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## **Ergosterol in fruit - a marker for mould toxins?**

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Ergosterol (also known as provitamin D2) is present in the cell membranes of fungi (including those of yeasts and moulds). Accordingly, ergosterol may occur naturally (such as in edible mushrooms, bread or cheese ripened with mould cultures) or may occur due to undesirable mould contamination. In light of these facts, the German Federal Institute for Risk Assessment (BfR) has investigated whether ergosterol can be used as a reliable 'marker substance' for a contamination of food with mould toxins, and whether the intake of ergosterol or vitamin D2 from food - specifically from fruit or fruit products - can cause adverse health effects for consumers.

With a few exceptions, such as cheese ripened with mould cultures (e. g. Camembert, Gorgonzola or Roquefort), mould is not desirable either on or in food products. It poses a potential health risk to consumers since some genera of mould can produce mould toxins (mycotoxins) under certain conditions. Ingestion of these toxins with the food can have serious consequences for human health. Limited data from scientific publications on ergosterol content of fruit and fruit products allows neither derivation of tolerance or maximum levels nor a reliable estimation of whether ergosterol content is an appropriate marker for mycotoxin contamination in food.

Irradiating food with UV light to increase vitamin D content is permitted for certain foods that naturally contain ergosterol (such as edible mushrooms or baker's yeast) within the European Union. This UV irradiation results in the transformation of ergosterol into vitamin D2 (ergocalciferol). This can then be converted into the biologically active form of vitamin D (calcitriol) by human metabolism in liver and kidneys. An excessive and higher than required intake of vitamin D can lead to consequences for health, including cardiac arrhythmias, restricted kidney function and calcium metabolism disorders (hypercalcaemia). In the opinion of the BfR, there is no evidence that the consumption of UV-irradiated food produced in compliance with EU legislation poses health concerns. Furthermore, there are currently no indications that the ingestion of ergosterol or vitamin D2 from foods - particularly from fruit or fruit products - could lead to adverse health effects such as a vitamin D overdose (hypervitaminosis).

### **1. Subject of the assessment**

The German Federal Institute for Risk Assessment (BfR) has addressed the question of whether findings and/or research data on the occurrence of ergosterol in fruit and fruit products are available that can be used to determine tolerance values for ergosterol in fruit and fruit products. The BfR also evaluated whether maximum values for ergosterol in fruit and fruit products could be formulated from a toxicological perspective. This assessment is intended to aid the work of the expert committees of the German Food Code Commission (DLMBK) in deriving a suitable phrasing for their guidelines.

## 2. Results

No information is available to the BfR concerning ergosterol content in fruit or fruit products that permits the determination of tolerance values for these food groups. There is merely a limited amount of data available from scientific publications on the ergosterol content of fruit and fruit products. No data are available from own investigations or from research projects of the BfR nor from monitoring programmes of official laboratories.

The BfR has found no indications that the intake of ergosterol or vitamin D2 from foods - and fruit or fruit products in particular - could lead to adverse effects (such as to hypervitaminosis). Nor are there any indications that the intake of food irradiated with UV light in compliance with EU legislation raises health concerns.

Beyond this, a potential correlation between ergosterol and mycotoxin concentrations in food is discussed. However, the BfR considers the available data on potential co-contamination with mycotoxins as insufficient for deriving maximum levels for ergosterol in fruit and fruit products.

Accordingly, the question of whether ergosterol content could be utilised as a suitable marker for contamination of food - as for example fruit juices - with mycotoxins or as a marker for the quality of fruit and fruit products in general cannot be conclusively answered on the basis of available data.

## 3. Rationale

### 3.1. Description of the agent

Ergosterol is present in the cell membranes of fungi and in significantly smaller concentrations also in yeasts. The biosynthesis of ergosterol depends on a wide range of factors, such as the species of fungi, the age of the fungi, the available nutrients and the presence of oxygen (Ghiretti *et al.*, 1995).

In physiological terms, ergosterol mediates selective membrane permeability in intact cells. Accordingly, the detection of the substance is utilised as a marker for undesirable mycotic growth in tomato products as a hygiene parameter (Kadalkal & Tepe, 2019).

The nutritional relevance of ergosterol (provitamin D2) results from its function as a precursor for the biosynthesis of vitamin D2, a prohormone that is in turn converted into biologically active vitamin D (calcitriol; see also section 3.3.1). Calcitriol is crucial to the maintenance of calcium and phosphate homeostasis and promotes i.a. bone mineralisation and neuromuscular coordination.

In the following section, the BfR answers specific questions on this topic.

3.2 *Question 1: Are findings and/or data available to the BfR about the occurrence of ergosterol in fruit and fruit products which could be applied to determine tolerance values and that could be made available to the DLMBK?*

The BfR has no data on the ergosterol content of fruit or fruit products from own investigations, research projects or monitoring programmes of official laboratories. The catalogue of matrix codes (*Auftragsdatenverarbeitungs (ADV)-Katalog*) maintained by the German Federal Office of Consumer Protection and Food Safety (BVL) lists a code for the parameter “Ergosterin 5,7,22-Ergostatrien-3b-ol Provitamin D2”, but no data were collected in national monitoring between 1995 and 2017.

This agrees with personal communications from experts working in German official laboratories for the assessment of fruit juices and fruit products. Only the Bavarian State Office for Health and Food Safety (LGL, Würzburg) is currently collecting data on the ergosterol content of various products such as tomato juices, berry preserves and smoothies made with berries. The results will be published in the LGL's 2019 Annual Report.

Beyond this, only limited published data on the ergosterol content of fruit and fruit products is available to the BfR. Decloedt *et al.* quantified the presence of ergosterol in beverages such as fruit and vegetable juices. No ergosterol was detected in most of the juice samples. Ergosterol concentrations of 17 and 29 µg/100 ml, respectively, were measured only in pomegranate juice and mixed vegetable juice (Decloedt *et al.*, 2017). Kadakal *et al.* (2005) investigated ergosterol in raw apple juice at different stages of decay, finding that ergosterol concentrations increased significantly with increasing decay (decay rate at 0%, 30%, 60% and 100% - ergosterol concentrations of 0.7, 23.9, 63 and 111 mg/l). An analysis of grape varieties also revealed a correlation between ergosterol concentrations and degree of decay (Porep *et al.*, 2014). There are also a number of papers on tomato products which Kadakal and Tepe discuss in their overview article “Is ergosterol a new microbiological quality parameter in foods or not?” (Kadakal & Tepe, 2019).

In conclusion, no information is available to the BfR concerning ergosterol content in fruit or fruit products that permits the determination of tolerance values for these food groups.

3.3 *Question 2: Would it be relevant for this purpose and, if necessary, is it possible to define maximum levels for ergosterol in fruit and fruit products from a toxicological perspective?*

In order to answer this question, both the toxic potential of ergosterol as well as its nutritional relevance is discussed. Furthermore, a potential co-contamination with mycotoxins is another aspect addressed here. The results of discussions held by the BfR Committee for Wine and Fruit Juice Analyses (Wufak) are also presented in the context of ergosterol as a potential marker for the contamination of fruit juices with mycotoxins.

#### 3.3.1 Hazard characterisation of ergosterol

Many foods contain ergosterol which is the primary sterol in the fungal cytoplasmic membrane. Ergosterin concentrations of 2.4-8.2 mg/100 g have been detected in button mush-

rooms, for example (Seeburg, 2014). Since ergosterol does not occur or occurs only in very low concentrations in the tissue of higher plants, it is considered to be a reliable marker for fungal infection (yeasts, moulds).

UV irradiation converts ergosterol (provitamin D<sub>2</sub>) photochemically into vitamin D<sub>2</sub> (ergocalciferol). After intake, the compound 25-hydroxy-vitamin D<sub>2</sub> (25-(OH)D<sub>2</sub>) is first formed in the liver from the prohormone vitamin D<sub>2</sub>. The biologically active form - 1 $\alpha$ ,25-dihydroxy-vitamin D (calcitriol) - is then produced in the kidneys and exhibits its effects primarily via the vitamin D receptor. High doses of the vitamin D<sub>2</sub> produced, for example, photochemically from ergosterol could therefore lead to an increase in the blood concentration of 25-(OH)D (Jasinghe *et al.*, 2005; Koyyalamudi *et al.*, 2009; Wilson *et al.*, 2017).

An increase in the plasma concentration of 25-(OH)D to values higher than 400 nmol/l can cause hypervitaminosis, however, even lower concentrations can also be associated with toxic effects. Potential consequences of a vitamin D intoxication include hypercalcaemia, cardiac arrhythmias and restricted kidney function (EFSA, 2012; Koul *et al.*, 2011).

As a result, a tolerable upper intake level (UL) of 100  $\mu$ g per day for adults, 25  $\mu$ g per day for infants (0-1 years), and 50  $\mu$ g per day for toddlers and younger children (1-10 years) has been derived. The NOAEL (no observed adverse effect level) for vitamin D is 250  $\mu$ g per day for healthy adults (EFSA 2012, 2018). The threshold for vitamin D intoxication is 1,000  $\mu$ g to 2,500  $\mu$ g per day over 1 to 2 months for adults with normal function of the parathyroid gland. According to the Joint Expert Commission of the BfL and BfArM (German Federal Institute for Drugs and Medical Devices) on Substance Classification, vitamin D intoxication cannot be caused solely by the consumption of common foods, including those foods irradiated with UV light (see also section 3.3.2) (BfL/BfArM 2017).

In relation to the question stated above, there are no indications from a toxicological perspective that the intake of ergosterol or vitamin D<sub>2</sub> from foods - and fruit or fruit products in particular - could lead to adverse effects (such as to hypervitaminosis). The desirable blood serum level for the marker 25-(OH)D of 50 nmol/l in blood is not achieved by over half of the population (BfL/BfArM 2017). Furthermore, the conversion rate of ergosterol into vitamin D<sub>2</sub> and then to 25-(OH)D is also dependent on a wide range of factors (UV spectrum, irradiation dose, bioavailability, etc.).

### 3.3.2 Ergosterol in food

Based on available data, it is not possible to evaluate the extent to which elevated concentrations of ergosterol in food resulting from fungal contamination affect the endogenous vitamin D status. The intake of vitamin D<sub>2</sub> from food, such as chocolate products, for example, is potentially higher than assumed as a result of fungal contamination (Kühn *et al.*, 2018) which should therefore be taken into account in the exposure assessment of vitamin D.

Furthermore, novel foods that exhibit elevated concentrations of vitamin D<sub>2</sub> as a result of irradiation by UV light have been approved by the European Commission in recent years.

These include UV-treated baker's yeast, UV-treated bread and UV-treated fungi (EU Commission, 2017). The technical process of UV irradiation can result in vitamin D2 concentrations of up to 700 µg per 100 g of food (Urbain *et al.*, 2011). However, the Commission has set maximum levels for vitamin D2 in the final product. EFSA assumes that even if maximum levels and maximum usable quantity are reached the UL for vitamin D would nonetheless not be exceeded in adults or children. No toxicological studies are available for the UV-treated food mentioned above. However, there are no indications that the intake of food irradiated with UV light in compliance with EU legislation raises health concerns (EFSA, 2014; 2015).

### 3.3.3 Relationship between ergosterol and mycotoxins

Some mould genera (e.g. *Fusarium*, *Penicillium* and *Aspergillus*) include toxin-forming moulds that produce mycotoxins as secondary metabolites. The contamination of food with these substances can have severe health consequences for the consumer.

Like the formation of ergosterol, the biosynthesis of these toxins is influenced by a series of exogenous factors (e.g. temperature, water activity, pH value) and varies by strain and toxin. Depending on temperature, *Aspergillus flavus* produces either aflatoxin (30 °C) or cyclopiazonic acid (25 °C), for example (Gqaleni *et al.*, 1997).

Therefore, contamination with moulds can also lead to a contamination with mycotoxins. This is the reason for the discussions about whether the detection of ergosterol in food could not only be used as a hygiene parameter but also as a marker for mycotoxin contamination (Kadalkal & Tepe, 2019). Accordingly, several studies investigated a potential correlation between ergosterol concentrations and the formation of mycotoxins in various foods (including Pietri *et al.*, 2004; Karaca & Nas, 2006; Ekinici *et al.*, 2014; Yassihuyuk *et al.*, 2014). However, the data currently available are not sufficient to enable an evaluation of whether ergosterol content could be utilised as a suitable marker for food contamination - as is the case with fruit juices and mycotoxins, for example - or as a marker for the general quality of foods other than tomato products (Kadalkal & Tepe, 2019).

In 2018, the *European Fruit Juice Association* (AIJN) was the first body to set a maximum ergosterol concentration of 0.76 mg/l as a quality parameter for tomato juice in its *Code of Practice*. According to information provided by *Schutzgemeinschaft Fruchtsaftindustrie e.V.* (Association for the protection of the interests of the Fruit Juice Industry - SGF), this value is derived from the applied maximum level set for ergosterol in tomato puree in Italy.

### 3.3.4 Results of discussions held by the BfR Committee for Wine and Fruit Juice Analyses on ergosterol as a quality parameter for wine and fruit juices.

Sessions held by the BfR Committee for Wine and Fruit Juice Analyses (Wufak) in 2014 and 2015 included discussions among Committee members about the use of ergosterol as a quality parameter for fruit juices. In particular, industry representatives reported on the debate and subsequent introduction of ergosterol content as a quality parameter for tomato juice in the *Code of Practice* issued by the *European Fruit Juice Association* (AIJN). As men-

tioned above, the *European Fruit Juice Association* (AIJN) was the first body to establish a maximum ergosterol concentration of 0.76 mg/l as a quality parameter for tomato juice in its *Code of Practice* in 2018.

For other products such as grape juice, various parameters (gluconic acid, glycerol and ergosterol) were discussed as potential markers for mycotoxin contamination. In terms of winemaking, the use of noble rotten grapes for certain selections was noted, whereby a deliberate infection with certain moulds such as *Botrytis cinerea* would lead to an expected higher concentration of ergosterol in the final product.

However, the members of Wufak had only limited data and findings as a basis for discussion, thus the suitability of ergosterol content as a marker for the contamination of fruit juices with mycotoxins, or as a general quality marker, could not be conclusively evaluated.

#### Further information on the topic of vitamins from the BfR website

A-Z index of vitamins:

[https://www.bfr.bund.de/en/a-z\\_index/vitamins-130216.html](https://www.bfr.bund.de/en/a-z_index/vitamins-130216.html)



BfR "Opinions app"

## 4. References

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### About the BfR

The German Federal Institute for Risk Assessment (BfR) is a scientifically independent institution within the portfolio of the Federal Ministry of Food and Agriculture (BMEL) in Germany. It advises the German federal government and German federal states ("Laender") on questions of food, chemical and product safety. The BfR conducts its own research on topics that are closely linked to its assessment tasks.

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