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A short study on the formation of styrene in cinnamon

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Introduction

Styrene is the starting material used in the production of styrene-based polymers which are used in many food packaging applications, and it can be detected in a variety of food products (1). This incidence can partly be explained by the migration into food from the packaging (2, 3), especially for dairy products for which polystyrene packaging is frequently used. An indirect contamination through air during food storage (4) has been also demonstrated.

Any incidence of styrene in food is not related to an exogenous contamination since styrene is also a compound which occurs naturally in foods. It has been identified in many different raw agricultural commodities such as fruits, cereals, coffee beans and beef meat (5) as well as in processed foods such as olive oil (6) or beer (7). The formation of styrene in food can be explained by different mechanisms: It can be a by-product of yeasts (7) or molds (8) naturally present on food or it can be an intermediate metabolite of foodstuffs.

Independently from its origin, the level of styrene in food products is generally low and in the range of 1 to 60 µg/kg (1). The average dietary intake was estimated to be in the range of 2–3 µg/person/day by a total diet study (9) in the UK. A more recent study (10) estimates the daily intake via food to be in the range of 0.2–1.2 µg and that the inhalation route accounts for more than 90% of the overall exposure to styrene. The WHO used a guideline value of 7.7 µg/kg body weight per day for the tolerable daily intake (11). Based on those data, the Scientific Committee on Food of the European Commission considers that the intake via food does not contribute to a risk to human health (12).

Among vegetal foods, cinnamon represents a special case. The level of styrene in fruits is generally very low (<3 µg/kg). In cinnamon, concentrations as high as

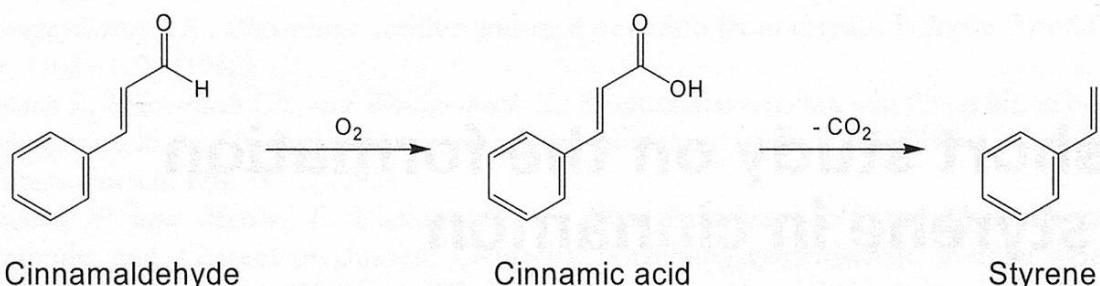


Figure 1 Possible reaction pathway for the formation of styrene from cinnamaldehyde

40 mg/kg have been reported (5, 13). The structure of styrene is contained in the cinnamaldehyde that is the major flavouring compound of cinnamon. The formation of styrene in cinnamon could well be explained by an oxidative degradation of the flavouring component. Cinnamaldehyde is prone to oxidation, yielding cinnamic acid. This compound may subsequently produce styrene via decarboxylation (figure 1). Such a mechanism has not yet been proved, but the formation of 4-vinylguaiacol from ferrulic acid (14) follows a comparable reaction pathway.

The characteristic smell of styrene was observed many times during the transport of cinnamon quills. To avoid a degradation of the quality during the transportation by sea, the storage conditions are today regulated with specific norms (15). A very high humidity or/and temperature seem to be the factors, which can influence the formation of styrene in cinnamon bark.

The objective of this work was to study the formation of styrene in cinnamon in order to assess whether the styrene level could be a quality indicator for this spice or not.

Experimental

Samples

16 cinnamon samples, either as stick or as powder, were bought directly in different Swiss shops. Cinnamon sticks were all "Ceylan cinnamon" (*Cinnamomum zeylanicum* Nees) with the exception of one decorative stick which was a "Chinese cinnamon" (*Cinnamomum cassia* Bl. or *Cinnamomum aromaticum* Nees). The variety of cinnamon powder samples was not indicated on the labels and was therefore not identified. Based on the country of origin labelling, the variety of cinnamon could however be deduced as *Cinnamomum cassia*, mostly cultivated in China, Indonesia, Laos and Vietnam. Ten irradiated powder samples were obtained from a spice importer (J. Carl Fridlin Gewürze AG, Hünenberg, Switzerland). These samples were irradiated with doses of 10 KGy (^{60}Co source). They were research samples, which were not destined to be marketed. During the study time all samples

were stored in the dark at ambient temperature; the humidity was not controlled as the fluctuation is usually low in the laboratory.

Samples of essential oils were obtained from the Swiss market.

Additionally, fresh sample of branches and leaves were collected from a cinnamon tree (*Cinnamomum verum* J.S. Presl. or *Cinnamomum zeylanicum* Nees) in the tropical greenhouse of the botanical garden in St-Gall. The fresh samples were stored under refrigeration (-20°C) until further use.

Extraction and analytical method

Sticks of cinnamon were first finely pulverised with a spice mill. The fresh samples (bark or leaves) were finely chopped with a knife. Samples sold as powder were treated directly. Cinnamon powder (0.5 g) was extracted three times with 2 ml of toluene (99.8 %, *for trace analysis* from Merck or *nanograde* from Mallinckrodt) under nitrogen at ambient temperature during 15 min in an ultrasonic bath. The yellow-green colour of the extract was removed by passing the toluene solution on a Fluorisil® Bond Elut LRC cleanup column (Varian). Styrene and cinnamaldehyde were measured by GC-MS analysis. Two instruments were used: a Trace 2000 series from Thermo-Finnigan or a Hewlett-Packard GC 5890 series II equipped with a HP 5972 MSD detector. The column was either a ZB-1 (Zebron) or a HP 5MS (Hewlett-Packard) with the dimension of $30\text{ m} \times 0.25\text{ mm i.d.}$ and a coating film thickness of $0.25\text{ }\mu\text{m}$.

Experimental conditions were kept identical on both instruments. 1 μl of the extract was injected (splitless) on the column. The carrier gas (helium) inlet pressure was 75 kPa. The oven temperature was maintained at 40°C during 5 min and then increased up to 200°C with a ramp of $12^{\circ}\text{C}/\text{min}$. Mass spectrometry involved electron ionization. Single ion monitoring (SIM) was used at the following m/z: 51, 78 and 104 for styrene, and 103 and 131 for cinnamaldehyde. The retention time of styrene was measured at 8.29 min on the ZB-1 column and at 8.52 min on HP 5MS. The styrene concentration in the samples was calculated by extrapolation from a calibration curve (nine solutions) obtained by dilution of four different stock solutions (concentrations: 5–22 mg/l). The final concentrations were distributed between 0 and 0.25 mg/l. The styrene was from Across organics (99.5 %, p.a. GC, stabilised with 10–15 ppm t-butylcatechol). The detection limit (LOD at 95 % confidence level) and the quantification limit ($\text{LOQ}=3 \times \text{LOD}$) of styrene in the toluene solution were calculated at 4 $\mu\text{g/l}$ and 12 $\mu\text{g/l}$ respectively.

Essential oils were diluted in toluene (1 mg oil in 50 ml toluene) prior to GC-MS analysis.

Artificial weathering of samples

24 samples of a single cinnamon powder were stored under defined environmental conditions. Each sample was conditioned in a special sealed container where the oxygen level and the relative humidity were adjusted. The containers were

stored during 17 days at specified temperature and then analysed with the method described above. Weathering was carried out with each combination of the environmental parameters listed in table 1. Experiments combining an R.H. of 53 % and a temperature of 60 °C were not performed because this specific R.H. is not adapted for temperatures above 35 °C.

Table 1
Environmental parameters combined in the weathering experiments.
 The indication in brackets refers to the technique used to reach the specific value

Relative humidity (%)	Temperature (°C)	Oxygen level (%)
0 (silica gel)	20	0 (argon)
53 (supersaturated solution of $Mg(NO_3)_2$)	35	22 (air)
100 (distilled water)	60	100 (oxygen)

Results and Discussion

Styrene in cinnamon is usually measured by headspace GC. The method developed in this study has the drawback of a solvent extraction but is quite sensitive with a quantification limit at 10 µg/kg. Depending on the toluene quality and source, blanks sometimes showed a peak at the retention time of styrene, but it was of constant intensity in time and insignificant compared to the peak of samples. It could easily be subtracted from the sample chromatogram. A representative chromatogram is depicted in figure 2. The recovery was not tested by the usual addition of deuterated styrene. Extraction experiments on real samples showed that at the 4th toluene extraction the styrene concentration is already below the detection limit. It was then deduced that the recovery was complete after three extractions. All samples were analysed at least twice. The repeatability of the extraction was assessed on the powder sample Nr 6 (table 2); the relative standard deviation on the extracted quantity of styrene after three extractions was measured at 2.7 %.

Commercial samples

The styrene concentration measured in commercial samples varies from 0.3 to 1.9 mg/kg cinnamon (table 2). At this concentration, pungent styrene cannot be smelled as it is easily covered by the strong fragrance of cinnamaldehyde whose concentration is much higher. These measured levels are also quite low compared to the results of a previous campaign (13) and study (5) where styrene was assessed at 7 and 39 mg/kg, respectively. This means for cinnamon samples, which were adequately produced and stored, that the usual styrene concentration is much lower than initially expected and is probably less than 2 mg/kg.

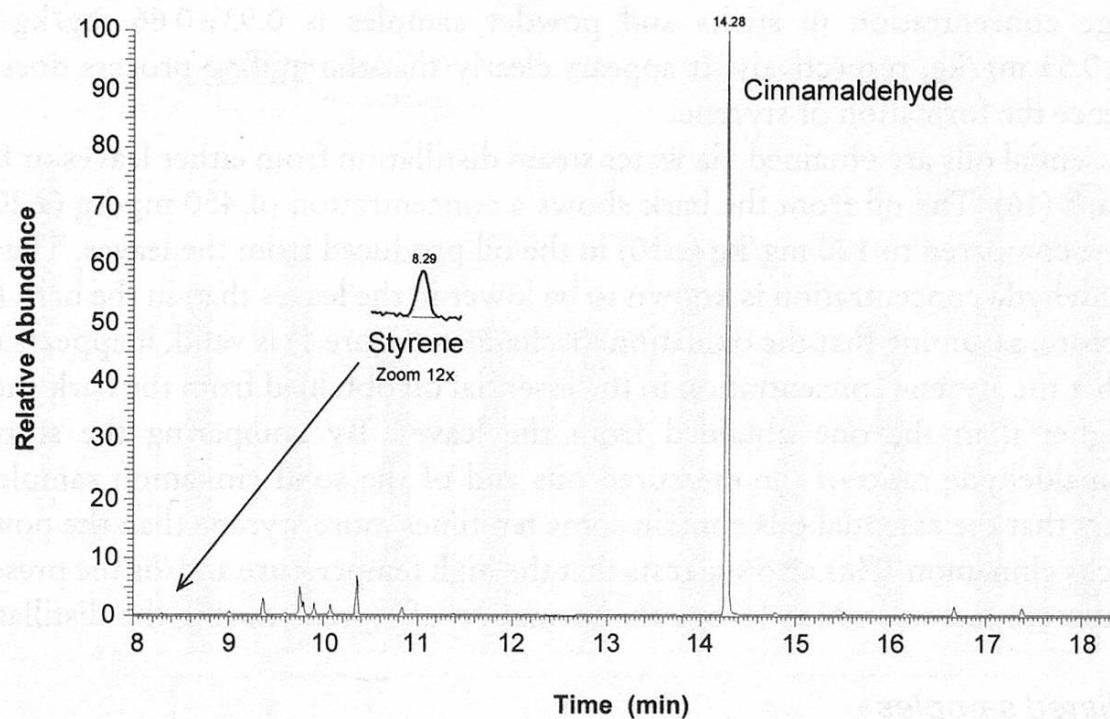


Figure 2 Chromatogram of the first toluene extraction of a cinnamon powder sample

Table 2
Styrene concentration in different commercial cinnamon spices and cinnamon products

Product form	Sample Nr	Country of origin	Concentration (mg/kg)
Stick	1	n/a	0.61
	2	Sri Lanka	0.31
	3	Sri Lanka	0.61
	4	Sri Lanka	0.47
	5 ^a	n/a	0.31
	6	Sri Lanka	1.71
	7	Sri Lanka	1.53
	8	Madagascar	1.88
Powder	9	Sri Lanka	0.57
	10	Madagascar	0.26
	11	Sri Lanka/China	0.44
	12	India	1.06
	13	Sri Lanka	0.33
	14	n/a	1.42
	15	Sri Lanka/Indonesia	1.61
	16	India	1.21
Essential oil	17 ^b	Madagascar	450
	18 ^c	Madagascar	120

^aornamental cinnamon quill; ^boil from bark; ^coil from leaves

Sticks and powder samples show a very uniform styrene concentration. The average concentration in sticks and powder samples is 0.93 ± 0.66 mg/kg and 0.86 ± 0.53 mg/kg, respectively. It appears clearly that the milling process does not influence the formation of styrene.

Essential oils are obtained via water steam distillation from either leaves or from the bark (16). The oil from the bark shows a concentration of 450 mg/kg (± 20) of styrene compared to 120 mg/kg (± 10) in the oil produced from the leaves. The cinnamaldehyde concentration is known to be lower in the leaves than in the bark (17). Therefore, assuming that the oxidation mechanism (figure 1) is valid, it appears normal that the styrene concentration in the essential oil obtained from the bark should be higher than the one obtained from the leaves. By comparing the styrene/cinnamaldehyde ratio of the measured oils and of the solid cinnamon samples, it appears that the essential oils contain some ten times more styrene than the powder or sticks cinnamon. This also suggests that the high temperature and/or the presence of water are factors, which favour the formation of styrene during the distillation.

Irradiated samples

Spices are often irradiated to reduce microorganism contamination and extend the shelf-life of the products. The high energy beam creates electron radicals in the material which further react with oxygen from the air to give peroxy radicals which can be quite stable in dried material. Peroxy radicals can also react into hydroperoxides. Both chemical groups are strong oxidants and it is reasonable to think that γ -irradiation could ease the oxidation of cinnamaldehyde into cinnamic acid. To verify this hypothesis ten special powder samples were obtained directly from a spice importer. Half of each sample was irradiated with a 10 kGy dose and analysed for its styrene concentration for comparison with the non irradiated sample. Measurements were carried out ten months after the irradiation phase for both irradiated and non-irradiated samples. The measured concentrations depicted in figure 3 indicate clearly that γ -irradiation has absolutely no influence on the formation of styrene in cinnamon. The difference measured between the two corresponding samples is smaller than the experimental precision. The assumption that irradiation would favour styrene formation can therefore be ruled out.

It is not possible to make a strong deduction on the possible role of microorganism in the styrene formation from these measurements. At the time of irradiation, the samples were already about two years old and their storage history (i.e. temperature and humidity) was unknown. However, if styrene is assumed to be a by-product of a microorganism occurring naturally on cinnamon, it would be logical to expect a higher level of styrene in the non-irradiated samples as the production of styrene was slowed down on irradiated samples. The fact that no difference is observed gives some weak indication that this hypothesis is rather unlikely.

Two samples were measured already two months after the irradiation. At that period as well, no significant difference in styrene was observed between non-irra-

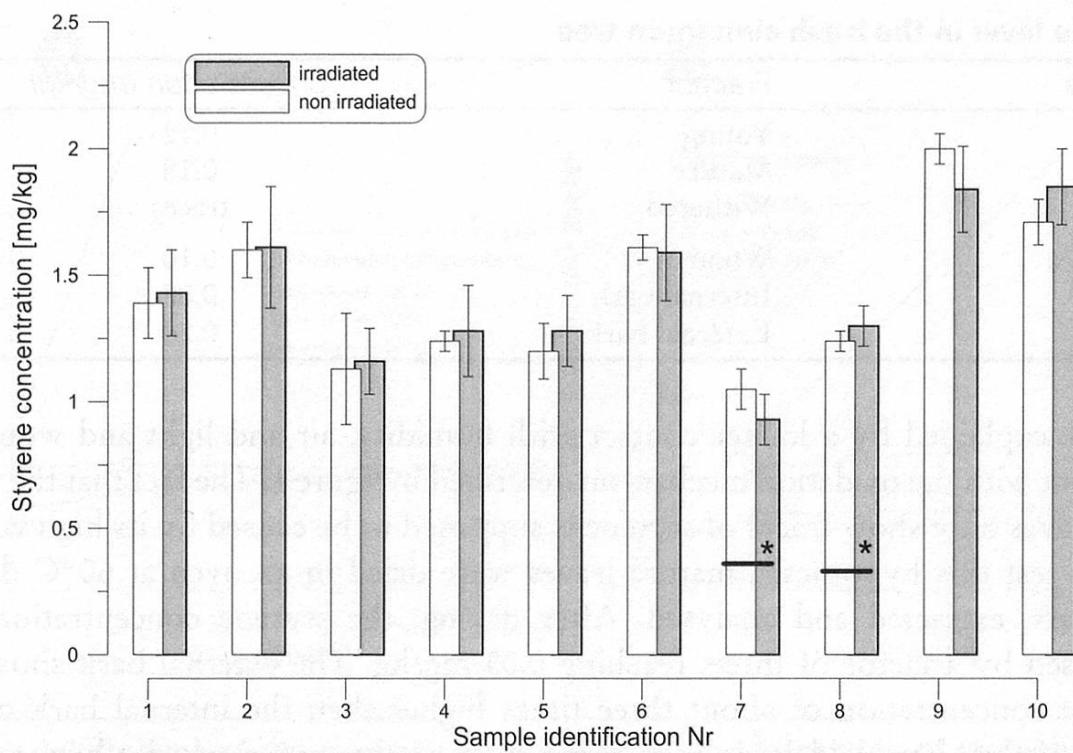


Figure 3 Styrene concentration in different cinnamon powders before and after irradiation (ten months)

The line on top of the bar is the standard deviation measured in three independent experiments.

*Level measured two months after the irradiation

diated and irradiated samples: concentrations of 0.33 and 0.36 mg/kg (standard deviation: 0.01) were measured in cinnamons Nr 7 and 8, respectively. The fact that the level of styrene has increased by a factor of about three during the eight months interval is not abnormal and supports the assumption of a slow oxidation process.

Fresh samples

Fresh samples of a cinnamon tree were collected and analysed for styrene content. Three different types of leaves were picked out and analysed separately: young leaves (small, green and shining), mature leaves (large, green with already some brown tips) and withered leaves (yellow, faded colour). The branches were cut and peeled in order to clearly separate the external bark and the internal bark from the wood. All fresh samples were first finely chopped with a knife before toluene extraction. Measured levels are indicated in table 3. Due to a limited amount of material collected, only one measurement was performed on each sample. It appears clearly that styrene is present in all parts of the cinnamon tree. It can therefore be considered as a natural constituent, originating from the plant metabolism. Mature leaves present a higher concentration than young leaves. This observation could

Table 3
Styrene level in the fresh cinnamon tree

Sample	Fraction	Concentration (mg/kg)
Leaves	Young	0.12
	Mature	0.18
	Withered	traces
Branches	Wood	0.10
	Internal bark	0.08
	External bark	0.26

well be explained by a longer contact with humidity, air and light and would be coherent with the oxidation mechanism described in figure 1. The fact that the withered leaves only show traces of styrene is supposed to be caused by its high volatility. To test this hypothesis, mature leaves were dried in an oven at 60°C during 48 hours, extracted and analysed. After drying, the styrene concentration had decreased by a factor of three, reaching 0.05 mg/kg. The external bark showed a styrene concentration of about three times higher than the internal bark or the wood. If the cinnamaldehyde concentration does not vary markedly between the external and internal bark, this difference of concentration could be explained, as for the leaves, by an increased exposure to the environment.

Compared to the fresh samples, the styrene concentration in the commercial samples increased by an average factor of 11 (average concentration in the commercial samples is 0.895 mg/kg). As only the internal bark is used to produce the spice, a concentration of 0.1 mg/kg could be taken as a reference value. This suggestion is of course to be taken with caution due to the limited number of fresh samples analysed and to the lack of connection between the fresh and commercial samples.

Eugenol (1-allyl-3-methoxy-4-hydroxybenzene) is the principal aroma substance in the leaves of the cinnamon tree with 70 to 85 % in the oil (17). It is also an efficient antioxidant and it could well play a role in the formation of styrene by hindering the oxidation of the cinnamaldehyde. Analyses were carried out with SIM at m/z 164 and 149 in order to detect this substance. Although eugenol could be assessed under the analytical conditions chosen (retention time 15.5 min on ZB-1 column), it was not detected in any of the samples tested. This negative result was not further investigated. It was supposed that the toluene treatment does not adequately extract the substance.

Influence of the atmosphere

The importance of atmosphere control during sea transportation of cinnamon quills in containers is well known and "Good Transportation Practices" are available today (15). Cinnamon should be stored under controlled atmosphere, i.e. in ventilated containers, at a temperature between 15–19°C and under a R.H. between 60–70 %. The influence of these environmental factors was checked in artificial

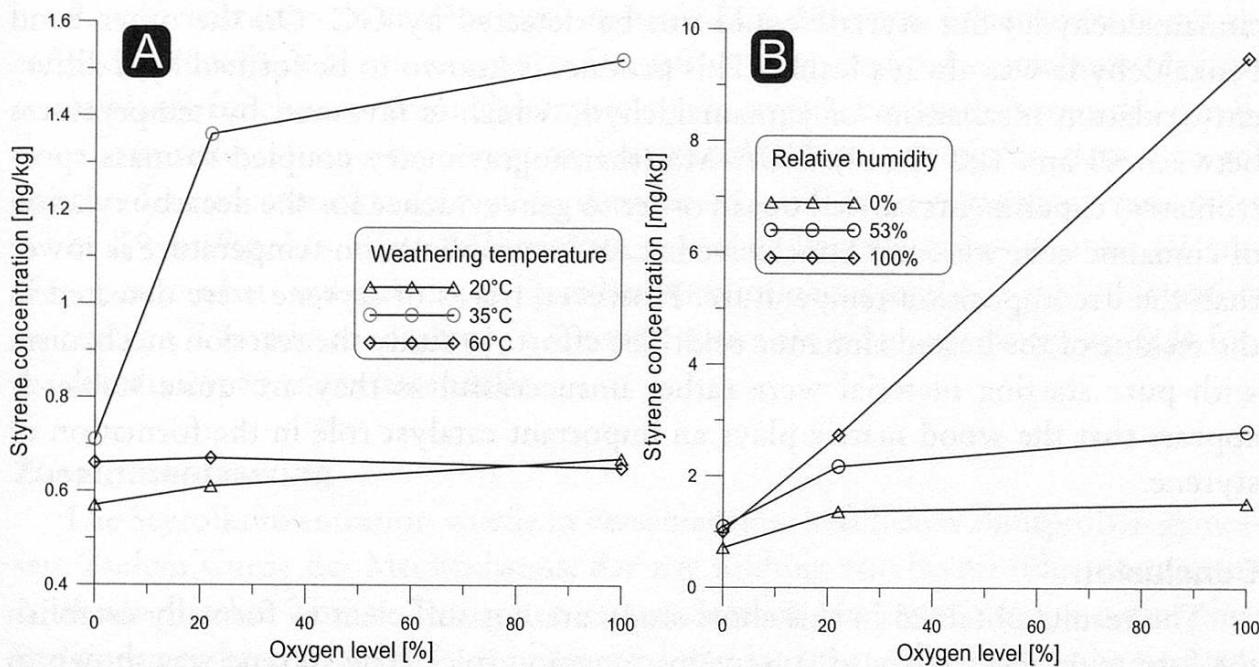


Figure 4 Influence of atmospheric parameters on the formation of styrene during storage of cinnamon powder

A Effect of temperature in function of oxygen level at 0% R.H.

B Effect of R.H. in function of oxygen level at a temperature of 35 °C

weathering experiments. The results of 17-days weathering experiments of sample 9 (table 2) are depicted in figure 4. They show that for samples stored at ambient temperature (20 °C), the formation of styrene is only slightly favoured by the oxygen level and humidity. The variation is weak but significant. The samples can however be considered as stable under these conditions. If the temperature is increased up to 35 °C, the effect of atmospheric conditions becomes definitively marked: both oxygen and water concentration in the atmosphere lead to an increase in the concentration of styrene (up to the factor ten) during the storage time. The weathering at 60 °C leads to a styrene concentration similar to a storage at 20 °C. No variation is observed by increasing the oxygen level. It is very unlikely that cinnamon is more stable at 60 °C than at 20 °C. It is more probable that the evaporation of styrene at high temperature becomes the process which controls the concentration. Without being a formal proof, all the data are in agreement with the proposed oxidative mechanism (figure 1). They indicate also that cinnamon can be efficiently protected, even under tropical conditions (high temperature, humidity), if the spice is stored under exclusion of oxygen.

There was an attempt to confirm the reaction mechanism leading to the formation of styrene (figure 1). Some simple experiments were performed with pure references of cinnamaldehyde and cinnamic acid at high temperature (45–100 °C) under oxygen. During the heating of cinnamaldehyde solutions the formation of cinnamic

acid was noticeable (precipitation; solubility of cinnamic acid is about 80 mg/ml cinnamaldehyde) but styrene could not be detected by GC. On the other hand benzaldehyde was always found. This product is known to be formed by a different oxidation mechanism of cinnamaldehyde which is favoured by temperatures between 60 and 100 °C (18). TGA-MS (thermogravimetry coupled to mass spectrometry) experiments carried out in order to gain evidence for the decarboxylation of cinnamic acid were not conclusive because its sublimation temperature is lower than the decomposition temperature. However, traces of styrene were detected in the residue of the heated cinnamic acid. The efforts to study the reaction mechanism with pure starting material were rather unsuccessful as they are quite stable. It appears that the wood matrix plays an important catalyst role in the formation of styrene.

Conclusion

The results obtained in this short study are not sufficient to formally establish the formation mechanism of styrene in cinnamon spice. The styrene was shown to be already present at low level in the living plant and factors such as humidity or oxygen concentration correlate with the styrene concentration. These observations give some significant hints which connect well with an oxidative mechanism of cinnamaldehyde within the plant. However, processes such as milling or irradiation which are known to promote the matrix oxidation show no effect on the final concentration of styrene. This is puzzling and suggests that the reaction mechanism is certainly more complex than the one depicted in figure 1. Especially, the direct decarboxylation is questionable as it does not function with pure cinnamic acid. A comprehensive study of the mechanism was out of the scope of this work.

In order to use the styrene concentration as an indicator of the quality of the spice, it would be necessary to better understand all the influencing factors. The data obtained from this survey suggest however that 2 mg/kg is probably a quite normal concentration which reflects an ideal production process and adequate storage conditions of the spice.

Summary

The level of styrene was analysed in different commercial cinnamon samples and the mechanism of formation was studied. Analysis of fresh samples of cinnamon show clearly that styrene is produced in the tree and that it reaches in the inner bark concentrations around 0.1 mg/kg. The concentration in samples collected from the market was measured between 0.3 and 1.9 mg/kg. The effects of γ -irradiation and storage conditions were also studied. The results indicate that the irradiation does not influence the styrene formation. Humidity, connected with temperature is the factor which controls the formation of styrene in cinnamon.

Résumé

La concentration du styrène a été mesurée dans différents échantillons commerciaux de cannelle et le mécanisme de formation a été étudié. Les analyses réalisées sur la plante fraîche montrent clairement que le styrène est produit sous des conditions naturelles et que sa concentration est d'environ 0,1 mg/kg dans l'écorce interne. La concentration du styrène dans les échantillons commerciaux varie entre 0,3 et 1,9 mg/kg. Les effets de l'irradiation- γ et de l'entreposage ont aussi été étudiés. Les résultats indiquent que l'irradiation n'influence aucunement la formation du styrène. L'humidité combinée à la température est le facteur qui contrôle la formation du styrène dans la cannelle.

Zusammenfassung

Die Styrolkonzentration wurde in verschiedenen käuflichen Zimtproben gemessen. Zudem wurde der Mechanismus, der zur Bildung von Styrol führt untersucht. Analysen frischer Zimtrindenproben zeigen deutlich, dass Styrol unter natürlichen Bedingungen bereits im Baum gebildet wird und im Innern der Rinde eine Konzentration von 0,1 mg/kg erreicht. Die Styrolkonzentrationen in den käuflichen Proben liegen zwischen 0,3 und 1,9 mg/kg. Die Auswirkungen von γ -Bestrahlungen und Lagerbedingungen wurde ebenfalls untersucht. Die γ -Bestrahlung hat keinen Einfluss auf die Styrolkonzentration. Hingegen erwiesen sich die Feuchtigkeit und die Temperatur als bestimmende Faktoren für die Bildung von Styrol in Zimt.

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Key words

Cinnamon, styrene, cinnamaldehyde, oxidative degradation

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