht wurden. Eine der üblichen Extraktion folgende Behandlung des Extrakts mit Dichloromethan zur Entfernung von störendem Koffein führt zu einer Verbesserung der Wiederfindung sowie zu einer Senkung der Nachweisgrenze um den Faktor drei auf 0,2 µg/kg.

Résumé

Une méthode modifiée se basant sur l'IAC-HPLC avec une détection par fluorescence est décrite. Plusieurs centaines d'échantillons ont déjà été examinés avec cette méthode pour la détermination de l'Ochratoxine A dans le café. Une extraction habituelle suivant le traitement de l'extrait avec du dichlorométhane pour l'élimination de la caféine gênante conduit à une amélioration de la reproductibilité ainsi qu'à un abaissement du seuil de détection d'environ un facteur trois (0,2 µg/kg).

Summary "Caffeine as Disruptive Element in the Ochratoxin A Analysis"

Several hundreds of analyses were already performed with the described method for the analysis of OTA in coffee, which is based on IAC-HPLC with fluorescence detection. After the usual extraction of the sample, an additional extraction with

dichloromethane was performed in order to remove disturbing caffeine. This treatment of the sample enhances the recovery and lets the limit of detection drop by a factor of three to 0.2 µg/kg.

Key words
Ochratoxin A, Analysis, Coffee, Spices

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GC/MS-MID Determination of Safrole in Soft Drinks

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Introduction

Safrole [5-(2-propenyl)-1,3-benzodioxole] is a compound belonging to the class of alkenyl benzenes. It is classified as "H/M" (high/medium) in the Nettox priority list (1) as far as myristicin [4-methoxy-6-(2-propenyl)-1,3-benzodioxole], apiole [4,7-dimethoxy-5-(2-propenyl)-1,3-benzodioxole], eugenol [2-methoxy-4-(2-propenyl)-phenol], methyl eugenol [1,2-dimethoxy-4-(2-propenyl)-benzene], elemicin [1,2,3-trimethoxy-5-(2-propenyl)-benzene], estragole [1-methoxy-4-(2-propenyl)-benzene] and other natural substances having chemical structures to be considered related to possible toxicological effects.

Safrole content in non alcoholic beverages is limited to 1 mg/kg by CE Directives N. 88/388 and 91/71 concerning "flavourings for use in foodstuffs and source materials for their production". The possibility to detect concentrations lower than 0.1 mg/l has a great impact on the recognition of convenience goods for which the lower content of safrole constitutes a quality parameter for safety reason.

The analytical methods available for a GC-based quantitative determination of safrole (2–4) suggest non-standardisable extraction procedures by solvent or/and steam distillation. These operations are critical points in relation to recovery values, repeatability and detection limits. Moreover, no validation data are reported for these methods. Our experience shows RSDs values in all cases higher than 8.0 % at concentrations lower than 100.0 µg/l.

Zubillaga and Maerker (5) suggest the use of a "dry column" procedure followed by TLC or HPLC isolation before GC analysis. Nevertheless this method is applicable only at safrole levels of 100 mg/kg.

The present communication reports an improved analytical method considering the standardisation of the extraction procedures, allowing the evaluation of very low safrole concentrations. The method foresees a preliminary extraction by hydrodistillation using a Clevenger-type apparatus, followed by GC/MS-MID (Multiple ion detection) analysis.

Furthermore we verify the applicability of that method on a commercial soft drink beverage ("cola" type).

Materials and methods

Standard and reagents

The following standards were used for our analytical purpose:

A Safrole (Aldrich, Milan, Italy) standard solutions at concentration between 500 and 50 mg/l in methanol: aliquots ranging between 25.0 and 2.5 mg of safrole, accurately weighed, are dissolved in 50 ml with methanol.

B Camphor (Aldrich, Milan, Italy) standard reference solution at 500 mg/l: about 25.0 mg of camphor, accurately weighed, are dissolved 50 ml with methanol.

C Limonene standard: Aldrich (Milan, Italy)

Identification of the linear concentration range

Several 500 ml volumes of an aqueous solution simulating the beverage (i.e. containing the same amount of sugars and organic acids) were placed in a Clevenger-type apparatus: to each of them 0.1 ml of safrole solutions at concentrations ranging of 500 to 50 mg/l (A in "Standard and reagents"), 0.1 ml of camphor standard solution (B in "Standard and reagents"), 1 ml of limonene (stripping agent and recovery solvent for safrole) and about 20 g of sodium chloride were added. These solutions contained 100.0 µg/l of camphor and safrole between 10.0–100.0 µg/l . These solutions were hydrodistilled in a Clevenger-type apparatus for two hours and the organic fractions collected were analysed by GC/MS-MID. The graph was constructed reporting in abscissa the ratio between safrole peak area (AS) and camphor peak area (AC), both expressed in "area counts". In ordinate safrole standard solutions concentrations (µg/l) are reported.

The volume of limonene recovered, not lower than 0.85–0.90 ml, does not influence the quantitative data obtained for safrole because the calibration graph depends on the area ratio safrole/camphor. Moreover, it's been verified that each safrole/camphor ratio in limonene was strictly reproducible in the overall range of linearity (average RSDs lower than 0.1%). Evidently that depends on the high volatility of both safrole and camphor, which gives high comparable recoveries.

Soft drink extraction

A 500 ml volume of the soft drink was placed in a Clevenger-type apparatus together with 1 ml of limonene, about 20 g of sodium chloride, 0.1 ml of camphor

standard solution (B in "Standard and reagents") and 0.1 ml of methanol. The mixture was extracted for two hours and the organic fraction collected was analysed by GC/MS-MID.

Recovery experiments

Recovery experiments were performed in triplicate by spiking the soft drink samples with safrole at levels between 30.0–80.0 µg/l.

GC/MS-MID analysis conditions

Analyses were performed on a Shimadzu mass spectrometer QP 5050A equipped with a gas chromatograph Shimadzu GC-17A; GC conditions were: an SPB™-5 fused-silica capillary column, 30 m × 0.25 mm i.d., 0.20 µm film thickness (Supelco, Milan, Italy); column temperature, 60° C (1 min) to 230° C (20 min) at 2° C/min; injector temperature, 220° C; injection mode, split; split ratio 1/20; injection volume 0.2 µl; carrier gas (He) at 1 ml/min.

MS conditions were: GC/MS interface temperature, 250° C; acquisition parameters, MID. Selected ions were m/z 95–108–152 for the internal standard (camphor) and m/z 161–162–163 for safrole.

Results and discussion

Table 1 reported the internal validation data for the proposed method: the recovery values ranged between 80–93 % (RSDs: 2.7–5.1 %). The limit of detection (LOD) was 10.0 µg/l based on a conventional signal/noise ratio of 3:1. The limit of quantification (LOQ) was 30.0 µg/l.

Table 1
Internal validation data for the proposed GC/MS method for quantitative determination of safrole in "cola type" beverages

Limit of detection (LOD) (mg/l)	10.0[a]
Limit of quantification (LOQ) (mg/l)	30.0
Recovery	80–93 % (RSDs = 2.7–5.1 %)
Linear concentration range (mg/l)	10.0–100.0 (Y = 111.7X, R^2 = 0.9985)

[a] Calculated as three times the baseline noise.

In figure 1 the linear concentration range for the method is reported: under the operating conditions described, safrole response was linear over a concentration range of 10.0–100.0 µg/l (Y = 111.7X, R^2 = 0.9985).

Moreover the method was applied to a commercial "cola" beverage giving a result lower than LOQ.

In conclusion the recoveries obtained with spiked samples and the other validation data give evidence of the accuracy and applicability of the proposed method: it

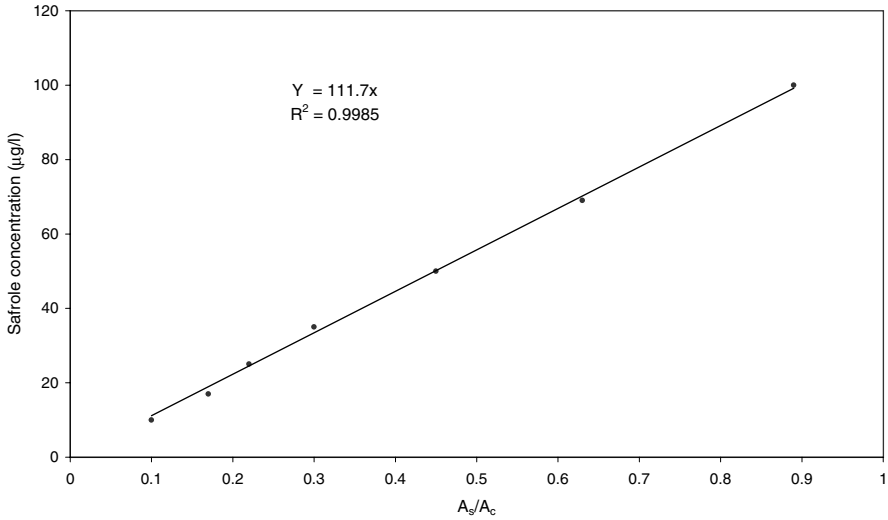

Figure 1 **Calibration curve for safrole quantitative determination over a linear concentration range of 10.0–100.0 µg/l obtained under the operating conditions described in the "Materials and methods" section**
A_S = safrole peak area
A_C = camphor (internal standard) peak area

is shown to be rapid, suitable to detect low concentration values and simpler than the current GC methods mentioned in bibliography.

These considerations suggest the use of that method as suitable for routine analysis on foodstuffs.

Summary

Safrole [1,3-benzodioxole, 5-(2-propenyl)-] is a limited substance according to the CE Directives N. 88/388 and 91/71 concerning "flavourings for use in foodstuffs and to source materials for their production". At today the GC analytical methods proposed for the quantitative determination of safrole are critical as far as concerning reproducibility, recovery values and detection limits. This note describes an improved analytical method to quantify safrole in "soft" drinks. Average recoveries of safrole from samples spiked at levels from 30.0 to 80.0 µg/l ranged from 80 to 93 % with good reproducibility (RSD = 2.7 at 30.0 µg/l). Limits of detection (LOD) and quantification (LOQ) were 10.0 µg/l and 30.0 µg/l respectively. The proposed method is also suitable for routine analysis.

Zusammenfassung

Safrol (1,3-Benzodioxol, 5-[2-Propenyl]-) ist eine Substanz gemäss EWG-Richtlinien 88/388 und 91/71 über «Aromen zur Verwendung in Lebensmitteln und Ausgangstoffe für ihre Herstellung». Bis heute sind die zur quantitativen Bestimmung von Safrol vorgeschlagenen GC Analysemethoden hinsichtlich Wiederholbarkeit, Wiederfindung und Nachweisgrenzen kritisch. Der vorliegende Bericht beschreibt eine verbesserte Analysenmethode zur quantitativen Bestimmung von Safrol in alkoholfreien Getränken. Die mittlere Wiederfindung von Safrol bei Proben von 30,0–80,0 mg/l liegt zwischen 80 und 93 % bei einer guten Wiederholbarkeit (RSD = 2,7 bei 30,0 mg/l). Die Nachweisgrenze (LOD) und der mengenmässige Nachweis (LOQ) betragen 10,0 mg/l bzw. 30,0 mg/l. Die vorgeschlagene Methode ist auch für Routineanalysen geeignet.

Résumé

Le safrole (1,3-benzodioxol, 5-[2-propenyl]-) est une substance régie par les Directives CE n. 88/388 et 91/71 concernant les «arômes à utiliser pour les denrées alimentaires et les matières premières pour leur production». Les méthodes d'analyse par chromatographie gazeuse proposées à ce jour pour la determination quantitative du safrole posent des problèmes aux niveaux de la reproductibilité, des taux de récupération et des limites de détection. Cet article décrit une méthode analytique améliorée pour quantifier le safrole contenu dans les boissons non alcoolisées. La moyenne de récupération du safrole sur la base d'échantillons dopés, à des concentrations comprises entre 30,0 et 80,0 mg/l allait de 80 à 93 % avec une bonne reproducibilité (RSD = 2,7 à 30,0 mg/l). Les limites de détection (LOD) et de quantification (LOQ) étaient de 10,0 mg/l et de 30,0 mg/l respectivement. La méthode proposée convient également pour les analyses de routine.

Key words: Safrole, Soft drinks, GC/MS determination

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Identification de composés monoterpéniques, sesquiterpéniques et benzéniques dans un lait d'alpage très riche en ces substances

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Introduction

Les terpènes, métabolites secondaires des plantes, sont réputés tant pour leurs activités biologiques, notamment leurs effets désinfectants et cicatrisants chez les plantes et répulsifs à l'égard de leurs prédateurs (1), que pour leurs propriétés odor(ifér)antes, en particulier chez les plantes médicinales et celles utilisées à des fins culinaires. Ce sont les composants dominants des huiles essentielles. Dans le domaine alimentaire, les terpènes sont présents surtout dans les produits d'origine végétale, mais peuvent avoir d'autres origines, notamment microbiologiques (levures (2), limonène par *Lb. acidophilus* (3), pinène (4)). Depuis quelques années, ils suscitent l'intérêt des chercheurs qui étudient les produits animaux, en particulier le lait et les produits laitiers (5–11). Présents dans les plantes consommées par les animaux, ils se retrouvent en nombre dans le fromage après un transfert dans le rumen puis dans le lait (11–13).

Leur intérêt dans ces produits est double. D'une part, ils peuvent servir de traceurs pour déterminer l'origine géographique (régions de montagne *versus* de plaine) et le type d'alimentation des animaux en ce qui concerne les produits carnés et laitiers. La composition tant qualitative que quantitative de ces composés varie en effet avec l'origine botanique de l'herbe consommée. Ils sont plus nombreux dans

les dicotylédones, avec pour conséquence une présence plus marquée dans les pâturages de montagne (8, 12–16). Ils sont également plus abondants dans l'herbe fraîche que dans le foin (17) en raison de leur forte volatilité (5). Enfin, l'apport de concentrés, par dilution de l'herbe dans la ration, diminue la concentration en terpènes du lait (18, 19).

D'autre part, ils pourraient être susceptibles d'influencer l'arôme des fromages. A l'état pur, ils présentent des odeurs caractéristiques décrites avec les termes «fruité, suave, frais, vert, d'herbe, de résine, de citron, de camphre, de conifère», selon les composés considérés, bien qu'ils semblent être présents dans ce type de produits en concentrations trop faibles pour avoir un rôle direct significatif (12). Cependant, il est possible qu'ils aient un effet indirect sur l'arôme des fromages, *via* une influence sur certaines activités microbiennes productrices d'arôme (12, 20).

La richesse de l'herbe en composés benzéniques varie également selon les zones de pâturage (21). Ces composés sont donc aussi susceptibles de jouer un rôle de traceurs d'origine dans les fromages s'ils ne sont pas dégradés en d'autres constituants. Peu de ces molécules ont fait l'objet d'une identification exacte dans les produits laitiers, en raison de leur faible concentration et de leur difficile extraction, leur origine même semblant parfois être problématique (33).

L'objectif du présent travail est donc d'établir une liste aussi exhaustive que possible des composés monoterpéniques, sesquiterpéniques et benzéniques que l'on peut rencontrer dans le lait afin de disposer de références pour des travaux d'identification ultérieurs. Ces composés ont en effet été identifiés dans un lait d'alpage particulièrement riche en ces substances en utilisant une technique de préconcentration des volatils par piégeage et désorption, l'espace de tête dynamique (DHS), couplée à une technique de séparation par chromatographie en phase gazeuse avec détection par spectrométrie de masse (GC/MS). Ce travail fait partie d'une étude plus vaste sur l'influence de la végétation des pâturages sur les caractéristiques sensorielles du fromage d'Abondance, dont certains résultats ont déjà été publiés (12, 13, 21–24). Ainsi, l'analyse d'un grand nombre d'échantillons de laits d'origines diverses a déjà fait l'objet d'une autre publication (13).

Matériel et méthodes

L'échantillon de lait étudié a été prélevé dans une zone d'alpage de la zone AOC Abondance (Haute-Savoie, Alpes françaises) dont la végétation était particulièrement riche en monoterpènes, sesquiterpènes et composés benzéniques (21, 22). Il a été conservé à −40° C pendant environ un an dans un flacon en verre fermé par un bouchon en téflon jusqu'au moment de l'analyse.

L'extraction des composés volatils a été réalisée par purge and trap (Tekmar, LSC 3000) sur une cartouche de tenax après décongélation de l'échantillon pendant une nuit à +4° C. Dix ml de lait ont alors été introduits dans une cellule en U (sparger) de 25 ml sans fritté (Agilent Technologies, art. no 5182-0849). La purge a été réalisée à 40° C pendant 40 min sous un courant d'hélium de 46 $ml.min^{-1}$. La désorption a été réalisée à 225° C pendant 2 min. La température du MCS (moisture control system)

a été maintenue à 225° C durant toute la durée de l'extraction. L'injection a été réalisée après cryoconcentration à –150° C.

La séparation des composés volatils sélectivement recherchés pour la présente étude – au détriment des plus légers perdus par saturation du piège dans de telles conditions – a été réalisée avec un chromatographe en phase gazeuse (Hewlett Packard 6890) équipé d'une colonne capillaire de silice fondue de type DB5 (60 m × 0,32 mm de diamètre intérieur, 1 µm d'épaisseur de film de phase, RTX5 Restek, Evry, France). Le débit d'hélium dans la colonne était de 2 $ml.min^{-1}$ à 40° C. Le four a été maintenu à 40° C pendant 5 min, puis la température du four a augmenté de 3° $C.min^{-1}$ jusqu'à 230° C où elle a été maintenue pendant 2 min.

Les composés volatils ont été détectés par un spectromètre de masse (MSD Hewlett Packard 5973) en impact électronique à 70 eV. La température de l'interface entre le chromatographe et le spectromètre de masse était de 280° C. L'acquisition des ions par le détecteur de masse a été faite entre 29 et 207 amu.

L'identification finale des composés détectés a été réalisée en utilisant i) le logiciel Masslib, ii) les données de la littérature (8, 10, 25–27), avec en particulier la consultation des spectres publiés par *Adams* (27) pour l'identification des sesquiterpènes, iii) la base de données constituée au laboratoire à partir d'analyses comparées de plus de 500 chromatogrammes de fourrages, de laits et de fromages de diverses origines et natures, sur la base des bibliothèques de spectres Wiley et NIST, ainsi que iv) l'injection de composés de référence. Le calcul des indices de Kovats (IK) a facilité la comparaison avec les données existantes.

Résultats et discussion

Le tableau 1 présente la liste des monoterpènes détectés, le tableau 2, celle des sesquiterpènes et le tableau 3, celle des composés benzéniques. L'identification par comparaison avec notre base de données interne est indiquée dans la quatrième colonne de chaque tableau. Une formule brute est proposée lorsque le composé ne peut pas être identifié avec certitude. L'identification par comparaison avec le logiciel Masslib®, par comparaison avec la littérature, et par injection de composés de référence purs, est donnée dans les 5e, 6e et 7e colonnes respectivement.

Les figures 1a et b présentent les portions du chromatogramme où sont localisés les pics significatifs pour cette étude. Les autres pics visibles sur ce chromatogramme, mais qui ne sont pas numérotés, correspondent à d'autres classes chimiques (alcools, composés carbonylés, esters etc.) sans intérêt pour ce travail. Un blanc (ou témoin) a encore confirmé que les composés trouvés dans ce lait ne sont pas des artéfacts qui pourraient être générés par exemple lors de la thermodésorption du piège utilisé (tenax).

La conservation de l'échantillon utilisé pendant une année à l'état surgelé n'ôte pourtant en rien à la validité des résultats présentés. Une étude complémentaire en cours de publication (Salmon et Buchin, INRA de Poligny, 2002) a en effet démontré que la présence des composés intéressants pour ce présent travail (*analyse quali-*

tative) n'est nullement influencée par de telles conditions de stockage. Seuls les *aspects quantitatifs* sont susceptibles de varier: la tendance générale observée est une diminution des concentrations des composés terpéniques et sesquiterpéniques et, inversement, une augmentation des concentrations des benzéniques. Cette augmentation est d'ailleurs accord avec les observations faites par *Bosset et al.* lors du stockage prolongé de fromages à l'état surgelé dans certains types d'emballage (33).

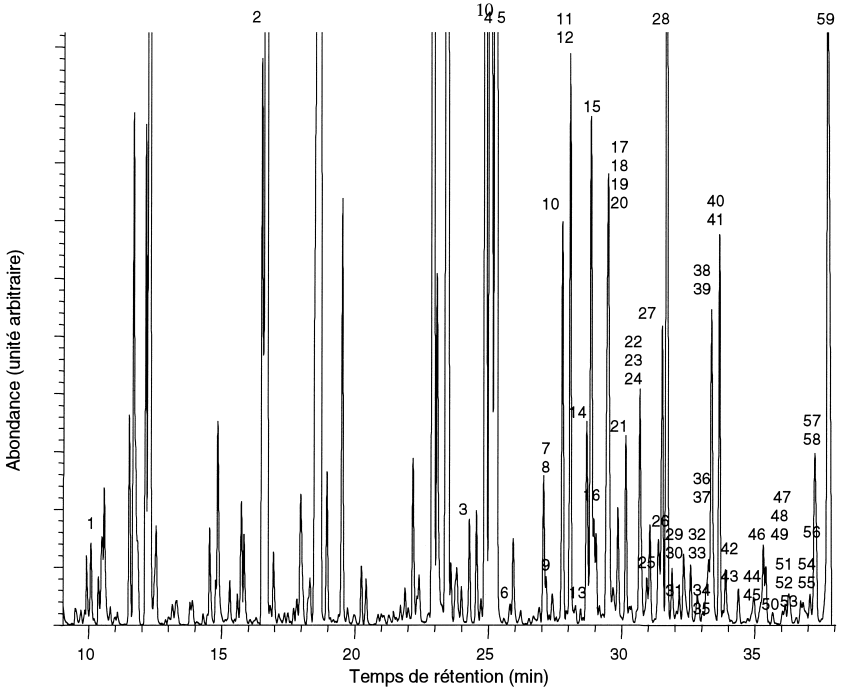

Figure 1A **Première portion du chromatogramme GC-MS correspondant aux composés monoterpéniques, sesquiterpéniques et benzéniques dans le lait d'alpage étudié entre 10 et 38 min (début)**
Légende: la numérotation des pics correspond à celle des tableaux 1 à 3

Monoterpènes

La plupart des 34 monoterpènes extraits ont été identifiés car présents et extractibles en quantité suffisante pour obtenir des spectres de masse interprétables (tableau 1). A l'exception des diméthyl octènes et octadiènes, la majorité sont des hydrocarbures cycliques, parmi lesquels se trouvent trois composés benzéniques (cymènes). Chacun de ces composés possède une fragmentation et un indice de

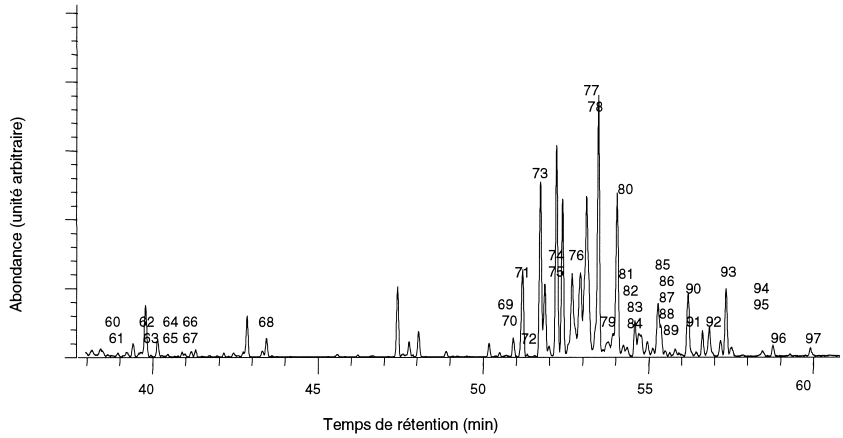

Figure 1B **Seconde portion du chromatogramme GC-MS correspondant aux composés monoterpéniques, sesquiterpéniques et benzéniques dans le lait d'alpage étudié entre 38 et 60 min (suite et fin)**
Légende: la numérotation des pics correspond à celle des tableaux 1 à 3

Kovats caractéristiques qui facilitent leur identification. Certains de ces composés ont déjà été décrits par différents auteurs non seulement dans les plantes pâturées par les animaux (8, 26), mais aussi dans des produits comme le lait (19) et différents types de fromages (7, 10, 14, 28). Ces composés seraient ainsi transférés du fourrage au lait, *via* l'animal, puis au fromage (11, 12). Il semble que quelques terpènes oxygénés soient également présents dans l'échantillon, dont trois ont fait l'objet d'une tentative d'identification indiquée par un (t) dans les tableaux 1 à 3. Les quantités extraites de ces composés sont très faibles, en raison soit d'une faible teneur naturelle dans l'échantillon, soit d'un taux d'extraction de la matrice trop bas. De ce fait, ils ont rarement été décrits dans le lait et les produits laitiers, bien qu'ils soient très présents dans les plantes pâturées (8, 22, 26). Par ailleurs, deux composés à treize carbones ont été mis en évidence (IK de 1359 et 1412).

Sesquiterpènes

Les sesquiterpènes (tableau 2) sont en général très peu présents tant en diversité qu'en quantités, voire absents dans le lait. Le lait analysé est donc particulièrement riche en sesquiterpènes, comparé aux laits habituellement analysés au laboratoire dans les mêmes conditions. Il faut pourtant remarquer que le fourrage donné au troupeau qui a produit ce lait est lui aussi exceptionnellement riche en ces composés, ce qui laisse supposer un transfert plus ou moins proportionnel de ces composés de l'herbe au lait par l'animal.

Tableau 1
Monoterpènes identifiés dans le lait

No pic	*IK*	*Ions spécifiques utilisés*	*Identification par base de données du laboratoire*	*Identification par Masslib (% similitude)*	*Littérature et IK correspondants*	*Composés de référence injectés et IK correspondants*
3	888	93-121-136	cyclofenchène			
6	912	93-121-91-136	2,2,7-triméthyl bicyclo (2.2.1) hept-2-ène			
7	931	93-121-136	tricyclène		tricyclène 936 (8) 911 (26)	
9	933	67-55-95-68-82-110-123	2,6-diméthyl 1,7-octadiène	2,6-diméthyl 1,7-octadiène (α-citronellène) (95 %)		
10	943	93-77-79-121-136	α-pinène	α-pinène (98 %)	α-pinène 945-948 (8) 933 (10) 929-940 (25)	α-pinène 943
11	947	55-67-69-82-95-123-138	3,7-diméthyl 1,6-octadiène	3,7-diméthyl 1,6-octadiène (99 %)		
12	948	136-121	β-fenchène			
14	958	93-79-95-121-107-136	α-fenchène		α-fenchène 959 (8)	
15	960	93-121-79-107-136	camphène	camphène (97 %)	Camphène 962-965 (8) 959 (10) 938-948 (25)	
19	970	70-55-69-140-83	3,7-diméthyl 2-octène *cis/trans*	3,7-diméthyl 2-octène (96 %)		
21	981	93-	α-myrcène		myrcène 987-991 (25)	
23	989	93-121-107	β-pinène		β-pinène 990-993 (8) 987 (10) 968-990 (25) 992 (26)	
24	990	95-69 coélué avec 123-138	2,6-diméthyl 2,6-octadiène	triméthyl 1,5-heptadiène ou 2,6-diméthyl 2,6-octadiène (t) (78 %)		
25	994	93-	β-myrcène		β-myrcène 985-987 (8) 992 (10)	
28	1006	93	δ-2-carène (t)			2-carène 1009

No pic	*IK*	*Ions spécifiques utilisés*	*Identification par base de données du laboratoire*	*Identification par Masslib (% similitude)*	*Littérature et IK correspondants*	*Composés de référence injectés et IK correspondants*
29	1009	95-67-81-138-123	camphane ou p-menth-2-ène	p-menth-2-ène (t) (83 %)		
30	1010	93 coélué avec 121-136	δ-4-carène		4-carène 1010-1013 (8) 1001 (25)	
31	1013	93-77-91-136	l-phellandrène	carveol (t)	α-phellandrène 1011-1014 (8) 1012 (10) 997-1006 (25) 1003 (26)	
32	1020	93-77-79-121-136	δ-3-carène	δ-3-carène (94 %)	δ-3-carène 1021-1025 (8) 1019 (10) 1004-1014 (25)	3-carène 1023
34	1026	121-93-136-77-79-105	α-terpinène	isoterpinolène (t) (80 %)	α-terpinène 1025 (8) 1025 (10) 1009-1065 (25)	α-terpinène 1025
35	1028	95-67-138-82-109	3-méthyl butylène cyclopentane			
36	1030	119-134-91	*m*-cymène	*m*-cymène (t) (83 %)	*m*-cymène 1026 (10) 1023-1026 (25)	
37	1032	95-67-138-123	*p*-menth-1-ène	carvomenthène (t) (80 %)		*p*-menth-1-ène 1032
38	1034	119-134-91	*p*-cymène	*p*-cymène (t) (96 %)	*p*-cymène 1024-1027 (8) 1032 (10) 1024-1038 (25) 1022 (26)	*p*-cymène 1033
40	1039	68-93-67-79-121-107-136	dl-limonène	limonène (98 %)	limonène 1037-1040 (8) 1038 (10) 1022-1038 (25) 1029 (26)	limonène 1038
41	1041	93-136	*cis*-ocimène		*cis*-ocimène 1025-1030 (8) 1050 (10) 1037 (25) 1041 (26)	
42	1044	71-108-84-154-139	1,8-cinéole		1,8-cinéole 1039-1045 (8) 1030-1033 (25) 1030 (26)	

No pic	*IK*	*Ions spécifiques utilisés*	*Identification par base de données du laboratoire*	*Identification par Masslib (% similitude)*	*Littérature et IK correspondants*	*Composés de référence injectés et IK correspondants*
49	1068	93-136-77-121	γ-terpinène	γ-terpinène (95%)	γ-terpinène 1061-1067 (8) 1067 (10) 1055-1088 (25) 1060 (26)	γ-terpinène 1068
57	1099	121-93-136	α-terpinolène	α-terpinolène (97%)	α-terpinolène 1094-1096 (8) 1097 (10) 1082-1092 (25) 1089 (26)	
70	1359	69-81-95-109-191-163	mélange: Z-α-damascone (t) + Z-β-damascenone (t)	mélange de 2 (dont structure clovène ou longipinène) (t)	Z-α-damascone 1354 (27) Z-β-damascenone 1359 (27)	
71	1365	81-95-69-93-109-163	esters: carvomenthyl acétate (t) + ester du citronellol (t)	structure sesquiterpène (t)	Carvomenthyl acétate 1351 (27)	
73	1376	81-135-95-123-191	thymol acétate ou citronellyl acétate (t)		thymol acétate 1354 (27) citronellyl acétate 1355 (27)	
78	1412	69-81-95-163-123-109	voisin de la E-*epi*-α-damascone		E-*epi*-α-damascone 1390 (27)	
79	1422	95-124-109	type isobornyl butyrate			

Légende: No pic: cf. figure 1; IK = indice de Kovats; Ions spécifiques = ions majoritaires du pic; Composés de référence = composés purs injectés dans les mêmes conditions et présentant le même spectre de masse; (t) = Tentative d'identification

Tableau 2
Sesquiterpènes identifiés dans le lait

No pic	*IK*	*Ions spécifiques utilisés*	*Identification par base de données du laboratoire*	*Identification par Masslib (% similitude)*	*Littérature et IK correspondants*	*Composés de référence injectés et IK correspondants*
69	1351	81-95-136-69-109	sesquiterpène (t)	structure longipinène ou néoclovène (t)		
72	1367	161-189-91-105	C 15 H24	structure bicyclique (sesquiterpène ?) (t)		
74	1395	81-95-109-67-121-163	β-patchoulène + ester		β-patchoulène 1380 (27)	
75	1396	161-189-204	voisin du clovène			
76	1399	109-161-204	sesquiterpène (PM = 204) ou voisin du davanafurane (t)			
77	1407	80-81-123-161	mélange avec β-bourbonène (PM = 204)		β-bourbonène 1431 (8) 1415 (10) 1385 (26) 1384 (27)	
80	1424	109-81-67-95-121-163	voisin du *epi*-cédrane			
81	1432	93-161(coélué)	C 15 H24	sesquiterpène (t)		
82	1437	81-95-109-67-123-206	composé sesquiterpénique à noyau cédrane (PM=206)			
83	1439	148-106-93-91-79-189	voisin du italicène ou *iso*-italicène (PM=204)		italicène 1401 (27) *iso*-italicène 1397 (27)	
84	1441	119-93-91-105-161	1,7-di-*epi*-α-cedrène (PM=204)		1,7-di-*epi*-α-cedrène 1397 (27)	
85	1448	94-91-105-93-133-161	longifolène (PM=204)		longifolène 1402 (27)	longifolène (1447)
86	1451	93-133-106-161-189	β-*trans*-caryophyllène ou α-*cis*-bergamotène (PM=204)	*iso*-caryophyllène ou caryophyllène VI (t) (80 %)	β-*trans*-caryophyllène 1476-1479 (8) 1451 (10) 1434-1440 (25) 1424 (26) 1418 (27)	β-*trans*-caryophyllène (1455)

No pic	IK	Ions spécifiques utilisés	Identification par base de données du laboratoire	Identification par Masslib (% similitude)	Littérature et IK correspondants	Composés de référence injectés et IK correspondants
					α-*cis*-bergamotène 1407 (26) 1415 (27)	
87	1452	119-93-161	mélange α-*trans*-bergamotène (PM=204) +α-guaiène (PM=204) +aromadendrène (PM=204) (t)		α-*trans*-bergamotène 1438 (26) 1436 (27) α-guaiène 1439 (27)	α-cedrène (1452)
88	1454	81-93-95-79-149-107-163	mélange avec β-humulène (PM=204)		β-humulène 1440 (27)	
89	1456	81-95-69-119-161	C 15 H24	mélange de 2 (dont 1sesquiterpène) (t)		
90	1471	163-107-81-93-121	voisin du *epi*-cedrane (PM=206)		*epi*-cedrane 1441 (27)	
91	1481	125-83-95-196	mélange avec muuroladiène (PM=204) (t)		muuroladiène 1460 (27)	
92	1493	161-122-107-91-133-204	7-*epi*-α-sélinène	α-panasinsène (t) (85 %)	7-*epi*-α-sélinène 1517 (27)	α-humulène (1490)
93	1497	191-161-189-135-105-175	mélange avec α-néoclovène (PM=204)	sesquiterpène (t)	α-néoclovène 1454 (27)	
94	1499	119-105-91-132-204	β-chamigrène (PM=204)		β-chamigrène 1478 (26) 1475 (27)	
95	1500	119-132-105-145-202	*ar*-curcumène (PM=202)		*ar*-curcumène 1483 (26) (27)	
96	1530	105-161-93-94-204	α-muurolène (PM=204)	α-muurolène (t)	α-muurolène 1493 (27)	
97	1557	159-202-128-144	*cis*- ou *trans*-calaménène (PM=202)	calaménène (t)	*cis*- ou *trans*-calaménène 1526 (26) 1532 (27)	

Légende: No pic: cf. figure 1; IK=indice de Kovats; Ions spécifiques=ions majoritaires du pic ; Composés de référence=composés purs injectés dans les mêmes conditions et présentant le même spectre de masse; (t)=Tentative d'identification; PM=Poids moléculaire

Il est cependant probable que seule une faible quantité des sesquiterpènes ingérés se retrouve dans le lait, bien qu'il soit en fait difficile de comparer les quantités présentes dans les deux produits. L'extraction dynamique de ces composés à partir du lait est en effet rendue difficile en raison de leur faible volatilité et de leur grande solubilité dans la matière grasse. Cette dernière retient sans doute une grande partie des sesquiterpènes dans la matrice, alors que cette difficulté n'existe pas ou que dans une moindre mesure avec des fourrages à matrice cellulosique.

Dans la zone du chromatogramme correspondant aux temps de rétention des sesquiterpènes (fig. 1), plus de 60 pics sont présents, parmi lesquels 24 ont pu être identifiés avec plus ou moins d'exactitude comme sesquiterpènes. Afin d'en faciliter l'identification, les spectres ont été comparés à ceux obtenus dans le fourrage correspondant. Cependant, la plupart des spectres des composés du lait sont d'une qualité médiocre à cause des faibles quantités de produits extraits et de nombreuses coélutions, ce qui rend l'identification difficile. Par ailleurs, la comparaison avec les données de la littérature a fait apparaître des indices de Kovats sensiblement différents d'une référence à l'autre. La comparaison avec les spectres publiés par *Adams* (27), qui a servi de base à la plupart des identifications, a ainsi montré des différences pouvant atteindre près de 50 unités malgré l'utilisation du même type de colonne, une DB5. Les valeurs publiées par *Cornu et al.* (26) sur le même type de phase sont en accord avec cet auteur, peut-être pris comme référence pour leur interprétation de spectres. La comparaison de nos résultats avec ceux de *Mariaca et al.* (8) a montré des écarts plus faibles malgré l'utilisation par ces auteurs d'une phase différente, une DB1. Les indices de Kovats calculés dans la présente étude sont en revanche voisins de ceux de *Viallon et al.* (10) obtenus également sur une DB5.

Parmi les 24 composés sesquiterpéniques extraits du lait, douze seulement sont présents avec certitude dans le fourrage correspondant (29). Cela tendrait à montrer que le transfert des terpènes du fourrage vers le lait constaté par *Viallon et al.* (11) et par *Bugaud et al.* (12) concerne les quantités globales de ces composés, mais pas l'ensemble de ceux-ci. Pour certaines molécules, il semble qu'il puisse se produire des isomérisations, des dégradations ou des bioconversions (30–32) lors du passage dans l'animal, comme cela a été déjà évoqué par *Bugaud et al.* (12) dans le cas des monoterpènes oxygénés. De plus, l'hypothèse susmentionnée de la biosynthèse de terpénoïdes par des microorganismes ne doit pas être écartée (2–4).

Composés benzéniques

La diversité des composés benzéniques (tableau 3) de l'échantillon analysé est très grande, puisque 41 composés ont été détectés. La plupart (n=38) sont des hydrocarbures monocycliques possédant de 0 à 5 atomes de carbone substitués. Si l'identification des substituants est relativement aisée par comparaison de leur spectre de masse à des spectres de référence connus, les isomères de position sont en revanche difficiles à déterminer en raison d'une (trop) grande similitude de leur

Tableau 3
Composés benzéniques identifiés dans le lait

No pic	*IK*	*Ions spécifiques utilisés*	*Identification par base de données du laboratoire*	*Identification par Masslib (% similitude)*	*Littérature (25) et IK correspondants*
1	662	78	benzène		
2	769	91	toluène (t)		
4	900	91-106-105	*m*-xylène		*m*-xylène 862-875
5	908	91-106-105	*o*-xylène		*o*-xylène 884-898
8	932	105-120	*iso*-propyl benzène	1-méthyl, éthyl benzène (98 %)	*iso*-propyl benzène 917
13	953	117-118-91	2-propenyl benzène		
16	962	91-120	propyl benzène	propyl benzène (97 %)	propyl benzène 965
17	969	105-120-91	éthyl, méthyl benzène	1-éthyl, x-méthyl benzène (t) (97%)	3éthyl, 1-méthyl benzène 954 ou 1-méthyl, 4-éthyl benzène 955
18	970	105-106-77	benzaldéhyde		
20	976	105-120-119	1,2,4-triméthyl benzène		1,2,4-triméthyl benzène 984-990
22	989	105-120-91	éthyl, méthyl benzène	x-méthyl, x-éthyl benzène (t)	
26	1000	117-118	1-propenyl benzène		
27	1002	105-120-119	1,3,5(t)-triméthyl benzène	triméthyl benzène (t) (99 %)	1,3,5-triméthyl benzène 961-1002
33	1022	132	méthyl, isopropenyl benzène		
36	1030	119-134-91	*m*-cymène	*m*-cymène (t) (83 %)	*m*-cymène 1023-1026
38	1034	119-134-91	*p*-cymène	*p*-cymène (t) (96 %)	*p*-cymène 1024-1038
39	1034	105-120	1,2,3(t)-triméthyl benzène	triméthyl benzène (t) (87%)	1,2,3-triméthyl benzène 996-1012
43	1051	117-118	2,3-dihydro indène		
44	1060	115-116	indène		
45	1061	105-134	méthyl, propyl benzène	mélange propyl méthyl benzène, benzène acétaldéhyde et autres… (t)	
46	1065	105-134	méthyl, propyl benzène		2méthyl, propyl benzène 1065

No pic	*IK*	*Ions spécifiques utilisés*	*Identification par base de données du laboratoire*	*Identification par Masslib (% similitude)*	*Littérature (25) et IK correspondants*
47	1067	119-134	diéthyl benzène		
48	1068	91-105-134	butyl benzène		butyl benzène 1067-1068
50	1073	119-134-105-91	diéthyl benzène		
51	1078	105-134	méthyl, propyl benzène		
52	1078	105-77-120	1-phényl éthanone		1-phényl éthanone 1065-1071
53	1081	120-119-91	méthyl benzaldéhyde		
54	1087	119-134	éthyl, diméthyl benzène		4-éthyl,1,2-diméthyl benzène 1094
55	1090	119-134	éthyl, diméthyl benzène	mélange de 2 (dont méthyl-(méthyl éthyl) benzène ?) (t)	
56	1096	119-134	éthyl, diméthyl benzène		
58	1099	132-117	méthyl, propenyl benzène (t)		
59	1106	105-77-136-51	benzoate de méthyle		
60	1129	119-134	éthyl, diméthyl benzène		
61	1134	119-134-91	éthyl, diméthyl benzène		
62	1157	117-132	éthyl, éthenyl benzène (t)		
63	1162	133-134-105-91	phényl propionaldéhyde		
64	1169	91-105	benzène+5C (t)		pentyl benzène 1168
65	1170	119-91-134	1-phényl 2-propanone (t)		
66	1179	134-133	benzaldéhyde+2 méthyl (t)		4-éthyl benzaldéhyde 1176
67	1180	105-77	benzoate d'éthyle		benzoate d'éthyle 1170
68	1209	128-	naphtalène		naphtalène 1196-1228

Légende: No pic: cf. figure 1; IK=indice de Kovats; Ions spécifiques=ions majoritaires du pic; Composés de référence=composés purs injectés dans les mêmes conditions et présentant le même spectre de masse; (t)=Tentative d'identification

spectre de masse. L'outil de choix, mais non disponible pour la présente étude, pour élucider les positions de substitution serait la détection par FT-IR.

Dans certains cas (xylènes, cymènes), les indices de Kovats sont suffisamment différenciés pour reconnaître le composé en se référant aux données de la littérature. Mais dans d'autres cas (par ex. éthyl, méthyl benzène ou éthyl, diméthyl benzène), les indices de Kovats correspondants sont trop proches pour permettre l'identification de la molécule. Seule l'injection de composés de référence est alors informative à condition pourtant que la totalité des isomères soient présents (ou puissent être introduits par enrichissement) dans l'échantillon, car c'est alors l'ordre d'élution qui est déterminant. Plusieurs hydrocarbures polycycliques ont été mis en évidence: l'indène et le 2,3-dihydro indène à 9 atomes de carbone ainsi que le naphtalène (10 atomes de carbone). Les composés polycycliques plus lourds ne sont probablement ni extraits, ni désorbés par la technique de purge and trap.

Quelques composés benzéniques carbonylés (aldéhydes, cétones, esters), contenant jusqu'à 9 atomes de carbone, sont également détectés.

Si la présence de tels composés a déjà été décrite dans les laits (16) ou différents types de fromages (6, 14, 28), une telle diversité n'a jamais été mentionnée. Dans les laits et les fromages, les composés benzéniques sont en grande partie des composés carbonylés qui proviennent du catabolisme microbien des acides aminés aromatiques tyrosine et la phénylalanine. L'origine des hydrocarbures benzéniques dans ces produits est plus difficile à déterminer. D'après *Bosset et al.* (33), ils pourraient en partie provenir d'une contamination extérieure, en particulier pendant la conservation des échantillons à l'état surgelé si ces derniers ne sont pas conservés dans des récipients parfaitement étanches. Malgré la tendance à l'augmentation des quantités en composés benzéniques au cours de la conservation des laits, il semble cependant que la plupart des composés soient déjà présents avant le stockage de l'échantillon de lait à l'état surgelé (Salmon et Buchin, en préparation). Il faut par ailleurs remarquer que les plantes (essentiellement les *Apiaceae*) du pâturage dont le présent échantillon est issu étaient elles-mêmes exceptionnellement riches en hydrocarbures benzéniques (21), tant en quantités qu'en diversité. Cette constatation étaye donc l'hypothèse d'une probable origine végétale de ces composés.

Conclusion

Le présent travail a permis de repérer dans un lait d'alpage un grand nombre de composés monoterpéniques ($n=34$), sesquiterpéniques ($n=34$) et benzéniques ($n=41$) et d'en proposer une identification plus ou moins précise allant d'une identification certaine à une simple proposition de formule brute. Dans certains cas intermédiaires, une tentative d'identification, indiquée dans les tableaux 1 à 3 par (t), a été réalisée, qui demande une confirmation ultérieure. Cette liste de composés constitue une première approche dans la constitution d'une banque de données de référence tels qu'indices de rétention sur colonne de type DB-5 et spectres de masse en vue de futurs travaux d'analyse qualitative (identification) sur ce type de pro-

duits. Il est en effet des plus probables que ces composés, en complément d'autres grandeurs mesurables, soient toujours plus souvent recherchés comme traceurs ou marqueurs d'une origine géographique de produits laitiers et carnés de montagne.

Résumé

Le présent travail établit une liste aussi complète que possible des composés monoterpéniques ($n=34$), sesquiterpéniques ($n=24$) et benzéniques ($n=41$) trouvés dans un lait d'alpage de la région d'Abondance (Alpes françaises) particulièrement riche en ces substances. L'analyse de ces composés a été faite par chromatographie en phase gazeuse sur une colonne de type DB5 avec détection par spectrométrie de masse après une extraction dynamique des volatils avec un système de préconcentration de type Tekmar 3000. L'identification des composés a été effectuée en utilisant i) le logiciel Masslib, ii) les données de la littérature, en particulier les spectres des sesquiterpènes publiés par *Adams*, iii) la base de données constituée au laboratoire à partir de fourrages, de laits et de fromages ainsi que iv) l'injection des composés de référence disponibles. Cette liste constitue une précieuse banque de données pour de futurs travaux en vue de la traçabilité des produits laitiers et carnés de montagne.

Zusammenfassung

In der vorliegenden Arbeit wurde eine möglichst vollständige Liste von Monoterpenen ($n=34$), Sesquiterpenen ($n=24$) und Benzolderivaten ($n=41$) zusammengestellt, welche sich in einer Alpmilch aus dem Abondance-Tal fanden, die sehr reich an diesen Substanzen war. Die gaschromatografische Analyse wurde auf einer DB5-Säule durchgeführt mit massenspektrometrischer Identifikation. Für die dynamische Extraktion der flüchtigen Stoffe wurde ein Voranreicherungssystem Typ Tekmar 3000 eingesetzt. Zur Identifikation der Substanzen wurden eingesetzt i) die Software Masslib, ii) Literaturdaten, im speziellen die Sesquiterpenspektren von *Adams*, iii) eine eigene Datenbank aus Substanzspektren von Futtermitteln, Milchen und Käsen sowie iv) eingespritzte Referenzsubstanzen. Diese Liste ist eine wertvolle Datenbank für zukünftige Arbeiten im Bereich der Ursprungsbestimmung von Milch- und Fleischprodukten aus dem Berggebiet.

Summary "Identification of Monoterpene, Sesquiterpene and Benzene Derivatives in a Highland Milk very rich in these Compounds"

The present work lists as completely as possible monoterpene ($n=34$), sesquiterpene ($n=24$) and benzene derivatives ($n=41$) found in a milk from highlands of the Abondance area (French Alps) particularly rich in such substances. These compounds have been analyzed by gas chromatography on a DB5-like column, coupled to mass spectrometry after a dynamic headspace extraction with a preconcentration system Tekmar 3000. The identification of the compounds was made using i) Masslib software, ii) literature data, in particular sesquiterpene spectra

published by *Adams*, iii) the data base built in the author's laboratory on the basis of forage, milk and cheese analyses, and iv) the injection of reference compounds when available. This list forms a valuable data base for future works aimed at the traceability of milk and meat products from mountain regions.

Key words
Terpene, Sesquiterpene, Benzene derivative, Milk, Highland

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Analytical Methods for the Determination of the Geographic Origin of Emmental Cheese. Free Fatty Acids, Triglycerides and Fatty Acid Composition of Cheese Fat*

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Introduction

Cow's milk fat is composed of triglycerides consisting of approx. 400 different fatty acids whose composition in milk and milk products is mainly influenced by the forage (1). During summer or by addition of vegetable fats to the fodder, the concentration of long chain unsaturated fatty acids is increased and that of short and medium chain fatty acids (C6-C16) is decreased (2–4). In the case of triglycerides, the group C50-C54 increased as the fodder changed from hay to grass (4) or by addition of vegetable fats (2, 5). Similar results were obtained for the composition of fatty acids and triglycerides by comparing milk fats from cows grazing in the lowlands and highlands (6). In the highlands, the level of unsaturated long chain fatty acids (C18:1, C18:2 and C18:3) was higher, and that of the saturated short and medium chain fatty acids lower (6, 7). Also, the level of short (C28-C36) and medium chain triglycerides (C42-C46) decreased from lowlands to highlands whereas that of long chain triglycerides (C50-C54) increased. The stage of lactation (8, 9) also influenced the composition of milk fat. The content of free fatty acids depended on the lipolytic activity of the microbial flora during cheese making and ripening. Lactic acid bacteria have a very low lipolytic activity (10) whereas the dif-

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ferent propionic acid bacteria used in the manufacture of Emmental have much higher and differentiated activities (11, 12).

The fatty acid and triglyceride composition could therefore help in discriminating dairy products manufactured in regions of different altitudes or to determine what typical fodder compositions were fed to the cows. The level of free fatty acids may also make it possible to discriminate Emmental cheese making according to the lipolytic activity of the propionic acid bacteria used.

The current work reports an attempt to use free fatty acids and composition of triglycerides and fatty acid to determine the geographic origin of Emmental cheese. It forms the first part of a broad screening test in a 3-year study on the authenticity of Emmental cheese and its geographic traceability (13, 14). During this step, a great number of analytical methods have been tested with respect to their discriminating potential using only two to three cheese samples per region (14–18). It is therefore obvious that the analytical results obtained from such a modest number of cheese samples per region can only give trends which should be confirmed later if they appear valuable for discriminating cheeses produced in Switzerland from those produced in other countries.

Materials and methods

Origin and selection of the cheese samples

The main framework of this study and the sampling have been described in detail in (14). Table 1 summarises the origin, the date of manufacture and the ripening time of these winter Emmental samples as well as the average altitude of the production zones.

Table 1
Origin and ripening time of the 20 cheese samples investigated

Sample	*Region (country)*	*Date of manufacture*	*Ripening time (months)*	*Average altitude (m) of the production sites*
AL 1-3	Allgäu (D)	20.12.2000	4	800
BR 1-3	Bretagne (F)	16.02.2001	2.5	100
CH 1-6	Switzerland (CH)	26.12.2000	4	570
FI 1-2	Middle Finland (FI)	05.02.2001	3	150
SA 1-3	Savoie (F)	08.02.2001	3	470
VO 1-3	Vorarlberg (A)	02.02.2001	3	780

Methods used for the determination of free fatty acid, triglyceride and fatty acid composition of milk fat

The gas chromatographic (GC) determination of the free fatty acids (FFA) was performed according to a method developed by *De Jong and Badings* (19), modified at the FAM. The FFA were extracted in sulfuric acid medium by a solution of

diethylether/heptane 1:1 (v/v). They were retained on an anion-exchange (aminopropyl-) column, the neutral lipids were eluted with hexane/2-propanol 3:2 (v:v) and the FFA with diethyl ether containing 2% of formic acid. For the triglycerides and the composition of fatty acids, the fat was extracted according to an IDF standard (20). For the determination of the triglycerides, the isolated fat was dissolved in heptane and directly injected into the GC according to *Collomb et al.* (21). The fatty acid composition of fat was determined by GC after transesterification to methylesters according to *Collomb and Bühler* (1).

Statistical methods

The averages and standard deviations were calculated for each value. Descriptive statistics, analysis of variance (ANOVA) and pairwise comparisons of mean values with Fisher's LSD test were performed using Systat for Windows version 9.0 (SPSS Inc., Chicago, IL).

Results and discussions

Because of the limited number of cheese samples analysed per region, only compounds showing highly significant inter-region differences (ANOVA, $p \leq 0.001$) have been considered. Consequently, out of the compounds analysed, only two free fatty acids, five triglycerides and 29 fatty acids are discussed. Five groups of fatty acids (medium chain, polyunsaturated, C18:2, conjugated linoleic acids (CLA) and ω-3) showed the required significant differences. We also did not use trained classification techniques such a linear discriminant analysis as they require a large data set in order to be reliable.

Significant differences between the regions of lower and higher altitude

Highly significant differences ($p \leq 0.001$) were found between winter Emmental cheeses produced in the regions of lower altitude Bretagne (BR) and Finland (FI) (100–150 m) compared to those produced in the four other regions of higher altitude (470–800 m). Furthermore, silage was fed only in BR and FI. The concentrations of different fatty acids and triglycerides from cheeses produced in regions of lower altitudes were significantly higher (table 2) or lower (table 3) than those found in cheese from the four regions of higher altitude. Such observations agree with the literature quoted in the introduction.

The concentrations of only one triglyceride (C38) and of six minor C18:1 fatty acids were significantly higher in cheeses produced in BR and FI than in the four other regions of higher altitude (table 2). The concentration of the triglyceride C38 was the highest in cheese produced in BR (13.16 g/100 g), and that from FI (12.93 g/100 g) overlapped with that found in Switzerland (CH) (12.68 g/100 g), BR and Vorarlberg (VO) (12.71 g/100 g). *Precht and Heine* (22) found a mean value of this triglyceride generally higher in winter fat (12.7 g/100 g; $s_x = 0.32$; $n = 283$) than in summer fat (12.13; $s_x = 0.27$; $n = 309$). *Collomb et al.* (6) found similar values to those

Table 2
Compounds where the two lowland regions BR and FI showed significantly (P≤0.001) higher values than the regions bordering the Alps

Region[1] *(n=)*	*Overlapping*[2]	*AL (3)*		*BR (3)*		*CH (6)*		*FI (2)*		*SA (3)*		*VO (3)*	
		x	s_x	*x*	s_x	*x*	s_x	*x*	s_x	*x*	s_x	*x*	s_x
Triglyceride (g/100 g triglyceride)													
Triglyceride 38	fi	*12.42*^{D}	0.27	*13.16*^{A}	0.09	*12.68*^{BCD}	0.25	*12.93*^{AB}	0.05	*12.36*^{CD}	0.10	*12.71*^{BCD}	0.07
Fatty acids composition (g/100 g fat)													
C18:1 t12	fi/br	*0.163*^{AB}	0.005	*0.193*^{A}	0.006	*0.136*^{BC}	0.023	*0.197*^{A}	0.006	*0.116*^{BC}	0.025	*0.111*^{C}	0.027
C18:1 t13-14+c6-8	fi	*0.411*^{BC}	0.002	*0.547*^{A}	0.086	*0.393*^{C}	0.053	*0.539*^{AB}	0.015	*0.307*^{C}	0.051	*0.340*^{C}	0.030
C18:1 c11	fi	*0.467*^{BC}	0.029	*0.545*^{A}	0.012	*0.444*^{C}	0.032	*0.505*^{AB}	0.006	*0.455*^{BC}	0.015	*0.458*^{BC}	0.019
C18:1 c12	–	*0.143*^{B}	0.013	*0.208*^{A}	0.006	*0.123*^{B}	0.019	*0.181*^{A}	0.008	*0.124*^{B}	0.002	*0.116*^{B}	0.011
C18:1 c13	fi	*0.049*^{BC}	0.004	*0.064*^{A}	0.002	*0.048*^{C}	0.004	*0.058*^{AB}	0.004	*0.040*^{D}	0.002	*0.040*^{D}	0.005
C18:1 t16+c14	fi	*0.225*^{C}	0.010	*0.338*^{A}	0.027	*0.258*^{BC}	0.017	*0.291*^{AB}	0.004	*0.248*^{BC}	0.008	*0.232*^{C}	0.039

Caption: *x*=mean value; s_x=standard deviation

Production sites: A>B>C>D (=significantly different contents p≤0.001) or AB=A and B overlap by using an univariate discriminant analysis

[1] AL=Allgäu, BR=Bretagne, CH=Switzerland, FI=Finland, SA=Savoie, VO=Vorarlberg

[2] fi/br=the group "Finland"/"Bretagne" overlaps with at least one other group from the regions bordering the Alps

Table 3
Compounds where the two lowland regions BR and FI showed significantly ($P \leq 0.001$) lower values than the regions bordering the Alps

Region (n=)	*Overlapping*	*AL (3)*		*BR (3)*		*CH (6)*		*FI (2)*		*SA (3)*		*VO (3)*	
		x	s_x	*x*	s_x	*x*	s_x	*x*	s_x	*x*	s_x	*x*	s_x
Triglyceride / Free fatty acids													
Triglyceride 48 (g/100 g triglyceride)	br	9.38^{A}	0.15	8.96^{AB}	0.08	9.17^{A}	0.27	8.53^{B}	0.08	9.35^{A}	0.12	9.35^{A}	0.02
Free linolenic acid (mg/kg)	–	35.7^{B}	3.3	17.1^{C}	2.7	34.8^{B}	7.5	13.4^{C}	2.6	35.5^{B}	0.3	51.3^{A}	8.9
Fatty acids composition (g/100 g fat)													
C13 iso	–	0.137^{AB}	0.005	0.088^{C}	0.004	0.124^{B}	0.013	0.075^{C}	0.003	0.157^{A}	0.007	0.142^{AB}	0.003
C14	–	10.33^{AB}	0.15	9.68^{C}	0.07	10.13^{B}	0.23	9.56^{C}	0.04	10.55^{A}	0.18	10.24^{AB}	0.19
C14 iso	–	0.240^{B}	0.005	0.203^{C}	0.001	0.240^{B}	0.018	0.185^{C}	0.004	0.304^{A}	0.008	0.245^{B}	0.006
C14 aiso	–	0.460^{AB}	0.020	0.384^{C}	0.006	0.419^{BC}	0.038	0.363^{C}	0.007	0.479^{A}	0.017	0.461^{AB}	0.013
C14:1 c	fi	0.913^{AB}	0.024	0.806^{D}	0.015	0.917^{A}	0.040	0.838^{BCD}	0.023	0.824^{CD}	0.021	0.905^{ABC}	0.033
C15	–	1.057^{A}	0.047	0.906^{B}	0.014	1.060^{A}	0.060	0.846^{B}	0.003	1.077^{A}	0.016	1.071^{A}	0.038
C15 iso	fi/br	0.262^{BC}	0.019	0.240^{BCD}	0.011	0.242^{CD}	0.023	0.213^{D}	0.003	0.322^{A}	0.010	0.279^{B}	0.013
C16 iso	br	0.331^{AB}	0.001	0.291^{CD}	0.007	0.326^{B}	0.017	0.277^{D}	0.004	0.339^{AB}	0.008	0.313^{BC}	0.006
C18:1 t10–11	fi	2.05^{A}	0.04	1.17^{D}	0.04	1.56^{BC}	0.29	1.20^{CD}	0.00	1.44^{BCD}	0.18	1.76^{AB}	0.05
C18:2 c9t13+(t8c12)	fi/br	0.172^{A}	0.016	0.175^{A}	0.009	0.146^{AB}	0.019	0.180^{A}	0.013	0.118^{B}	0.008	0.126^{B}	0.010
C18:3 c9c12c15	–	0.884^{A}	0.063	0.280^{C}	0.053	0.781^{AB}	0.162	0.372^{C}	0.032	0.673^{AB}	0.043	0.915^{AB}	0.075
C18:2 c9t11	fi	0.935^{A}	0.020	0.410^{D}	0.010	0.660^{B}	0.131	0.411^{CD}	0.010	0.623^{BC}	0.061	0.771^{AB}	0.020
C18:2 c9c11	fi/br	0.054^{A}	0.009	0.013^{C}	0.004	0.036^{B}	0.011	0.017^{C}	0.001	0.029^{BC}	0.005	0.045^{AB}	0.003
C22:5 (DPA) (n-3)	–	0.104^{AB}	0.006	0.051^{C}	0.003	0.101^{AB}	0.008	0.046^{C}	0.001	0.087^{B}	0.009	0.108^{A}	0.014
Groups of fatty acids (g/100 g fat)													
Sum C18:2	fi	3.29^{A}	0.03	2.44^{D}	0.05	2.75^{BC}	0.16	2.49^{CD}	0.16	2.67^{C}	0.07	2.97^{B}	0.08
Sum polyunsaturated FA[1]	–	4.61^{A}	0.09	3.06^{D}	0.11	3.97^{BC}	0.31	3.13^{D}	0.14	3.76^{C}	0.14	4.32^{AB}	0.17

Region (n=)	Overlapping	AL (3)		BR (3)		CH (6)		FI (2)		SA (3)		VO (3)	
		$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x
Sum CLA[2]	fi	*1.01*^{A}	0.03	*0.44*^{D}	0.01	*0.72*^{B}	0.15	*0.45*^{CD}	0.01	*0.67*^{BC}	0.06	*0.85*^{AB}	0.03
Sum ω-3[3]	fi	*1.42*^{A}	0.11	*0.61*^{D}	0.09	*1.25*^{AB}	0.22	*0.66*^{CD}	0.01	*1.04*^{BC}	0.08	*1.40*^{AB}	0.11

Caption: see table 2; FA=fatty acids; DPA=cis 7, cis 10, cis 13, cis 16, cis 19-docosapentaenoic acid according to reference (1).

[1] C18:2 -ttNMID to -c9c15, C18:3 -c6c9c12+-c9c12c15, C18:2 -c9t11 à C20:2 cc, C20:3 à C22:6

[2] CLA total (sum C18:2 -c9t11, -c9c11, -t9t11)

[3] C18:2 -t11c15+c9c15, C18:3 c9c12c15, C20:3 n-3, C20:5, C22:5 and C22:6

found in BR and FI in summer milk fat (13.4 g/100 g; $n=13$) produced in Switzerland at an altitude of 600–650 m and much lower values (12.0–12.5 g/100 g; $n=36$) at higher altitudes (900–2120 m). However, the concentration of this compound which ranged normally from 11.39 to 13.95 g/100 g ($n=592$) in milk fat (22), is very dependent on the fodder.

The concentrations of the six minor unsaturated fatty acids were the highest in BR and those of the fatty acids C18:1 -t12 and -c12 the highest in BR and FI. These results agreed better with the values generally measured in Switzerland for summer than for winter milk fat (1) and could be due to the influence on milk fat of the silage fed to the cows in FI and BR.

The concentrations of one triglyceride (C48), one free fatty acid (linolenic acid), fourteen fatty acids and four groups of fatty acids (C18:2, polyunsaturated, conjugated linoleic acid and ω-3) were significantly lower in cheese from BR and FI than in samples from the four other regions of higher altitude (table 3). The concentration of triglyceride C48 was the lowest in cheese from FI, and that from BR overlapped with that found in VO, Savoie (SA) and Allgäu (AL). *Precht and Heine* (22) found a mean value for this compound generally higher in winter fat (9.13 g/100 g; $s_x=0.38$; $n=283$) than in summer fat (9.07; $s_x=0.39$; $n=309$). *Collomb et al.* (6) found a trend towards higher values (8.15 to 8.60 g/100 g, $n=49$) as a function of the altitude but the results were not significantly different. In this study, the lower concentration found in BR (8.96 g/100 g) and FI (8.53 g/100 g) compared to that found in other countries (9.17–9.38 g/100 g) could be attributed to the influence of silage and/or to the altitude (lower diversity of plant species in hay (23)). Here again, the concentration of the triglyceride C48 in milk fat normally lies in a much broader range (7.91 to 10.65 g/100 g; $n=592$) (22) than that found in this study.

Except for the C18:2 c9t13+(t8c12), the fatty acids present in a lower concentration in cheeses from BR and FI than in those from other countries were essentially iso, anteiso and unsaturated long chain fatty acids. The concentration of myristic acid (C14) is generally higher in winter fat than in summer fat. That of the iso and anteiso compounds gives generally similar values in the two seasons. That of the unsaturated long chain fatty acids is generally higher in summer milk fat than in winter milk fat (1) and increased with the altitude (6, 7, 23). However, the concentration of fatty acids normally varies within a broader range than that found in this study. For example, *Precht and Molkentin* (24) found in 100 milk fats values ranging from 8.90 to 13.57 g/100 g for the myristic acid and from 0.34 to 1.06 for linolenic acid (C18:3 c9c12c15). For these two compounds, the values found within the six regions considered ranged from 9.56 to 10.55 g/100 g fat for the myristic acid and from 0.28 to 0.88 g/100 g fat for the second compound. The low concentration of esterified as well as free linolenic acid in cheeses from BR and FI can be attributed to a low concentration of this compound in silage fat. The lowest concentration of free linolenic acid in BR (17.1 mg/kg) and FI (13.4 mg/kg) compared to that found in other countries (34.8–51.3 mg/kg) may have the same cause. Another explanation

may be the different lipolytic activity of the propionic acid bacteria used for cheese making. In a study carried out at the FAM, the concentrations of free linolenic acid ranged from 23 to 57 mg/kg Emmental cheese depending on the bacteria used (FAM, unpublished results). However in the current study, the sum of the thirteen free fatty acids analysed (without butyric acid) did not show any significant differences.

The concentrations of groups of fatty acids (∑ C18:2, ∑ polyunsaturated, ∑ CLA and ∑ ω-3) were the highest in AL and VO and the lowest in BR and FI. Table 4 presents the sum of the CLA and the content of the fatty acids C18:1 t10+t11 as a function of the altitude.

Table 4
Influence of altitude on the concentration of CLA and C18:1 t10-11 in Emmental cheese

Forage type	*Region (altitude)*	*CLA (g/100 g fat)*	*C18:1 t10-11 (g/100 g fat)*
Maize and grass silage, hay	Bretagne (100 m)	0.44	1.17
Grass silage, hay	Finland (100–150 m)	0.45	1.20
Hay	Savoie (470 m)	0.67	1.44
Hay	Switzerland (570 m)	0.72	1.56
Hay	Vorarlberg (780 m)	0.85	1.76
Hay	Allgäu (800 m)	1.01	2.05

The contents of both sums of fatty acids increased as a function of the altitude. The elevated concentration of the sum of the t10 and t11 fatty acids is likely to be mainly due to that of the trans vaccenic acid (C18:1 t11) from which the CLA is endogenously synthesised (25). According to *Precht and Molkentin* (26), trans vaccenic acid normally represents approximately 90% of the total of the two acids t10 and t11, which cannot be separated using our chromatographic conditions. These authors showed in feeding tests – where cows stayed in the barn or on pasture or were fed with rape oil, rapeseed wholemeal or pellets – that the increase in the CLA concentration in milk fat usually correlates with the concentration of trans fatty acids. *Collomb et al.* (23, 27) found significantly different values for the sum of CLA and C18:1 t10+t11 fatty acids in milk fat produced from cows grazing in lowlands (600–650 m), mountains (900–1210 m) and highlands (1275–2120 m). The concentrations of CLA, compounds well-known for their positive effects on health (28), increased from 0.87 to 1.61 and to 2.36 g/100 g fat (maximum value: 2.87 g/100 g fat) as a function of the altitude. The concentration of the fatty acids C18:1 t10+t11 increased similarly from 2.11 to 3.66 and to 5.10 g/100 g fat (maximum value: 5.67 g/100 g fat). In the current study, the values obtained were relatively low

compared to those of summer milk fats in high altitude but corresponded to values for summer milk fats from AL and VO.

The lower concentration of the long chain polyunsaturated fatty acids found in BR and FI can be correlated with a silage fodder fat poor in polyunsaturated fatty acids (23). The concentration of the ω-3 compounds ranged from 0.61 to 1.42 g/100 g cheese fat. *Collomb et al.* (23) found increasing values as a function of the altitude (1.39 to 2.09 g/100 g fat). The low value found in BR (0.61 g/100 g fat) and FI (0.66 g/100 g) is due to the low level of linolenic acid (C18:3 c9c12c15), the main ω-3 compound of milk fat.

Significant differences between Finland and other countries

Table 5 presents the concentrations of compounds which were the highest or the lowest in FI cheeses compared to those produced in other countries.

The concentrations of the triglycerides C40 and C54 and those of the fatty acids C18, C18:1 t9, C20 and C20:1 c11 were the highest in FI cheeses. *Precht and Heine* (22) found lower mean values for the triglycerides C40 and C54 in winter fat (C40:9.78 g/100 g; s_x=0.31; n=283; C54:3.99 g/100 g; s_x=0.87; n=283) than in summer fat (C40:10.01; s_x=0.28; n=309; C54:6.58; s_x=0.82; n=309). For the triglyceride C40, *Collomb et al.* (6) found significantly higher values in lowlands (10.4 g/100 g; n=13) compared to the highlands 9.84–10.0 g/100 g; n=24) and much lower values for the triglyceride C54 in the lowlands (4.83 g/100 g) compared to the highlands (7.48–7.78 g/100 g). In this study, the high concentrations of the triglycerides C40 (10.34 g/100 g) and C54 (5.01 g/100 g) in FI cheeses were rather in the range of the values generally obtained for summer fats. These two triglycerides probably consist on a high extent of oleic acid (C18:1 c9) as confirmed by the highest level of this acid (results not tabulated) found in cheese fat from FI (16.72 g/100 g fat) compared to that found in cheese from the other regions (14.22–15.44 g/100 g fat). The concentration of stearic acid (C18) was the highest in cheese from FI (10.20 g/100 g fat) compared to that from the other regions (7.31–8.42 g/100 g fat). This result could be attributed to the high level of oleic acid in grass silage combined with a high biohydrogenation activity in the rumen of the cow (23). The normal range in milk fat lies between 6.12 and 12.50 g/100 g (24).

The concentration of the triglyceride C46 and that of palmitic acid (C16) as well as of other minor fatty acids difficult to interpret were the lowest in FI cheeses. *Precht and Heine* (22) found higher mean values for the triglyceride C46 in winter fat (7.55 g/100 g; s_x=0.51; n=283) than in summer fat (6.82 g/100 g; s_x=0.47; n=309). For this triglyceride *Collomb et al.* (6) found significantly higher values in lowlands (6.67 g/100 g) than in the highlands (6.08–6.31 g/100 g). In this study, the low concentration of the C46 triglyceride (7.07 g/100 g) in FI cheese were once again rather in the range of summer fat and could be due to the silage fodder based on grass. The lower content of the fatty acid C16 found in FI cheese (24.66 g/100 g) supports this

Table 5
Compounds where only FI was significantly different (P≤0.001) from all other regions

Region (n=)	*AL (3)*		*BR (3)*		*CH (6)*		*FI (2)*		*SA (3)*		*VO (3)*	
	x	s_x	*x*	s_x	*x*	s_x	*x*	s_x	*x*	s_x	*x*	s_x
Higher value												
Triglyceride 40 (g/100 g triglyceride)	9.68^{B}	0.12	9.90^{B}	0.10	9.77^{B}	0.24	10.34^{A}	0.04	9.56^{B}	0.05	9.62^{B}	0.06
Triglyceride 54 (g/100 g triglyceride)	3.92^{B}	0.14	3.70^{B}	0.20	3.73^{B}	0.37	5.01^{A}	0.13	3.64^{B}	0.12	3.49^{B}	0.15
C18 (g/100 g fat)	7.81^{BC}	0.16	8.42^{B}	0.25	7.55^{BC}	0.62	10.20^{A}	0.55	7.31^{C}	0.16	7.33^{BC}	0.28
C18:1 t9 (g/100 g fat)	0.213^{B}	0.016	0.231^{B}	0.024	0.199^{B}	0.025	0.292^{A}	0.013	0.209^{B}	0.017	0.192^{B}	0.008
C20 (g/100 g fat)	0.141^{B}	0.008	0.122^{C}	0.006	0.141^{B}	0.009	0.171^{A}	0.009	0.145^{B}	0.008	0.134^{B}	0.002
C20:1 c11[1]	0.052^{AB}	0.003	0.038^{C}	0.002	0.047^{B}	0.003	0.057^{A}	0.002	0.044^{BC}	0.002	0.039^{C}	0.007
Lower value												
Triglyceride 46 (g/100 g triglyceride)	7.74^{AB}	0.18	7.46^{B}	0.06	7.72^{AB}	0.18	7.07^{C}	0.13	7.81^{A}	0.12	7.68^{AB}	0.01
C16 (g/100 g fat)	27.17^{A}	0.49	27.91^{A}	0.78	27.78^{A}	0.75	24.66^{B}	0.26	27.05^{A}	0.27	27.73^{A}	0.92
C17 (g/100 g fat)	0.540^{A}	0.014	0.519^{A}	0.070	0.581^{A}	0.072	0.265^{B}	0.163	0.600^{A}	0.073	0.667^{A}	0.063
C17 iso (g/100 g fat)	0.062^{A}	0.007	0.068^{A}	0.009	0.068^{A}	0.007	0.036^{B}	0.003	0.079^{A}	0.001	0.074^{A}	0.014
C17 aiso (g/100 g fat)	0.212^{A}	0.015	0.207^{A}	0.003	0.221^{A}	0.010	0.16^{B}	0.006	0.222^{A}	0.006	0.217^{A}	0.007
Sum medium chain FA[2]	46.00^{A}	0.79	45.59^{A}	0.87	46.29^{A}	1.11	41.66^{B}	0.22	46.27^{A}	0.54	46.27^{A}	1.23

Caption: see table 2

[1] FI not significantly different from AL

[2] C12 to C16:1 c according to reference (1)

interpretation and corresponds to a concentration found for summer milk fat in lowlands regions (23).

Significant differences between Bretagne and other countries

Table 6 presents the concentrations of compounds which were the highest or the lowest in BR cheeses compared to those produced in other countries.

The concentration of free butyric acid, and minor fatty acids difficult to interpret, were respectively the highest or the lowest in BR. Butyric acid is produced by butyric acid bacteria which are commonly found in silage.

Conclusion

Free fatty acid, triglyceride and fatty acid (FA) composition of Emmental cheese fat were investigated to find potential markers of geographic origin. The fat composition of milk and milk products is strongly correlated with the forage fed to the herd. The latter depends on the season, on regional parameters such as altitude, geology, type of agriculture and on further non-regional parameters such as type of concentrates used and addition of vegetable oils. The cheeses investigated in this study all originated from winter productions. In "Bretagne" and "Finland", feeding with silage is permitted, whereas it is prohibited in the other regions. The differences in altitude and silage feeding are the most likely explanation for the highly significant differences ($P \leq 0.001$) found between these two regions and the others. For groups of fatty acids such as the C18:2, polyunsaturated FA, conjugated linoleic acid and ω-3 FA, "Bretagne" and "Finland" showed the lowest values. The butyric acid content was the highest in those regions. It is thereafter only possible to discriminate between the three groups FI, BR and the remaining regions.

Moreover, these differences may partly disappear during summer production while fodder is based mainly on fresh grass. Consequently, it will not be possible to interpret any fatty acids composition during the transition phases between summer and winter production, which represents almost half a year's production. Finally, the composition of the forage changes during a season. Any addition of a new type of cereal or vegetable fat will modify the fatty acid profile of the milk. Thereafter, apart for the higher content of trans vaccenic acid and conjugated linoleic acids in summer Emmental cheese produced at higher altitude, the fat composition is not adequate for discriminating between the various geographic origins of Emmental cheese.

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Table 6
Compounds where BR was significantly different (P≤0.001) from all other regions

Region (n=)	*AL (3)*		*BR (3)*		*CH (6)*		*FI (2)*		*SA (3)*		*VO (3)*	
	x	s_x	*x*	s_x	*x*	s_x	*x*	s_x	*x*	s_x	*x*	s_x
Higher value												
Free butyric acid (mg/kg)	*87.2*^{B}	16.3	*425.3*^{A}	41.4	*85.9*^{B}	17.9	*169.5*^{B}	175.0	*83.3*^{B}	5.1	*88.5*^{B}	3.0
C16:1 c (g/100 g fat)	*1.21*^{B}	0.03	*1.37*^{A}	0.04	*1.24*^{B}	0.06	*1.07*^{C}	0.01	*1.19*^{BC}	0.06	*1.16*^{BC}	0.05
Lower value												
C20:1 c9 (g/100 g fat)	*0.119*^{A}	0.007	*0.091*^{B}	0.003	*0.119*^{A}	0.007	*0.125*^{A}	0.006	*0.118*^{A}	0.008	*0.112*^{A}	0.008
C20 (g/100 g fat)	*0.141*^{B}	0.008	*0.122*^{C}	0.006	*0.141*^{B}	0.009	*0.171*^{A}	0.009	*0.145*^{B}	0.008	*0.134*^{B}	0.002

Caption: see table 2

Summary

Free fatty acids, triglycerides and fatty acid composition in cheese fat were investigated on 20 Emmental cheese samples from six regions in Europe to find the best parameters for discriminating between their different geographic origins. These samples originated from a winter production. "Bretagne" and "Finland", where silage feeding is permitted and which are located close to sea level, were easily differentiated from the other regions. The concentration of some groups of polyunsaturated fatty acids such as C18:2 , conjugated linoleic acids and ω-3 was significantly lower. However, the ranges of values obtained in this study were always smaller than those found in broader studies. The butyric acid content was higher in the two regions with silage feeding. These differences may however partially disappear during summer production. The other regions showed almost no significant differences. The high variability of the fatty acid composition due to both seasonal and feeding effects leads to the conclusion that theses methods are not adaquate for discriminating between the geographic origins of Emmental cheeses.

Zusammenfassung

In 20 Emmentalerproben (Winterproduktion) von sechs europäischen Regionen wurden die freien Fettsäuren, die Triglyzeride und die Zusammensetzung der Fettsäuren des Käsefettes studiert. Ziel dieser Untersuchung war, aussagekräftige Parameter zu finden, um diese Käse nach ihrer geographischen Herkunft zu unterscheiden. Die Käse der Regionen «Bretagne» und «Finnland», wo die Verfütterung von Silage erlaubt ist und die in der Nähe des Meeres liegen, sind von den anderen leicht zu unterscheiden. Dabei waren einige mehrfach ungesättigte Fettsäuren wie C18:2, konjugierte Linolsäuren und ω-3 in signifikant geringerer Konzentration vorhanden. Doch ist die Spannbreite der Werte in dieser Studie immer schmaler als in umfangreicheren Studien. Der Buttersäuregehalt war dort höher als in den Käsen der Regionen ohne Silageverfütterung. Diese Unterschiede können während der Sommerproduktion teilweise verschwinden. In den Käsen der anderen Regionen konnten nur geringe Unterschiede festgestellt werden. Die geographische Herkunft der Emmentaler Käse kann mit diesen Methoden aufgrund der grossen saisonal und fütterungsbedingten Variabilität der Fettsäurezusammensetzung nicht unterschieden werden.

Résumé

Les acides gras libres, les triglycérides et la composition en acides gras de la matière grasse du fromage ont été étudiés dans 20 échantillons d'emmental de productions hivernales provenant de six régions d'Europe pour trouver les meilleurs régresseurs susceptibles de discriminer leur origine géographique. Les régions de «Bretagne» et «Finlande», où l'affouragement au silo est autorisé et qui sont proches du niveau de la mer, sont facilement différenciées des autres. La concentration de quelques groupes d'acides gras polyinsaturés tels que C18:2, linoléiques conjugués

et ω-3 était significativement plus faible que dans les autres régions. Cependant les plages de valeurs trouvées étaient toujours moins étendues que celles publiées lors d'études plus larges. La teneur en acide butyrique y était plus haute que dans les régions sans ensilage. De telles différences peuvent pourtant s'atténuer durant les productions estivales. Les autres régions n'ont montré que peu de différences. La forte variabilité de la composition de ces acides, liée tant à la saison qu'à l'affourage-ment, rend ces méthodes inadéquates pour discriminer l'origine géographique de ces fromages.

Key words

Emmental, Fatty acid, Triglyceride, Authenticity, Traceability

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Honey Authenticity

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Introduction

Honey is one of the very few natural foods which is offered today. The composition and the main quality criteria for honey are summarised in the Swiss Food Manual (1) and also in different monographs (2, 3).

The Codex Alimentarius Standard and the EU Council Directive

The honey standards of the Codex Alimentarius (CA) (4) and of the European Community (EU) (5) have been revised recently. The changes in the standards and the analytical methods used for their determination, following the advice of the International Honey Commission, were recently reviewed (6).

The standards of the Codex Alimentarius and the EU are very similar. The Codex Alimentarius honey standard is more detailed, containing references to quality factors such as heavy metals, pesticides and adulteration.

The definition of honey in both standards is the same:

"Honey is the natural sweet substance, produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature."

According to the CA standard the essential composition and quality factors are:

"3.1 Honey sold as such shall not have added to it any food ingredient, including food additives, nor shall any other additions be made other than honey. Honey shall not have any objectionable matter, flavour, aroma, or taint absorbed from foreign matter during its processing and storage. The honey shall not have begun to ferment or effervesce. No pollen or constituent particular to honey may be removed except where this is unavoidable in the removal of foreign inorganic or organic matter.

Table 1
Honey standard of the Codex Alimentarius Standard and EU Honey Directive: important new quality criteria

Composition criteria	*Value*
Sugar content	
Fructose and glucose content (sum of both)	
– blossom honey	not less than 60 g/100 g
– honeydew honey, blends of honeydew and blossom honey	not less than 45 g/100 g
Sucrose	
– in general	not more than 5 g/100 g
– false acacia (*Robinia pseudoacacia*), alfaalfa (*Medicago sativa*), Banksia (*Banksia menziesi*), French honeysuckle (*Hedysarum*), red gum (*Eucalyptus camadulensis*), leatherwood (*Eucryphis lucida, Eucryphia milliganii*), Citrus spp.	not more than 10 g/100 g
– Lavender (*Lavandula spp.*), borage (*Borago officinalis*)	not more than 15 g/100 g
Moisture content	
– in general	not more than 20 %
– heather (Calluna), EU, CA; baker's, EU	not more than 22 %
– baker's honey from heather (Calluna), EU	not more than 25 %
Electrical conductivity	
– honey not listed below, and blends of these honeys	not more than 0.8 mS/cm
– honeydew honey and chestnut honey and blends of these except of those listed below	not less than 0.8 mS/cm
– exceptions: strawberry tree (*Arbutus unedo*), bell heather (*Erica*), eucalyptus, lime (*Tilia spp.*), heather (*Calluna*), manuka or jelly bush (*Leptospermum*), tea tree (*Melaleuca spp.*)	
Free acid	
– in general	not more than 50 meq/kg
– baker's honey (only EU Directive)	not more than 80 meq/kg
*Diastase activity** (Schade units)	
In general; except baker's honey (EU)	not less than 8
Honey with low natural enzyme content (e.g. citrus honey) and a HMF content of not more than 15 mg/kg	not less than 3
*HMF** (mg/kg)	
In general; except baker's honey (EU Directive)	40
Honey of declared origin from regions with tropical regions with tropical climates and blends of these honeys	80

* Determined after processing and blending

3.2. Honey shall not be heated or processed to such an extent that its essential composition is changed and/or its quality impaired."

In the EU council directive, Annex II the same essential composition and quality factors are mentioned, but the text is formulated differently. Thus according to both standards honey should be authentic.

The new honey compositional criteria of both standards are summarised in table 1. The meaning of these standards has been discussed recently (6). Compared to the previous standards there are some changes:

- The new maximum limit for the water content is fixed at 20 %, while the old standards (and also that of the Swiss Food Directive) were at 21 %.
- There is a change in the HMF and diastase limits: the limits of maximum 40 mg/kg HMF and minimum eight diastase units are valid after processing and blending, while the old CA standards had limits of 80 mg/kg and three diastase units.
- Under some circumstances both standards will allow the fine filtration of honey, removing all the pollen, as long as the honey is described as "filtered".

In the present standards the humidity limit is lowered to 20 %, which is the same as in the previous Swiss Food Directive. Indeed in the present directive it had to be harmonised with the old European limit of 21 %.

In both standards it is remarked that the HMF and the diastase limits are valid after processing and blending of honey. This can be interpreted to mean that these two quality factors are not valid during the whole shelf life of honey. Indeed, it has been a concern of the honey trade for a long time, mostly in non European countries that these two quality factors are often not fulfilled in retail honey during the shelf life of the honey, especially during storage at higher temperatures.

The removal of pollen will make the determination of botanical and geographical origin of honey much more difficult, if not impossible unless the law in each member state permits inspectors to enter packing plants to take raw material samples and insists that packers keep full records proving traceability.

The compositional criteria for honey of both standards are very similar. However, there are also some differences:

- The Codex Alimentarius standard refers only to retail honey and there is no special mention of baker's and industrial honey. This is planned to be added in a future addendum to the standard.
- In the EU directive the quality criteria hydroxymethylfurfural (HMF) content, diastase activity and honey acidity are intended for application by commercial partners and governments, while according to the CA standard they are only for voluntary application between trade partners.
- According to the EU directive honey is referred to as a product of *Apis mellifera*, the European honey bee, while the Codex Alimentarius honey is defined as a product of all honey bees.

The honey definition of the Codex Alimentarius is more correct, as different honeys are offered on the world market, with a predominance of course of *Apis mellifera* honey. Indeed, a major part of Asian honey is produced by the Asian bee, *Apis cerana*. The honey, produced by that bee has a composition similar to that of the *Apis mellifera* honey, but has a higher water content and no special compositional criteria have been established.

The methods used for the determination of the quality factors are described only in CA standards, while the EU directive refers to the Codex methods without naming them explicitly, as is to be expected under the principle of subsidiarity.

The Swiss Food Legislation will have to adapt to the new honey Codex Alimentarius standard and the EU honey directive. From a methodological point of view no adaptations are necessary for the Swiss Food Manual, as the same methods, which are used for the new quality factors according to the Codex Alimentarius, are already part of the present Swiss Food Manual. On the other hand, the Swiss Food directive has to be harmonised with the new CA standard and the EU directive.

Authenticity issues

A major concern of food control is to ensure that honey is authentic in respect of the legislative requirements.

The authenticity of honey has two different aspects:

– Authenticity in respect of honey production
– Authenticity in respect of descriptions: geographical and botanical origin, "natural", "organic", "raw" and "unheated" honey

In determining honey authenticity both aspects should be considered.

There are several reviews on different honey authenticity issues (7–10). The objective of this review is to examine all authenticity issues and the methods used to prove authenticity. In order to make the review more readable and compact, an attempt is made to concentrate on the most important issues, without giving too much detail. Researchers looking for greater details on methods and issues are advised to consult the cited references.

Authenticity of production

Processing by the beekeepers and the industry

Retail honey has always been subjected to some form of processing during honey production. Today, most if not all commercial honey is produced by centrifugation. An appellation such as "harvested in the cold" is a mislabelling, since honey is harvested naturally at temperatures between 25–32°C, which is similar to the temperature in the beehive (35°C). Filtering of honey is an important issue. In the regulations of many European beekeeping associations, and also of the Swiss ones, the use of honey filters is prescribed. Beekeepers should harvest honey by using filters with a mesh size not smaller than 0.2 mm in order to prevent pollen

removal. On the other hand, some packers, mostly in North America, will use smaller filters in order to filter out undesirable contaminants. According to the international honey legislation, such honey should be labelled as "filtered" (see Introduction).

However, the use of excessive heat for pasteurisation and liquefaction might have adverse effects on honey quality, e.g. loss of volatile compounds and reduction of enzyme activity. Pasteurisation to kill osmophilic yeast is carried out for 7.5 minutes at 63° or for 1 minute at 69°C, with rapid heating and cooling involved (11). Pasteurised honey should be labelled according to the Swiss food legislation, but appellations "pasteurised honey" are seldom seen, if ever, on retail honey pots.

The great majority of Swiss honey is harvested and marketed by the beekeepers. This honey is fresher and has taken less heat load than the non Swiss honey, which has undergone subsequent heat treatments for liquefaction and filling purposes. There is a significant difference between the HMF and the invertase and diastase activities of these two honey classes (12).

Honey crystallisation can be influenced on an industrial scale by the Dyce procedure, to produce the so called creamed honey (13). A certain amount of a fine crystallised honey is mixed to liquid honey and the crystals are allowed to grow at 14°C. This procedure stabilises the honey consistency. This procedure will not change honey authenticity, as no foreign matter has been added or taken away from honey.

Addition of sweeteners

As a natural product with a relatively high price, honey has been for a long time a target for adulteration. Correct beekeeping practice ensures that sweeteners used to feed bees should not adulterate honey. This implies improper feeding of sugar during the honey flow or addition of sugars to honey. The following sweeteners have been used: acid inverted sugar syrups, corn syrups, syrups of natural origin such as maple, cane sugar, beet sugar, molasses, etc. In recent years, there has been a major adulteration problem in the world, concerning mainly Chinese honey (14, 15). Presently these sweeteners are mainly bee feeding syrups, produced by the hydrolysis of maize, cane and beet sugar.

Harvesting of non-ripe honey, addition and removal of water

Normally, the water content of honey harvested in countries with a moderate climate is below 18 %. However, in some countries the harvested honey contains more than 20 % water, due to climatic or harvesting conditions. Only honeys having less than 17.1 % water are regarded as safe (16). The Codex definition prescribes that the bees dehydrate and store the honey and leave it in the honeycomb to ripen and mature. This implies that the combs will be capped. In normal beekeeping practice there may be a few cells around the edge of a comb that remain uncapped but essentially the maturing process must have been allowed to occur. There are two

instances which give rise to a water content high enough to require remedial action. In the United States some honeys may contain more than the 18.6 % water limit prescribed by United States Department of Agriculture standards, even though they are derived from capped combs. The small excess of water may be removed by centrifuge or vacuum evaporator. In China and probably some other countries a practice occurs which results in a product which is difficult to equate with the Codex definition of honey. The nectar is harvested before the bees have had time to "deposit, dehydrate, store and leave in the honey comb to ripen and mature". The water content may easily be over 25 %. This "green" honey will easily ferment, often before it has had time to reach the factory for vacuum evaporation. It therefore ferments, resulting in a product with an off-taste, high levels of dead yeast, glycerol and butanediol (17) and also ethanol (18). There is a loss of aroma compounds in the drying process. Indeed, there are modern drying technologies that would add back to honey the aroma compounds, lost after the drying procedure.

Addition of water is probably not a realistic adulteration practice because of the fermentation risk, unless the water content was very low and was adjusted back to some agreed level such as 18.6 % in the United States. Some packers would argue that it is legitimate to replace the small amounts of water lost during processing. The water content of honey can naturally be as low as 13.6 % and as high as 23 % depending on the source of the honey, climatic conditions and other factors. Fermentation does not usually become a problem in honeys with a water content less than 18 %. The crucial criterion is that the honey be produced in a way compliant with the Codex definition.

Authenticity of origin and misdescriptions

Misdescription of botanical source

Bees forage different plants, so that honey is always a mixture of different sources. When higher prices are paid for certain types of honey, beekeepers and honey packers will often designate the botanical source of the honey. The International Honey Commission is presently working on the establishment of quality criteria for the most important European unifloral honeys.

World-wide light honeys like orange blossom or acacia honey achieve higher prices than honey blends or other unifloral honeys. On the other hand, in different countries, other unifloral honeys will achieve higher prices, depending on customer preference. Blossom honeys (with some exceptions) should have an electrical conductivity of less than 0.8 mS/cm (table 1). Currently, the honey floral type is judged on bases such as sensory analysis, pollen and chemical analysis. As the pollen content is subject to considerable variation, judgement of the honey is based on a combination of several quality criteria.

In some central European countries like Germany, Switzerland and Austria honeydew honeys achieve generally higher prices than blossom honeys. Although

honeydew honeys contain microscopically visible "honeydew elements" like algae and fungi, there are no quantitative microscopic quality criteria. Honey is labelled as "forest" or "honeydew" or "fir" on the basis of sensory judgement and electrical conductivity measurements. For honeydew honeys the norm of a minimum of 0.8 milli Siemens/cm (mS/cm)has been adopted in the world standards (table 1). Fir honeys have generally conductivity values greater than 1 mS/cm (19,20). However, there are no internationally accepted quality criteria for the different types of honeydew honeys and such are fixed in individual countries.

Misdescription of the geographical and the topological source

Generally, in Western Europe honey imported from China or Latin America has a lower price than the locally produced honey. Differences persist also between countries in Europe and also between geographical regions. Thus there is a financial interest in mislabelling honeys. Pollen analysis and a number of chemical methods have been used for the characterisation of the geographical origin of honey.

In the routine control of honey the geographical origin is often checked by pollen analysis as it requires only inexpensive instrumentation. In many countries pollen analysis of the locally produced honeys is regularly carried out and the pollen specialists there have a precise knowledge of the pollen spectrum of the honeys of their country. In food control pollen analysis is very efficient for the differentiation of honeys produced in distinctly different geographical and climatic areas. If the geographical differences are less pronounced, the determination of the pollen spectrum will generally not yield a confident authenticity proof. Also it has to be borne in mind that melissopalynological methods will not meet the quality standards for a modern validated and quality assured analytical method. The methods are based on experience and are thus subjective and have not been tested by modern proficiency test trials. The use of computerised methodology for pollen analysis is very promising.

It may be possible to characterise honeys from different topographical regions within definite, relatively small geographical areas by the measurement of common chemical parameters and subsequent evaluation with modern statistical methods (see method section).

Misdescription of the entomological source.

The present EU Directive definition defines honey as derived from *Apis mellifera*, while according to the Codex standard honey is the product of all honeybees. *Apis mellifera*, originally indigenous to Africa and Europe, has been introduced into major exporting countries such as China, where honey is also produced from *Apis cerana*. This has created two problems which need resolution. It is necessary to characterise the honey from species other than *Apis mellifera* so that the honey from these species can be accepted in international trade either as indistinguishable from *Apis mellifera* honey (*Apis cerana*, the Asian bee) or with compositional limits of its

own (*Apis dorsata*, stingless bees). Indeed, it is planned that the honeys of bees others than *Apis mellifera* also be characterised and a Codex standard for these honey types be established.

Organic, raw or unheated honey

Recently a European (21) and a Swiss (22) regulation for the production of organic honey have been established. As with all organically produced food, the control of organic honey implies only the beekeeping procedures and not the honey quality. As a consequence, no testing regime can decide if a honey is organic or not. However, the presence of veterinary drug residues will definitely demonstrate that organic production methods have not been used and will thus expose the mislabelling of a sample as organic. An appellation of "natural" honey is a mislabelling, since honey is natural by definition.

Fresh honey has a very low hydroxymethylfurfural (HMF) level and will still contain its natural level of enzymes. Appellations "fresh" "raw" or virgin honey indicate that honey is fresh and unheated. In the EU an appellation "virgin honey" has been proposed (23). Such honeys should have a maximum HMF content of 25 mg/kg. There are also suggestions that the HMF level of raw, unheated honeys should be below 15 mg/kg, while the invertase activity should be higher than 10 Hadorn units (1, 24). Different European beekeeping associations have accepted the latter quality criteria for their specially labelled honeys.

Methods for testing the authenticity of honey due to effects during honey production

The methods for testing the different authenticity issues are summarised in table 2.

Addition of sugars

Depending on their origin, sugars added are divided into two types: C3 and C4. Sucrose and the natural sugars of honey belong to the C3 type while cane sugar and sugars produced from the hydrolysis of maize starch are of the C4 type. Many different methods have been proposed for detecting honey adulteration with sugar. However, most of these methods have not been found useful in practice (9). Here only the methods with proven or promising efficacy will be discussed.

Adulteration by cane and maize syrups

Isotope tests

Maize and sugarcane metabolise by the Hatch-Slack or C4 metabolic pathway. As a result, sugar syrups derived from them exhibit a $^{13}C/^{12}C$ ratio, expressed as a δ value, different from that of honey the sugar of which is derived via a C3 pathway. The δ value of C4 syrups is close to –10‰ while the average value for honey is –25.4‰. The original method for measuring the $^{13}C/^{12}C$ ratio (25, 26) has been

Table 2
Methods for testing of authenticity issues due to improper production, processing and storage

Method, principle	*Status, remarks*
Detection of sugar cane adulteration	
Detection of specific microscopically visible constituents of cane sugar	screening
determination of $^{13}C/^{12}C$ ratio	definite procedure
Detection of adulteration by maize syrups	
determination of $^{13}C/^{12}C$ ratio	definite procedure
determination of specific sugars	methods not conclusive
Detection of sugar adulteration by feeding sucrose	
measurement of sucrose, erlose content	screening
measurement of proline content	screening
determination of bisulfite sugar derivatives	used only in Russia, to be tested
Detection of beet sugar adulteration	
FTIR spectroscopy	research results, to be tested for routine use
Detection of improper water removal and of fermentation	
off taste, yeast count, glycerol and ethanol	limits are not established internationally
Detection of overheating and storage defects	
measurement of HMF, diastase and invertase activity	limits according to CA, EU-Directive or Swiss Food Manual

For references and discussion of methods see text.

improved with the introduction of a protein internal standard (27–29). The method currently used, with or without an internal standard, allows the detection of 7–10 % adulteration with cane sugar or maize syrups. Besides the measurement of the $^{13}C/^{12}C$ ratio, deuterium NMR (30, 31) can be determined to yield a greater certainty in the interpretation of the $^{13}C/^{12}C$ measurement. There are a number of other papers reporting the isotope ratios of honeys of different botanical and geographical origin (30–35).

Determination of sugars

This area has been recently extensively reviewed (36). Maize syrups like high fructose corn syrup (HFCS) often contain trace amounts of higher oligosaccharides, which are naturally not present in honey. The measurement of the maltose/isomaltose ratio (37, 38) proved some, if not complete efficacy for the determination of adulteration with HFCS syrups. However, this method has not been validated for

reliable determination of sugar syrup adulteration. The reason is that the composition of these syrups is constantly changing, thus making the detection of adulteration difficult, independent of the type of syrup. HFCS sugars have been determined by isolation of the specific oligosacharides by preparative HPLC, followed by quantitation by analytical HPLC (39). This method is very labour intensive and was not used for practical honey control. Separation of sugars by ion chromatography (40), GC (41, 42) or micellar electro-kinetic chromatography (43) have been used for the detection of HFCS sugars. Carbohydrate adulteration testing has also been carried out with ^{13}C-NMR spectroscopy (44). The determination of sugars has to be updated to be able to detect the actual oligosaccharide composition of the HFCS syrups.

Routine microscope and physico-chemical methods

The determination of routine parameters allows a cheap and quick screening of doubtful honeys, which then can be tested with more sophisticated and expensive techniques.

The detection of specific microscopically visible constituents of cane sugar can be used as a cheap screening method for the detection of this type of sugar adulteration (45). This test is cheap and simple and can be used as a screening test before carrying out the more expensive isotope test.

In sugar adulterated honeys some chemical parameters such as enzyme activities, HMF content, ash content, electrical conductivity and proline content are lowered. These changes might indicate possible adulteration, if the normal variation of these parameters in different honeys is taken into account when interpreting the test for adulteration. Indeed, proline was suggested as a quality criterion for honey with respect to sugar adulteration. It was proposed that natural honeys should have a proline content of more than 180 mg/kg (46). A lower proline content could mean that the honey has been adulterated with sugar. However, this value can be higher for certain honeys, as the proline content depends on the honey type. Also, it should be borne in mind that some of these parameters like HMF and enzyme activity will change on heating and storage. Routine analysis of the sugar spectrum by HPLC or GC can also give some information on possible adulteration. Honeys adulterated by feeding of sucrose to the bees have an increased concentration of sucrose and erlose (47). However, it should be borne in mind that sucrose decreases rapidly with time, and also the erlose concentration will change slowly upon storage (47). On the other hand, certain honeys such as citrus, acacia, rhododendron, honeydew and others have a higher natural content of this sugar (48).

Other methods

A number of other methods have been described for the proof of adulteration of honeys by sugars. However, most of them have not proved useful (9). An interesting method for the detection of adulteration by beet sugar by means of testing for

sugar bisulfite derivatives was developed in Russia (49) but up to the present time there is no experience with this method in other countries. Recently FTIR spectroscopy was used for testing honey adulteration by inverted beet sugar (50). This method is based on the honey dilution effect of added sugars and might not be the universal method needed to prove adulteration of all different types of honeys. Also, it has been used for model studies and its utility remains to be tested with different honeys.

Harvesting of honey with high humidity, addition of water

Harvesting of honey with high humidity, or subsequent addition of water to honey can result in honey fermentation and spoilage (see authenticity issues). Honey spoilage can be tested by a microscopical yeast count (51), by measuring glycerol (52), butanediol or ethanol (53). The last two methods based on enzymatic determinations are quick and inexpensive. A limit of 300 mg/kg glycerol has been proposed for blossom honey (17). Honeydew honeys have higher glycerol content (17) and a limit for these honeys has not yet been proposed. A limit of 150 mg/kg ethanol has been suggested for Spanish (53) and also for Italian (54) blossom honey.

Methods for testing authenticity in respect to descriptions

The different methods are summarised in table 3.

Table 3
Methods for testing of authenticity of botanical origin

Method, principle	*Status, remarks*
Pollen analysis classical and statistical assessment of data	used for routine control, to be used together with chemical parameters
Sensory analysis	specialised personnel necessary
Determination of routine parameters e.g. electrical conductivity, fructose and glucose content (fructose/glucose ratio)	used for routine control together with pollen and sensory analysis
Chemometric evaluation of routine parameters	used predominantly in research
Determination of aroma compounds volatiles: dynamic head space volatiles: SPME flavonoids: HPLC	all methods used predominantly in research, routine testing not carried out
Determination of other minor components amino acids trace elements	methods used in research, routine testing not carried out

For references and discussion of methods see text.

Botanical origin

Pollen analysis

Pollen analysis (melissopalynology) with light microscopy was the first method used to determine the botanical origin of honey. It has the advantage that it needs only inexpensive instrumentation. The drawback is that it needs highly specialised personnel and cannot for the time being be aided by computers for more efficient assessment of data. The International Commission of Bee Botany set the methodology (55) which is in the process of revision in the work programme of the European Honey Commission. Pollen analysis can be qualitative and quantitative. Generally, for the determination of the botanical and geographical origin of honey qualitative pollen analysis is carried out. Here the percentage representation of the different pollen types is determined. Pollen analysis is based on the individual knowledge and appreciation of each pollen analyst and is thus subjective to a certain extent. Recently pollen analysis ring trials for the determination of the precision were carried out (56). The precision for the determination of certain pollen types was comparable to the precision determined for the measurement of chemical parameters. The criteria for the percentages of the dominant pollens of different unifloral honeys were set 30 years ago (55). However, the pollen content of nectar varies to a very large extent. For example, according to this standard citrus honey should have at least 10 % of citrus pollen, rape honey is expected to contain more than 45 % rape pollen, while a chestnut honey must contain more than 90 % of chestnut pollen. These figures can also vary depending on the relative content of the accompanying pollen. Indeed, practical experience has shown that the present melissopalynological quality criteria for unifloral honey figures are not valid for all honeys. Thus, presently, pollen analysis is used in combination with the sensory and chemical analysis of the unifloral honeys.

Determination of routine physico-chemical parameters

The determination of quality parameters by modern analytical methods (57) is now routinely used for the determination of the botanical origin of honey. Of all quality parameters measurements of electrical conductivity and of the fructose and glucose content, providing the fructose glucose ratio, are most useful. The determination of electrical conductivity is the fastest method for routine honey control. Values for blossom and honeydew honeys have been recently accepted in the Codex honey standard and in the EU Directive for honey (5, 6) as a criterion of the differentiation between blossom and honeydew honeys. Also, electrical conductivity values of the most important unifloral honeys of the world have been compiled recently (7). However, by using linear discriminant statistical analysis of different honey quality parameters (sugars, electrical conductivity, optical rotation, nitrogen content) a good separation between unifloral honeys (58–60) can be achieved. Also, honeydew and blossom honeys can be distinguished by the same approach (12, 61).

However, it should be noted than these methods have been used for research purposes and their utility for routine purposes is doubtful.

Determination of other parameters

Although chemometric analysis will discriminate between different unifloral honeys, it will not differentiate between polyfloral and unifloral honeys by discriminant analysis using routine quality parameters, because of the great variation of parameters in polyfloral honeys. For this purpose the use of specific unifloral markers is necessary.

Determination of carbohydrates

Determination of the whole honey spectrum, especially of a great number of minor oligosaccharides by capillary gas chromatography (62, 63) and HPLC (39, 64–66) has some discriminant capacity for the differentiation of unifloral honeys. With normal chromatographic methods, capable of determining the main oligosacharides, it is not possible to differentiate the unifloral honeys, although there are some differences between unifloral honeys. Even using very thorough separation of the sugars the oligosaccharide patterns of different unifloral honeys seem very similar (67). As the minor sugars are the product of honey enzymes of bee origin, minor oligosaccharides are most probably not specific markers of unifloral honeys. The differences encountered in the different unifloral honeys are probably due to different invertase activities, as this enzyme causes most sugar transformations, also the building of most honey oligosaccharides. Moreover, the activity of this enzyme is very heat and storage dependent; this will inevitably cause a great variation of the concentration of honey oligosaccharides.

Determination of aroma compounds and phenolics

Aroma compounds and flavonoids have been used as specific markers for unifloral honeys. Extraction of honey aroma by organic solvents for the quantitative analysis of honey and subsequent determination of the honey aroma spectrum have shown differences between unifloral honeys (8, 9). However, the extraction of volatiles is not suitable for routine testing of the botanical origin of honey because it is too time consuming. Dynamic head-space analysis of honey aroma can be useful for routine authenticity testing of the botanical origin of honey and it has been used with promising results (68–70). From these results it seems probable that less volatile components are more typical for the different botanical sources of honey. Another new technique is the use of SPME (Solid Phase Micro Extraction) for the analysis of aroma compounds. It has the advantage over dynamic head space that it has an increased sensitivity and that less volatile aroma compounds can be analysed. This technique has been successfully used for the determination of honey volatiles of unifloral honeys (71, 72).

Phenolics are another class of compounds which have been used for the proof of botanical origin. Recently, the use of these compounds for the determination of the botanical origin of honey has been extensively reviewed (73). It seems that there are typical markers for some unifloral honeys, e.g. methyl anthranilate and hesperitin for citrus honey, kaempferol for rosemary honey, which can be used successfully for authenticity testing. On the other hand, many unifloral honeys do not have specific markers. Also, the fact that many phenolics originate from propolis, which also varies independently of the honey nectar origin, will make the search for new markers difficult.

Determination of other minor honey constituents

Unifloral honeys differ to a certain extent in their content of amino acids (74–76) and trace elements (77–79). Determination of a number of chemical parameters including water activity, free amino acid composition, reducing sugars, total sugars and pH and subsequent evaluation by chemometric methods allowed the differentiation between different Italian unifloral honeys (80).

Methods for testing the authenticity of geographical origin

Pollen analysis

Pollen analysis is also used to determine the geographical origin of honey. The possibilities of pollen analysis for the determination of the geographical origin of honey have been reviewed recently (81). Indeed, the differences of the pollen spectrum between honeys from quite different geographical and climatic zone are easy to detect. However, if the geographical zones are closer, differences are more difficult to distinguish. In such cases more sophisticated melissopalynological methods should be used. In recent years pollen analysis has been successfully used for the determination of honeys originating from close geographical zones by the use of special software for pollen analysis (82), statistical discriminant analysis (83–84) and cluster analysis (85). No ring trials concerning the determination of accompanying pollens and on the assessment of geographical origin using these pollens, have been carried out up to the present.

Determination of routine parameters

The determination of routine quality parameters like hydroxymethylfurfural (HMF) content and honey enzyme activity (invertase, diastase) reflects honey freshness. These parameters will differ when they are determined in locally sold and imported honey (12). Indeed, in Western Europe the locally produced honeys are mostly unheated and will reach the consumer during the honey production year while the imported honey has been heated and stored for longer periods before reaching the market.

Table 4
Methods for testing of authenticity of geographical origin

Method, principle	*Status, Remarks*
Classical pollen analysis	used for routine control, not tested with ring trials
Chemometric evaluation of routine chemical parameters	
e.g. pH, acidity, electrical conductivity, fructose, glucose etc.	used only in research investigations
Determination of other minor components	
amino acids	all methods used only in research investigations
trace elements	
flavonoids	

For references and discussion of methods see text.

By chemometric combination of different parameters such as water, proline, ash content, electrical conductivity, acidity (free and lactone), pH, HMF, diastase and sugars a good separation of honeys from different geographical regions of Spain was achieved (86–90). However, the practical importance of the above mentioned chemometric classifications is questionable as the botanical origin of honey was mostly not considered. Also, it should be borne in mind that some parameters such as HMF, diastase and the content of individual sugars are storage- and heat-dependent.

Determination of other parameters

The determination of trace elements is widely used in food authenticity studies, also in relation to the geographical origin (91). First studies with a radioactivation method in French and Hungarian honeys were carried out in 1974 (92). By a combination of several elements it was possible to differentiate between acacia honeys from the two countries. Analysis of trace elements by atomic spectrometry in Spain showed that these honeys could be classified by the content of trace elements (93, 94).

Analysis of amino acids is another promising method. In different studies it was shown that differences in the amino acid spectra could distinguish between honeys from different geographical origins (95–97). However, in these studies, as in many others, the differences in the botanical origin of the honeys studied were not considered.

Flavonoids, which are known to be markers of the botanical origin of honey, can also serve also as markers of the geographical origin of honey (73).

In recent work it was shown that pyrolysis GC is a promising technique for the differentiation of honey geographical origin (98). The drawback is that sophisti-

cated instrumentation is used and thus for the present time the method is unsuitable for routine honey control.

The analysis of contaminants such as heavy metals and insecticides can also give information on the geographical origin of honey. Indeed, honey has been proposed as a biological indicator for contamination with heavy metals (99, 100) and pesticides (101, 102) of certain geographical areas. However, the measurement of contamination parameters has not been especially used as a geographical indicator for honey.

Proof of other authenticity issues

By definition retail honey should not be overheated. Changes of honey quality resulting in overheating are not permitted and such honey has to be used as baker's or industrial honey. In the EU honey legislation there are limits for the HMF content and for the diastase activity (table 1). Overheated honey is detected by measuring HMF, diastatase and invertase activity (1, 57). HMF is the better criterion, as honey enzyme activities vary considerably depending on the honey type. Some honeys, such as citrus, acacia etc., have naturally low enzyme levels and this must be taken into consideration when interpreting the results.

Conclusions

Although there are powerful methods to prove honey adulteration, they have to be further improved in order to keep the image of honey as an authentic natural food.

Concerning the botanical origin of honey quality, criteria for the determination of unifloral honeys, based on a wide variety of commercially available unifloral honeys, should be developed. The International Honey Commission is working on a data bank for quality criteria of the most important European unifloral honeys. The use of SPME for the determination of honey volatiles and honey aroma should be developed as a tool for the determination of the botanical origin of honey.

Research on the determination of the geographical origin of honey is only at the beginning. There are promising methods which have to be tested with a much greater number of honeys. Here the chemometric evaluation of routine analytical data seems to be a promising method, which has to be further improved. Studies with stable isotope ratios other than carbon, such as the D/H ratio and the oxygen isotopes ^{16}O and ^{18}O may prove useful. However, it seems likely that some of the differences between honeys of different geographical origin are probably due to differences in the botanical origin. Model studies should be carried with the same unifloral honey coming from different geographical origins.

The present review can serve for the stimulation of further work on honey authenticity issues in the canton food laboratories. Projects on cheese and wine authenticity have been initiated in the framework of the Swiss Food Manual of the

Federal Health Office. A project on honey authenticity has been submitted in the same framework.

Summary

Honey is the only natural sweetener offered world-wide today, which has the status of being healthy for young and old. The authenticity of honey is defined by the Codex Alimentarius standard, the EU Honey Directive and the Swiss Food Directive. The Codex and the EU standards were recently revised. The changes in these standards, as well as their consequences for the Swiss Food Directive are discussed. The authenticity of honey has two different aspects: Authenticity in respect of honey production and authenticity in respect of descriptions such as geographical and botanical origin, "natural", "organic", "raw" and "harvested in the cold". The objective of this review is to examine the different authenticity issues and the methods used to prove the authenticity of honey, in order to enable a successful authenticity testing.

Zusammenfassung

Honig ist das einzige natürliche Süssungsmittel, welches weltweit das Image eines gesundheitsfördernden Lebensmittels für jung und alt hat. Die Honigauthentizität ist definiert im Codex Alimentarius Standard, der EU Honigverordnung und der schweizerischen Lebensmittelverordnung. Die Änderungen dieser kürzlich revidierten Codex und EU Honigverordnungen werden diskutiert sowie die Konsequenzen für die schweizerische Lebensmittelverordnung. Die Honigauthentizität hat zwei verschiedene Aspekte: Authentizität der Honigproduktion und Authentizität der Honigumschreibung betreffend die botanische und die geographische Herkunft sowie die Bezeichnungen «natürlich», «roh», «biologisch», «kaltgeschleudert». Das Ziel dieser Übersichtsarbeit ist, die verschiedenen Aspekte der Honigauthentizität und die Methoden, die für ihre Prüfung angewendet werden zu diskutieren, um eine erfolgversprechende Prüfung der Honigauthentizität zu ermöglichen.

Résumé

Le miel est le seul édulcorant naturel mondialement reconnu pour ses propriétés bienfaitrices sur la santé aussi bien pour les jeunes que pour les personnes âgées. L'authenticité du miel est définie au sein des normes du Codex Alimentarius, dans la directive de l'UE relative au miel ainsi que dans l'ordonnance sur les denrées alimentaires. Les modifications issues de la révision récente des normes du Codex Alimentarius et de la directive de l'UE ainsi que les conséquences pour l'ordonnance sur les denrées alimentaires font l'objet de discussions. L'authenticité du miel comporte deux différents aspects: premièrement, l'authenticité de la production de miel et, deuxièmement, l'authenticité de la dénomination du miel par rapport à l'origine botanique et géographique ainsi qu'aux désignations «naturel», «brut», «biologique» et «extrait à froid». L'objectif de cette étude permettant d'avoir une vue d'en-

semble consiste, sur la base des connaissances actuelles, à examiner les différents aspects de l'authenticité du miel et des méthodes utilisées pour sa détermination afin d'obtenir une détermination fiable.

Key words
Honey, Authenticity, Production, Origin, Misdescription

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Ausschreibung

Münchner Mykotoxin-Stipendium zur Förderung von Forschung und Fortbildung auf dem Gebiet der Mykotoxinologie

Die Gesellschaft für Mykotoxinforschung e.V. (www.mykotoxin.de) schreibt für den Zeitraum 2002/2003 das mit insgesamt

€ 10000.–

dotierte *Münchner Mykotoxin-Stipendium* aus.

Das Stipendium wird von der Stiftung zur Förderung des Fachgebietes Mikroökologie und Mykotoxinologie, vertreten durch Prof. Dr. Brigitte Gedek und Prof. Dr. Wolfram Gedek, der Gesellschaft für Mykotoxinforschung e.V. als Träger zur Verfügung gestellt. Es soll an das wissenschaftliche Wirken des Ehepaares Gedek während ihrer akademischen Tätigkeit an der Ludwig-Maximilians-Universität München erinnern.

Das Stipendium dient dem Zweck, einem/einer jungen Wissenschaftler/in die Finanzierung eines Forschungs- oder Fortbildungsaufenthaltes (Reise- und Aufenthaltskosten) auf dem Arbeitsgebiet der Mykotoxinologie an einer angesehenen Institution im In- oder Ausland zu ermöglichen.

Der/die Bewerber/in sollte zum Zeitpunkt der Bewerbung nicht älter als 40 Jahre sein und folgende Unterlagen der Bewerbung beifügen: Lebenslauf; bisheriger wissenschaftlicher Ausbildungsgang; detaillierte Beschreibung des Forschungsvorhabens bzw. Forschungszieles; Zustimmung der Institution, an der das Forschungsvorhaben bzw. die Fortbildung durchgeführt werden soll; Befürwortungsschreiben von zwei Hochschullehrern hinsichtlich der Förderungswürdigkeit des Bewerbers bzw. der Bewerberin; Publikationsliste.

Über die Vergabe entscheidet ein Kuratorium ausschliesslich nach wissenschaftlichen Qualitätsmerkmalen. Entscheidungskriterien, Zahlungsmodus sowie Berichtspflicht durch den Stipendiaten bzw. der Stipendiatin sind durch Satzung geregelt.

Bewerbungen sind bis spätestens **31. Juli 2002** zu richten an die
Gesellschaft für Mykotoxinforschung
Kuratorium Münchner Mykotoxin-Stipendium
c/o Herrn Dir. u. Prof. Dr. M. Gareis
Institut für Mikrobiologie und Toxikologie, BAFF
E.-C.-Baumann Strasse 20
D-95326 Kulmbach

Announcement

"Munich Mycotoxin Scholarship" (Münchner Mykotoxin-Stipendium) for the Promotion of Qualification and Research in the Area of Mycotoxinology

The Society for Mycotoxin Research (www.mycotoxin.de) is pleased to announce the research scholarship named "Münchner Mykotoxin-Stipendium" for the period 2002/2003. The scholarship is endowed with

€ 10000.–

This scholarship is financed by generous support from the Foundation for Promotion of Microecology and Mycotoxinology (Prof. Dr. Brigitte Gedek and Prof. Dr. Wolfram Gedek) and awarded by the Society for Mycotoxin Research. The scholarship is in remembrance of the scientific work of Profs. Brigitte and Wolfram Gedek during their active academic careers at the Ludwig-Maximilians-University of Munich, Germany.

The scholarship is intended to enable a mycotoxinology-orientated research/study visit (any country; travel and living costs) of a young scientist to a reknowned Institute.

The applicant should not be older than 40 years. Applications must include the following documents:

- Curriculum vitae including previous scientific education and achievements (list of publications),
- detailed description of the scientific programme,
- letter of agreement from the host Institute,
- letters of recommendation from two University Professors.

According to the statutes, the decision about the scholarship is made by a Scientific Committee exclusively on the basis of the scientific quality of the application. The successful applicant is required to report to the scholarship committee about the results and achievements obtained during the research/study visit.

The deadline for submission of applications for the period 2002/2003 is **31 July 2002**. Applications should be sent to:
Gesellschaft für Mykotoxinforschung,
Kuratorium Münchner Mykotoxin-Stipendium
c/o Herr Dir. u. Prof. Dr. M. Gareis
Institut für Mikrobiologie und Toxikologie, BAFF
E.-C.-Baumann Strasse 20
D-95326 Kulmbach

SGLH SSHDA

Schweizerische Gesellschaft für Lebensmittelhygiene (SGLH)

Die Schweizerische Gesellschaft für Lebensmittelhygiene (SGLH) hat sich im Interesse der öffentlichen Gesundheit die Förderung einer hygienisch sicheren Ernährung, die Bearbeitung fachspezifischer Anliegen der Lebensmittelhygiene sowie den Erfahrungsaustausch unter den Mitgliedern zur Aufgabe gemacht.

Diese Ziele sucht die SGLH mit der Durchführung von jährlichen Arbeitstagungen, praktisch orientierten Fachkursen, Fachvorträgen und Workshops zu erreichen. Sie schafft und fördert Arbeitsgruppen zur Behandlung aktueller Probleme der Lebensmittelhygiene und unterstützt aktiv das Ausarbeiten von Vorschlägen für Normen und Beurteilungskriterien mikrobiologischer Untersuchungen. Diese Ziele möchte die SGLH in enger Zusammenarbeit mit anderen Fachgruppierungen, Behörden, Lehranstalten und weiteren Interessenvertretern der Lebensmittelsicherheit realisieren.

Den Veranstaltungen sind jeweils Themen aus dem Bereich der Lebensmittelhygiene gewidmet, vor allem Fragen der hygienischen Behandlung von Lebensmitteln, der modernen mikrobiologischen Diagnostik und der Bekämpfung von Lebensmittelinfektionen und -intoxikationen. Das Verständnis aktueller Forschungsresultate und deren Umsetzung in die Praxis soll generell gefördert werden.

Die SGLH zählt 560 Mitglieder. Zu ihnen gehören insbesondere Vertreter der gewerblichen und industriellen Lebensmittelproduktion, Mitglieder schulischer Einrichtungen sowie Mitarbeiter der Überwachungsbehörden.

Der Mitgliederbeitrag beträgt Fr. 50.– für Einzelmitglieder und Fr. 200.– für Kollektivmitglieder.

Die «Mitteilungen aus Lebensmitteluntersuchung und Hygiene» sind das offizielle Publikationsorgan der SGLH. Der Preis für das Abonnement ist im Mitgliederbeitrag inbegriffen.

Weitere Informationen zu Leitbild und Schwerpunkten der SGLH-Aktivitäten sind auf der Homepage unter www.sglh.ch zu finden.

Werden auch Sie Mitglied der SGLH!

Anmeldung direkt via Internet mit dem Formular auf der Homepage www.sglh.ch.

Leo Meile, Präsident der SGLH, Labor für Lebensmittelmikrobiologie, ETHZ, CH-8092 Zürich, E-mail: leo.meile@ilw.agrl.ethz.ch

SGLH SSHDA

Société suisse d'hygiène des denrées alimentaires (SSHDA)

La société suisse d'hygiène des denrées alimentaires (SSHDA) œuvre dans l'intérêt de la santé publique par la promotion de la sûreté hygiénique des aliments, en informant sur les questions actuelles liées à l'hygiène des denrées alimentaires et en favorisant les échanges d'expérience entre ses membres.

La SSHDA assure la poursuite de ces buts par des journées de travail, des cours techniques orientés vers la pratique, des conférences spécialisées et des workshops. Elle crée et encourage la mise sur pied de groupes de travail consacrés à l'analyse des problèmes actuels et soutient activement l'élaboration de propositions de normes et de critères d'appréciation pour les analyses microbiologiques alimentaires. La SSHDA veut réaliser ces buts en étroite collaboration avec d'autres associations, les instances officielles, les universités, les écoles supérieures et d'autres personnes ou institutions œuvrant dans le domaine de la sûreté alimentaire.

Les activités de la société sont consacrées à des thèmes d'actualité liées à l'hygiène des denrées alimentaires, avant tout dans les domaines du traitement hygiénique des denrées alimentaires, des méthodes modernes de diagnostic microbiologique et de la lutte contre les intoxications et infections d'origine alimentaire. La compréhension des résultats de recherche actuels et leur application dans la pratique doivent être encouragées d'une manière générale.

La SSHDA compte 560 membres. Parmi eux se trouvent en particulier des représentants de l'industrie alimentaire et de la production alimentaire artisanale, des membres d'institutions de formation professionnelle, ainsi que de nombreux collaborateurs des instances officielles de surveillance.

La cotisation annuelle s'élève à Fr. 50.– pour les membres individuels et à Fr. 200.– pour les membres collectifs.

Les «Travaux de chimie alimentaire et d'hygiène» constituent l'organe de publication officiel de la SSHDA. Le prix de l'abonnement est compris dans le montant de la cotisation.

Des informations concernant la vision directrice et les centres d'intérêt de la SSHDA se trouvent sur Internet à l'adresse www.sglh.ch.

Devenez vous aussi membre de la SSHDA!

Inscription en qualité de membre de la SSHDA directement par Internet sur le site www.sglh.ch.

Leo Meile, président de la SSHDA, Labor für Lebensmittelmikrobiologie, ETHZ, CH-8092 Zürich, E-mail: leo.meile@ilw.agrl.ethz.ch