Comparative Abundance of Furan-2(5H)-one in Smoked Sausages Processed by Conventional Smoking and Smoke Flavouring

A Pilot Study of Two Industrial Smoking Technologies

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Jämförande förekomst av furan-2(5H)-one i rökta korvar framställda genom traditionell rökning och rökning med rökarom

En pilotstudie av två industriella röktekniker

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Abstract

Furan-2(5H)-one has recently attracted regulatory attention within the European Union due to concerns regarding genotoxicity. This pilot study compared the relative abundance of furan-2(5H)-one in sausages processed by conventional smoking and smoke flavouring. Three sausage groups were produced: conventionally smoked sausages, smoke flavouring-treated sausages, and a non-smoked control. Analysis was performed using headspace GC–MS. Furan-2(5H)-one was detected in both smoked treatments but not in the control group. The smoke flavouring treatment showed a substantially higher relative abundance than conventional smoking, and significant differences were observed between treatments ($p < 0.001$). The findings indicate that smoking technology may influence the abundance of furan-2(5H)-one in smoked foods and provide comparative data to an area where information remains limited.

Keywords: Furan-2(5H)-one, Liquid Smoke, Conventional Smoking, Sausage, GC-MS

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Abbreviations

Abbreviation	Description
EFSA	European Food Safety Agency
JECFA	Joint FAO/WHO Expert Committee on Food Additives
PAH	Polycyclic Aromatic Hydrocarbons
RISE	Research Institutes of Sweden AB
SFPP	Smoke flavouring primary products
SLU	Swedish University of Agricultural Sciences
TTC	Threshold of Toxicological Concern
UVCBs	Substances of unknown variable composition, complex reaction products or biological materials
WOE	Wight of evidence

1. Introduction

1.1 The smoking process

Preservation

The primary function of the smoking process has traditionally been the preservation of food. Alongside salting and drying, it represents one of the earliest methods employed for preserving meat and other food products. Archaeological evidence indicates that smoke was already used in food preparation during the Middle Stone Age. (Lazlo & Karl 1984).

Exposure of products to smoke results in a reduction in moisture content and leads to the enrichment of various smoke-derived compounds (Nizio et al. 2023). Consequently, the shelf life of smoked products is significantly extended. During the combustion of wood, compounds such as formaldehyde and phenolic substances are generated, contributing to the preservative effect of smoking. These constituents inhibit the growth of numerous microorganisms and suppress oxidative reactions. Experimental systems designed to replicate smoke composition have demonstrated antimicrobial, antioxidant, and antifungal activities. (Wendorff et al. 1993; Niedziela et al. 1998; Suñen et al. 2001; Bortolomeazzi et al. 2007). The shelf life of smoked products may also be influenced by several other factors, including conditions for smoke generation, storage temperature, and salt concentration.

Sensory attributes

In contemporary applications, however, smoking is mainly valued for enhancing sensory attributes and inducing structural changes within the product matrix. (Nizio et al. 2023). Smoking enhances the visual appearance of meat through browning reactions between amino acids in the muscle tissue and carbonyl compounds generated during the thermal degradation of wood carbohydrates. In addition, it provides a characteristic smoky aroma and flavour (Lazlo & Karl 1984).

1.2 Smoking techniques

1.2.1 Smoking temperatures

In terms of temperature, two principal types of smoking are mostly discussed: cold smoking and hot smoking. Hot smoking may be further subdivided into warm smoking and high-temperature hot smoking. (Nizio et al. 2023).

Cold smoking

Cold smoking is a long-term process, typically lasting from 1 to 14 days, performed at temperatures ranging between 15 and 25 °C and at a relative humidity of approximately 95% (Šimko 2005). Under these conditions, neither cooking nor protein coagulation occurs. Although most cold-smoked products require further thermal treatment prior to consumption, cold-smoked salmon is typically consumed without additional heat processing (Bannerman 1980). Due to cold smoking is performed at temperatures insufficient to inactivate pathogenic microorganisms, the process may permit bacterial survival and potential growth unless additional hurdles are applied, such as salting, drying, and refrigeration.

Warm smoking

Warm smoking is conducted at temperatures ranging from 25 to 50 °C and at a relative humidity of approximately 80%, with processing times varying from 4 hours to 2 days. This method results in partial denaturation of the raw material through heating and drying of the outer layer, while the interior largely retains the characteristics of the fresh product. This temperature range (25–50 °C) is favourable for bacterial growth and may therefore pose a microbiological risk. Consequently, strict control measures are required in food processing. However, these relatively low temperatures are associated with a reduced formation and uptake of harmful smoke-derived compounds (Yang & Chiang 2019).

Hot smoking

During hot smoking, the process preformed at higher temperatures, with smoke temperatures reaching approximately 70–80 °C. Under these conditions, the product undergoes cooking, and consequently no additional thermal treatment is required after smoking (Bannerman 1980). Several factors may influence the effectiveness of the hot smoking process. The most important include raw material handling, processing temperature, smoke characteristics, and the duration of smoking (Goulas & Kontominas 2005). Hot smoking results in minimal product weight loss and reduces processing time; however, due to the higher temperature, it may increase the uptake of harmful smoke-derived compounds.

1.2.2 Transfer of smoke constituents

During processing, temperature, humidity and smoke flow rate strongly influence the efficiency of smoke component sorption. During smoking, the smoke constituents settle on the product, driven by centrifugal and gravitational forces, and are absorbed in the thin water layer on the surface of the product. The rate of deposition of various constituents depends on whether they are in the dispersing or dispersed phase of the aerosol at the given temperature and on the condition of the surface layer. These factors govern the uptake of smoke constituents during both conventional smoking and the application of smoke flavourings (Stołyhwo & Sikorski 2005a; Sinkiewicz & Sikorski 2024).

1.2.3 Conventional smoking

Smoke generation

Smoke is traditionally produced by the smouldering of wood, wood chips, or other types of biomass. The process is regulated through a controlled and limited airflow. As a result, a consistent smoke concentration can be achieved, thereby reducing the risk of temperature spikes and fluctuations in smoke intensity. During the process, a sufficient volume of smoke-generating material must cover the heat source to maintain a consistent smoke stream and prevent ignition. In many smokehouses, a time-regulated feeding system is implemented to ensure process stability and continuity (Hui 2012).

The smoking chamber

Figure 1 shows a schematic diagram of the smoking chamber. Smoke is generated in a separate smoke generator, where a heat source facilitates the smouldering of a suitable smoke material. The generated smoke is then conveyed to the smoking chamber, where the product is exposed to controlled smoking conditions (Hui 2012).

Depending on the desired processing temperature (e.g., cold, warm, or hot smoking), the smoke temperature can be regulated. This regulation is commonly achieved through control of combustion intensity, airflow, or by incorporating a cooling system between the smoking generator and the smoking chamber (Nizio et al. 2023).

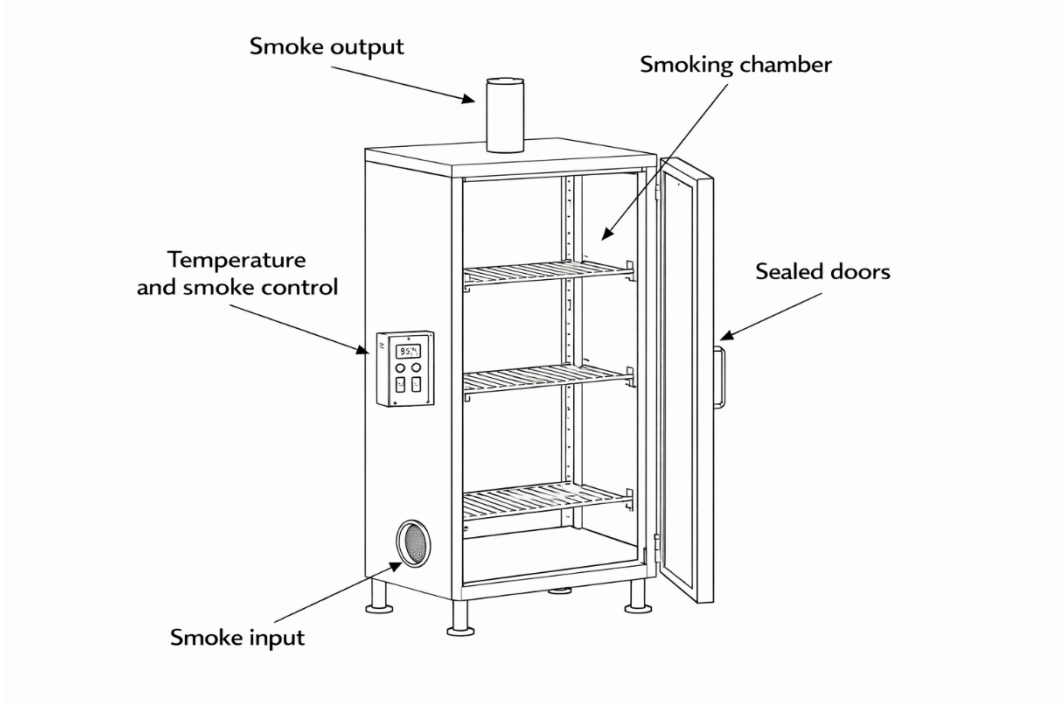


Figure 1: Principal diagram of a smoking house adopted from Nizio et al. (2023)

1.2.4 Smoke Flavourings

Liquid smoke, also referred to as smoke condensate, smoke flavourings, smoke aromas, or wood vinegar, is widely used in food processing as an alternative to conventional smoking (Tiilikkala et al. 2010). It can be applied directly to foods through mixing or injection, drenching, or used in smokehouses via spraying (atomisation) or reconstituted in water vapour during the cooking-phase. All these techniques enable controlled and consistent flavour development (Lingbeck et al. 2014).

Production of liquid smoke

Liquid smoke is defined as a purified condensate obtained from the controlled pyrolysis of biomass under limited oxygen conditions. It is produced on a large industrial scale and may be produced either directly through fast pyrolysis processes or as a by-product of charcoal production (Simon et al. 2005).

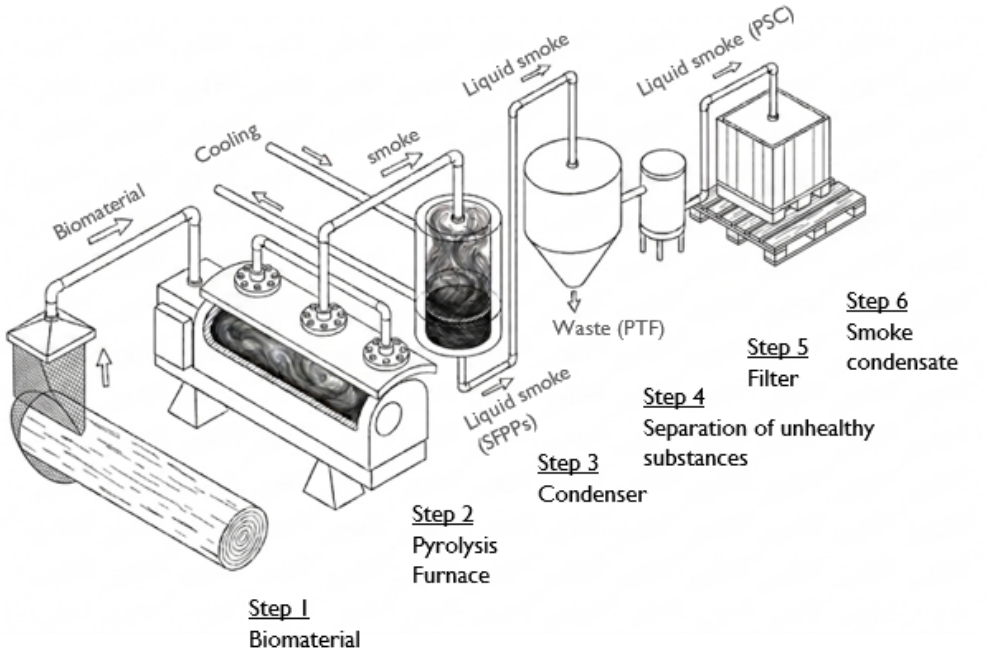


Figure 2: The figure illustrates the principle of the industrial liquid smoke production process. The process begins with a biomass (Step 1), which is heated in a pyrolysis furnace (Step 2). The generated smoke is then directed to a condenser (Step 3), where it is condensed (SFPPs). After condensation, harmful substances (PTF) are separated and removed (Step 4), after which the liquid is filtered (Step 5) to obtain the final smoke condensate (PSC+ fractions of PTF) (Step 6). Adopted from Kerry Group (2019)

The principle of the process is shown in Figure 2. The process begins with the smoke source (Step 1) and proceeds with heating in a reactor under controlled conditions (Step 2). Smoke gases are generated, which contain various volatile compounds (Xin et al. 2021). These gases are subsequently conveyed to a condenser, where they are cooled and liquefied (Step 3). The resulting dark brown liquid is then transferred to storage tanks to allow phase separation into an aqueous phase, known as the Primary Smoke Condensate (PSC), and an oil phase, referred to as the Primary Tar Fraction (PTF) (step 4). Under EU legislation, the PSC and PTF are legally referred to as Smoke Flavouring Primary Products (SFPPs) (Hui 2012; Lingbeck et al. 2014). The aqueous phase (PSC) forms the crude liquid smoke product, while the oil phase (PTF) contains accumulations of phenolic polymers and polycyclic aromatic hydrocarbons (PAHs), making it unsuitable for use as a food additive (Guillén et al. 2000). Different methods may be used to separate the PSC and the PTF (Hui 2012) and to further recover desirable compounds for taste and aromas from the PTF (Underwood & Graham 1989; Šimko 2005). The liquid smoke is then filtered and purified (Step 5) (Lingbeck et al. 2014) and the final smoke condensate is obtained, including PSC and fractions of PTF.

1.3 Composition of smoke

1.3.1 Raw Materials for Smoke Generation

Various materials are used in the food smoking processes. In the whisky industry, peat is burned during malt kilning to impart characteristic phenolic flavours (Da Porto et al. 2006). Regionally, materials such as twigs of juniper, spruce branches and heather may also be employed; however, their use is generally confined to traditional practices (Larsson 1982). Recently, rice hulls and coconut shells have also been employed (Wang et al. 2017).

Types of wood

In contemporary smoking of foods, hardwood, derived from deciduous species, remains the principal material for smoke generation. The tree species most commonly utilised include alder, beech, ash, maple, acacia, oak, and various fruit trees, such as cherry, apple, and walnut. They are typically applied in the form of wood chips or sawdust. In contrast, coniferous wood is generally unsuitable due to its high resin content, which may generate carcinogenic compounds during combustion. Different species of wood produce distinct chemical compounds; these differences influence both the sensory and physicochemical characteristics of the final product (Nizio et al. 2023).

Hardwood

Hardwood possesses a more favourable hemicellulose composition relative to other structural components, thereby contributing to improved smoke quality and overall smoking performance (Stumpe-Vīksna et al. 2008). The physical properties of the wood, particularly its hardness, are likewise critical. Owing to its greater density, hardwood combusts more slowly than softwood, resulting in a reduced rate of oxidation of the volatile compounds generated. Increasing wood density enhances the efficiency of forming smoke constituents relevant to the smoking process (Nizio et al. 2023).

Parameters for smouldering of wood

The smouldering of the wood is dependent on parameters such as the moisture content of the wood used (Tóth & Potthast 1984; Maga 1988), the temperature attained during combustion, and the oxygen concentration within the combustion chamber. (García-Falcón & Simal-Gándara 2005; Nizio et al. 2023).

1.3.2 Physical composition of smoke

Physically, smoke is a complex polydisperse aerosol composed of fine liquid and solid particles (0.08–0.15 μm in diameter) dispersed within a gaseous phase consisting primarily of air, carbon monoxide, carbon dioxide, water vapour, and other gaseous compounds (Šimko 2005; Sinkiewicz & Sikorski 2024). During smoking, more than 400 individual compounds have been reported to be deposited onto the product. The composition of smoke is influenced by several interacting process factors (Cardinal et al. 2006).

1.3.3 Chemical composition of smoke

Pyrolysis

Smoke may be generated through the thermal decomposition and partial oxidation of wood under oxygen-restricted conditions, a process referred to as pyrolysis (Simon et al. 2005). This process can be characterised as a series of thermochemical reactions, beginning with the primary pyrolytic degradation of structural lignocellulosic components, followed by multiple secondary transformations of the initial pyrolysis products. Wood is composed predominantly of cellulose (approximately 40–50%), hemicellulose (20–30%), and lignin (20–30%), and the thermal decomposition of these components generates a complex mixture of volatile compounds. These primary products subsequently undergo further reactions, leading to the formation of compounds such as furans, phenolic derivatives, and polycyclic aromatic hydrocarbons.(Tóth & Potthast 1984).

Temperature during pyrolysis

Temperature is one of the key parameters influencing the composition of smoke (Guillén & Ibargoitia 1996). This can be attributed to the fact that individual pyrolytic processes occur within distinct temperature ranges. Endothermic processes, such as water evaporation, occur at approximately 120–150 °C, while the exothermic degradation of hemicellulose (200–250 °C), cellulose (280–320 °C), and lignin (around 400 °C) takes place at progressively higher temperatures. As a result, different types of compounds are released at different temperatures (Reyes et al. 2021).

Pyrolysis of cellulose

The thermal degradation of cellulose appears to proceed via an initial acid-catalysed hydrolytic cleavage to glucose, followed by dehydration to form 1,6-anhydroglucose (β-glucosan). This is followed by a fragmentation step that produces acetic acid and related compounds, along with water and, in some cases, minor amounts of furans and phenolic compounds. Cellulose further degrades to

form a range of low-molecular-weight oxygenated compounds, including formic and acetic acid, acetaldehyde, hydroxy acetaldehyde, furfural, furanones, and anhydroglucopyranoses such as levoglucosan. Small quantities of methanol and formaldehyde may also be generated, although these are more commonly associated with lignin degradation (Mohan et al. 2006; Wang et al. 2017).

Pyrolysis of hemicellulose

The polymeric structure of hemicellulose varies according to its botanical origin. Hardwoods (deciduous) are primarily rich in xylan-based pentosans, whereas softwoods (conifers) contain higher proportions of glucomannans and other hexosans. Pentosan-rich hemicellulose readily degrades during pyrolysis to form furans (e.g. furfural) and aliphatic carboxylic acids, in contrast, hexosan components derived from hemicellulose in softwoods undergo degradation pathways that partly resemble those of cellulose. Hemicellulose is the least thermally stable of the major wood components and decomposes at lower temperatures than cellulose and lignin (Mohan et al. 2006; Wang et al. 2017).

Pyrolysis of lignin

The pyrolysis of lignin generates compounds that are regarded as key contributors to smoke flavour, particularly phenolic derivatives such as phenolic ethers, including guaiacol and syringol, together with their homologues and related compounds. The structural composition of lignin varies between hardwood and softwood species, reflecting differences in their respective monolignol units (Mohan et al. 2006; Wang et al. 2017).

Main groups of compounds in smoke

The major groups of compounds present in smoke include phenols, carbonyl compounds, organic acids, furans, alcohols, and hydrocarbons. Phenolic compounds are primarily responsible for the characteristic smoky flavour and also contribute to antioxidant and antimicrobial properties. Carbonyl compounds, such as aldehydes and ketones, play an important role in colour formation and flavour development. Organic acids, particularly acetic acid, contribute to the mild sour note of smoke and enhance its preservative effect by lowering the pH. Furans and furan derivatives provide sweet, caramel-like, and fruity flavour notes. In addition, smoke also contains tar, a condensed fraction that includes hydrocarbons and polycyclic aromatic hydrocarbons (PAHs), some of which are considered undesirable due to their potential carcinogenic properties (Maga 1988).

1.3.4 Harmful Compounds

Polycyclic Aromatic Hydrocarbons (PAHs)

An important aspect of smoking technology concerns the formation of harmful compounds. One such group is polycyclic aromatic hydrocarbons (PAHs), and their presence in the finished products may have implications for human health (Kafouris et al. 2020). PAHs are generated during the incomplete combustion of wood, and their formation is strongly influenced by process parameters such as temperature, oxygen availability, and fuel characteristics (Stołyhwo & Sikorski 2005b; Racovita et al. 2020).

To minimise PAH formation, key variables such as wood moisture content, air supply, and combustion temperature must be carefully controlled. Increasing the moisture content of the wood can reduce PAH formation by lowering combustion temperature. Additionally, the smoking method plays a crucial role, as direct smoking, where food is exposed directly to combustion gases, generally results in higher PAH levels compared to indirect smoking, in which the smoke is generated separately and cooled before contacting the product (Maga 1988).

Furans

Another group of compounds raising concern for human health in smoke products are furans. These compounds are formed from both hemicellulose and cellulose through secondary degradation pathways, although hemicellulose-derived pentoses are considered the primary precursors under typical smoking conditions (Montazeri et al. 2013; Snehanath et al. 2026). Additional furans may also be generated via Maillard reactions (Seok et al. 2015; Batool et al. 2023; Snehanath et al. 2026)

Due to their potential health implications, furans have received increasing scientific and regulatory attention. Despite advances in analytical methods, challenges remain in accurately determining furan levels across different matrices, contributing to uncertainties in exposure assessment and risk evaluation (Batool et al. 2023).

Furans have also gained increasing attention within the European Union due to their evaluation by the European Food Safety Agency (EFSA) in relation to potential health risks. One substance in particular that has been under scrutiny is furan-2(5H)-one [CAS 497-23-4]. Also referred to as butenolide or γ-crotonolactone, which, according to EFSA, raises safety concerns and is considered genotoxic in vivo (EFSA 2019b). Therefore, on the 17 May 2019, in order to protect human health, according to regulation (EU) 2019/799 furan-

2(5H)-one was removed from the list of approved flavourings found in Regulation (EC) No 1334/2008 (European Commission 2019).

2. Background

2.1 Legal background for smoked products within the European Union.

2.1.1 Legal background to conventional smoked products

All foods placed on the market within the European Union must be safe and are regulated under Regulation (EC) No 178/2002. Furthermore, the production of smoked foods and hygiene requirements in the smoking process are governed by Regulations (EC) No 852/2004 and (EC) No 853/2004. Within the European Union, maximum levels for polycyclic aromatic hydrocarbons (PAHs) are strictly regulated under Regulation (EU) 2023/915. However, certain conventionally smoked products are permitted higher concentrations under specific derogations, due to the impact that modifying conventional smoking practices would have on product characteristics.

In the EU, under Regulation (EU) 2026/1890) maximum levels have been established for the sum of furan, 2-methylfuran, and 3-methylfuran in processed cereal-based foods for infants and young children and in baby food. However, these maximum levels do not apply to smoked meat products, and no specific maximum level is established for furan-2(5H)-one under this regulation (European Commission 2026). Currently there is an ongoing process within the European Commission to develop a list of contaminants to be monitored in conventionally smoked foods (European Commission 2024k; 2025b)

2.1.2 Legal background to smoke aromas

In 1991, the European Commission concluded that smoke flavourings used in foods should undergo formal toxicological evaluation; this area was subsequently regulated 2003 under Regulation (EC) No 2065/2003. Within this regulation, the first submission of Smoke Flavouring Primary Products (SFPPs) for safety assessment by the European Food Safety Agency (EFSA) was established (European Commission 2003). Between 2005 and 2012, ten SFPPs were evaluated for potential safety concerns and later authorised under the Regulation (EU) No 1321/2013 for use in foods within the European Union (European Commission 2013).

EFSA's updated protocol for assessing genotoxicity

In 2021, EFSA issued updated scientific guidance for the submission of applications on Smoke Flavouring Primary Products (SFPPs) (EFSA 2021). Compared to the previous guidance (EFSA 2005), the new guidance included new requirements in terms of analytical characterisation and different methods for the assessment of exposure.

Smoke Flavouring Primary Products (SFPPs) are regarded as complex chemical mixtures or UVCBs (substances of unknown or variable composition, complex reaction products, or biological materials), and are therefore considered by EFSA to contain a substantial unidentified fraction (EFSA 2021). Due to this classification, these flavourings are subject to both whole-mixture and component-based assessments during the evaluation of genotoxicity (EFSA 2019a; 2021). More specifically, EFSA's updated protocol for assessing the genotoxicity (EFSA 2019a) of individual constituents includes comparison of estimated dietary exposure levels to the Threshold of Toxicological Concern (TTC) for DNA-reactive substances. Exceedance of this threshold indicates a potential safety concern with genotoxicity. The TTC is set at 0.0025 μg/kg-day (EFSA 2019a; 2021), and if the estimated exposure of a constituent exceeds this limit, EFSA considers that a concern for genotoxicity cannot be excluded. In such cases, the assessment is not progressed further, as genotoxicity must be ruled out before a full safety evaluation can be completed (EFSA 2021).

Removal of furan-2(5H)-one from approved flavourings in Regulation (EC) No 1334/2008

In EFSA evaluations, flavouring substances are grouped into subgroups based on structural similarity to enable group-based risk assessment. However, certain compounds, such as furan-2(5H)-one, may be assessed as stand-alone substances when their chemical reactivity or toxicological profile deviates from the subgroup, meaning their evaluation does not influence other structurally related compounds (EFSA 2019b). The substance furan-2(5H)-one has in particular been under scrutiny. According to EFSA, the substance raises safety concerns and is considered genotoxic in vivo (EFSA 2019b). Therefore, on the 17 May 2019 according to regulation (EU) 2019/799, the substance furan-2(5H)-one was removed from the list of approved flavourings found in Regulation (EC) No 1334/2008, in order to protect human health (European Commission 2019).

Denied re-authorisation of smoke flavouring primary products

The removal of furan-2(5H)-one from the list of approved flavouring substances (European Commission 2019) influenced the re-authorisation of all authorised commercial smoke flavourings (European Commission 2024a; b; c; d; e; f; g; h).

Required by Regulation (EU) No 1321/201, authorised SFPPs have to be re-evaluated every 10 years by EFSA. In 2022, eight SFPPs were re-submitted; none were re-authorised for use in the EU under Regulation (EU) No 2024/2067 due to concerns about genotoxicity (European Commission 2024a; b; c; d; e; f; g; h).

One of the rejected flavourings was Scan Smoke PB 1110 (SF-001) renamed in the application as 'ProFagus Smoke R714 (European Commission 2024c). The potential concern for genotoxicity was raised due to the presence of the substance furan-2(5H)-on (European Commission 2024c).

2.1.3 Discussion within the European Commission regarding furan-2(5H)-on as a contaminant in smoked foods.

During discussions among Member States on 24 April 2024 regarding the proposed rejection of eight authorised smoke flavouring primary products, several Member States requested a monitoring recommendation for the presence of furan-2(5H)-one and other substances in smoked meat, cheese and fish products. Furthermore, EFSA was to be given a mandate to perform a comparative risk assessment of smoking processes used to impart flavour to food (European Commission 2024k). On 19 September 2025, the Commission announced that EFSA is evaluating a list of contaminants to be monitored in conventionally smoked foods, with furan-2(5H)-one among the substances under consideration (European Commission 2025b).

To date, no EFSA scientific opinion or report confirming the completion of such an assessment has been published, nor has a draft list of contaminants to be monitored in conventionally smoked foods been presented.

In light of the ongoing discussions within the European Union regarding furan-2(5H)-one as a potential process contaminant in smoked foods, there is a need to investigate how different smoking processes influence the levels of this compound. As commercial smoke flavourings have been denied re-authorisation due to genotoxicity concerns related to furan-2(5H)-one, while assessments of conventionally smoked products are still lacking, a comparative study of the two

processes may contribute to the current scientific understanding of furan-2(5H)-one abundance in smoked foods.

3. Aim and purpose

3.1 Aim

The aim of this pilot study was to compare the relative abundance of furan-2(5H)-one in sausages processed by conventional smoking and by smoke-flavouring treatment.

3.2 Purpose

The purpose was to contribute comparative data to the ongoing discussion on smoke-derived contaminants and the safety assessment of different smoking processes.

4. Materials and Method

4.1 Materials

4.1.1 Procurement and handling

Wood chips

15 kilograms of wood chips consisting entirely of beech wood (moisture content 10 ± 2%), classified as class 8 (6–8 mm), were obtained from AWPOL, 33-122 Wierzchosławice, Poland. The wood chips were stored in the dark at room temperature until use.

Smoke Flavouring

Two 10 L containers of the full-strength smoke flavouring ProFagus Smoke R714 (batch no. 205012926; SF-001), produced from a wood mixture comprising 90% beech and 10% oak. It was obtained from ProFagus (Uslarer Straße 30, 37194 Bodenfelde, Germany). The smoke flavouring was stored in the dark at low temperature (4°C) until use.

Sausages preparation

The sausages were produced by Scan Sverige AB under the product name *Scan varmkorv* (Art. No. 608777; GTIN: 7300208777005). The product declaration stated the following ingredients: pork and beef (62%), water, pork and beef fat, potato flour, salt, onion, spices, spice extracts, antioxidant (E300), preservative (E250), collagen casing. The collagen casings used were supplied by Viscofan (models NDX cal. 17 and L035, transparent colour). The declared nutritional values per 100 g are presented in Table 1.

Table 1. Declared nutritional values of the sausages per 100 g.

Nutrient	Amount per 100 g
Fat	26
Saturated fat	7.5
Carbohydrates	5
Sugars	<0.5
Protien	10
Salt	2

4.2 Method

4.2.1 Sausage preparation

All sausages were manufactured according to standard industrial processing procedures using a Protech 6 batch-smoking system with separate heating and cooling, an A220 wood-chip smoke generator, and an AEROSOL+ EVO liquid smoke generator. Three sausage groups, each weighing 3.6 kg, were produced: conventionally smoked sausages, sausages treated with smoke flavouring reconstituted in water vapour, and a heat-treated control group without smoke exposure.

The group subjected to conventional smoking was initially pre-cooked at 50 °C for 5 min, followed by drying for 15 min. Subsequently, approximately 6 kg of wood chips was applied during a 45 min smoking process at a smokehouse temperature of 64.92 °C. Thereafter, the sausages were steam-cooked for 17 min and a core temperature of 78.08 °C was reached.

The group treated with smoke flavouring was pre-cooked and dried as previously described. Followed by simultaneous steam-cooking and smoke flavouring application using 3.56 kg of smoke flavouring reconstituted in 32.04 kg of water and vaporised prior to application. The treatment was carried out for 17 min until a core temperature of 78.04 °C was achieved.

The untreated control group was processed according to the same procedure as the smoke flavouring group (core temperature of 78,21 °C), with the exception that no smoke processing was performed.

Immediately after processing, all sausages were subjected to an additional drying step for 2 min and subsequently placed under refrigerated storage at 2 °C. After 12 h, the sausages were separately packaged according to processing method under modified atmosphere conditions, with 16 sausages per package. The packaged sausages were subsequently stored at 4 °C 28 days prior to analysis.

4.2.2 Analysis

The analytical method was developed and performed by Research Institutes of Sweden AB (RISE; organisation no. 556464-6874).

Sample preparation

Sausages were cut into 5 g cylindrical samples and subsequently sectioned lengthwise into six triangular-shaped segments. Each 5 g sample was placed in a

20 mL glass vial. Volatile compounds were extracted from the sample headspace using HiSorb™ high-capacity sorptive extraction probes coated with a PDMS/CWR/DVB sorbent phase (polydimethylsiloxane/carbon wide range/divinylbenzene). Headspace extraction was performed at 40 °C for 60 min.

Following extraction, the analytes adsorbed onto the HiSorb™ probes were thermally desorbed using a Centri® thermal desorption system (Markes International, Llantrisant, UK), focused on a cold trap, and subsequently transferred to the gas chromatograph. Sample handling and transfer of the HiSorb™ probes were performed using a PAL RSI robotic autosampler (CTC Analytics, Zwingen, Switzerland). Five biological replicates were analysed for each treatment group.

GC-MS

Chromatographic separation was performed using an Agilent 8890 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled to an Agilent 5977B single quadrupole mass spectrometer and equipped with a DB-5 capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The oven temperature was initially held at 30 °C for 5 min, increased to 50 °C at 4 °C min^{-1} and held for 5 min, then increased to 70 °C at 4 °C min^{-1}, followed by a final ramp to 230 °C at 10 °C min^{-1} and held for 5 min.

Identification of furan-2(5H)-one was confirmed by comparison with an authentic reference standard. Relative abundance was determined by integration of the peak area in the extracted ion chromatogram (m/z 84). Peak areas were expressed in area units (a.u.).

4.2.3 Statistical analysis

Mean values and standard deviations (SD) were calculated, and differences between treatments were tested by one-way analysis of variance (ANOVA) at a significance level of $p < 0.05$. Boxplots were created to show the data distribution. All analyses were carried out in Microsoft® Excel® for Microsoft 365 MSO (Version 2604 Build 16.0.19929.20172: Microsoft Corporation, Santa Rosa, CA) 64-bit.

5. Results

5.1. GC-MS

Representative extracted ion chromatograms (m/z 84) are shown in Figure 3. A peak corresponding to furan-2(5H)-one was observed in both smoked treatments, whereas no corresponding peak was detected in the non-smoked control sample.

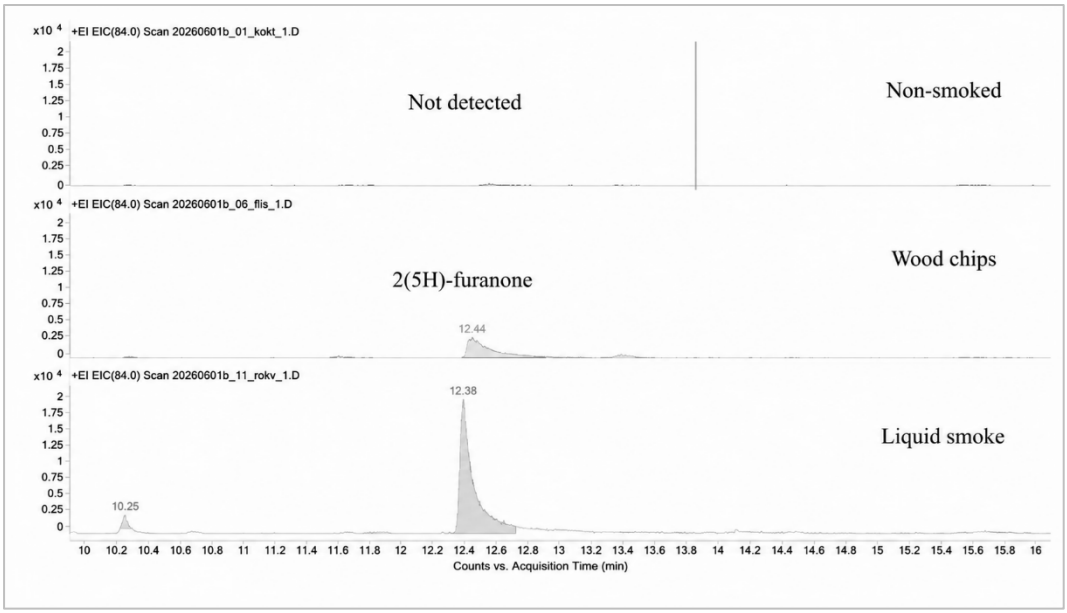


Figure 3. Extracted ion chromatograms (m/z 84) obtained by GC–MS analysis of non-smoked, wood chip-smoked and liquid smoke-treated sausages. A peak corresponding to furan-2(5H)-one was detected in both smoked treatments but not in the non-smoked control sample.

5.2. Statistical analysis

One-way ANOVA, mean values ± standard deviation (SD)

Statistical analysis of the GC–MS data revealed significant differences among the treatment groups (Table 2). One-way ANOVA showed highly significant differences in the relative abundance of furan-2(5H)-one between treatments ($F = 2005$, $p < 0.001$). The mean relative abundances (± SD) for sausages smoked with wood chips (23 450 ± 1 893) were substantially lower than those for sausages treated with liquid smoke (120 778 ± 4578), corresponding to an approximately five-fold higher relative abundance in the liquid smoke treatment. Furan-2(5H)-one was not detected (n.d) in the non-smoked control group.

Table 2. Relative abundance of furan-2(5H)-one expressed as GC–MS peak area (m/z 84) in sausages treated with wood chips, liquid smoke, and no smoke treatment. Values are presented as mean ± SD (n = 5).

Groups	*n*	*Mean*	*SD*
Wood Chips	5	23 450	1893
Liquid smoke	5	120 778	4579
non-smoked	5	n.d	-

ANOVA	
F	2005
p-value	< 0.001

Boxplot

Figure 4 illustrates the distribution of the relative abundance of furan-2(5H)-one within each treatment group. The boxplots show a clear separation between treatments and limited variation within groups.

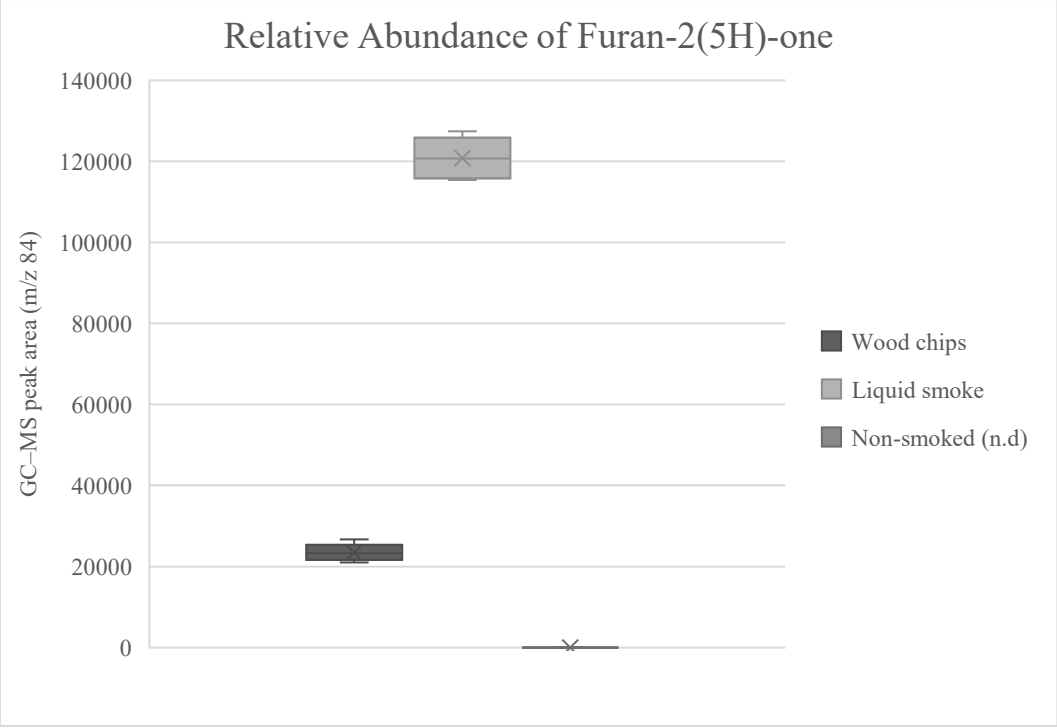


Figure 4. Boxplot of relative abundance of furan-2(5H)-one expressed as GC–MS peak area (m/z 84) in sausages treated with wood chips, liquid smoke, and no smoke treatment. Boxplots are based on five replicates per treatment (n = 5).

6. Discussion

The aim of this pilot study was to compare the relative abundance of furan-2(5H)-one in sausages processed by conventional smoking and by smoke-flavouring treatment.

6.1 Factors influencing furan-2(5H)-one in the different smoking treatments

The results suggest a difference between the smoking treatments, as sausages treated with smoke flavouring exhibited a higher relative abundance of furan-2(5H)-one than conventionally smoked sausages. Since smoke flavourings are produced from condensed wood smoke, compounds such as furan-2(5H)-one may be expected to occur in both conventionally smoked foods and foods treated with smoke flavourings. The absence of furan-2(5H)-one in the control group suggests that its abundance was associated with smoke exposure rather than the sausage formulation or heat treatment alone.

Furthermore, the application method may have contributed to the observed differences in furan-2(5H)-one abundance. Humidity, smoke composition and smoke flow influence the sorption of smoke constituents by food products (Šimko 2005; Sinkiewicz & Sikorski 2024). As the smoke flavouring was applied in water vapour during steam cooking, the transfer of water-soluble and volatile smoke-derived compounds may have been enhanced compared with conventional smoking, which relies on deposition from a comparatively drier smoke aerosol.

In addition, the higher relative abundance may reflect the production process of smoke flavourings, which can concentrate certain smoke-derived compounds compared with conventional smoking. However, as the initial concentrations of furan-2(5H)-one in the smoke flavouring and the generated smoke were not measured, the extent to which source concentrations contributed to the observed differences remains uncertain.

6.2 Methodological considerations and future research

Several methodological aspects should be considered when interpreting the results. Although the observed differences between treatments were pronounced, the findings should be considered within the scope of a pilot study and require confirmation in larger studies including multiple production batches to strengthen their generalisability. Furthermore, the study quantified relative abundance based on GC–MS peak areas rather than absolute concentrations. Consequently, the

results allow comparison between treatments but do not permit estimation of actual dietary exposure or direct comparison with toxicological thresholds. Finally, only one smoke flavouring, one conventional smoking setup and one food matrix were investigated. The findings should therefore not be regarded as representative of all smoke flavourings or conventional smoking processes.

Given the current regulatory interest in furan-2(5H)-one, these findings contribute to a field where comparative data between conventional smoking and smoke flavouring appear to be limited. This knowledge gap has also been recognised in recent discussions within the European Commission, where Member States requested comparative risk assessments between conventionally smoked foods and foods treated with smoke flavourings (European Commission 2024i; j; k; 2025a; b; c; u.å.). Although the toxicological significance of furan-2(5H)-one in smoked foods remains under evaluation, the observed differences between smoking processes may be relevant for future investigations of smoke-derived contaminants in food. Future research could include absolute quantification, a wider range of smoke flavourings and conventional smoking conditions.

7. Conclusion

The present pilot study suggests that smoking technology may influence the abundance of furan-2(5H)-one in smoked sausages. A substantially higher relative abundance was observed in sausages treated with the tested smoke flavouring than in conventionally smoked sausages, while the compound was not detected in the non-smoked control group. These findings indicate the need for further studies involving additional smoke flavourings, conventional smoking, food matrices and quantitative analyses.

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Populärvetenskaplig sammanfattning

Rökning har använts för att konservera och ge smak åt livsmedel i tusentals år. I dag används både traditionell rökning med trä och så kallade rökaromer, även kallade liquid smoke. Rökaromer framställs genom att röken från trä samlas upp, renas och används som ingrediens eller appliceras i rökskåp. Under senare år har flera rökaromer blivit föremål för diskussion inom EU efter att vissa ämnen i produkterna bedömts kunna innebära hälsorisker.

Ett av dessa ämnen är furan-2(5H)-one, som bildas när trä hettas upp och bryts ned under rökprocessen. Samtidigt är kunskapen begränsad om hur ämnet förekommer i traditionellt rökta livsmedel jämfört med livsmedel som behandlats med rökaromer.

Syftet med denna studie var därför att jämföra förekomsten av furan-2(5H)-one i varmkorv som röktes med bokflis och i motsvarande varmkorv som behandlades med rökarom producerad på bok. Som jämförelse användes även en korv som endast värmebehandlats utan rökning.

Resultaten visade att furan-2(5H)-one kunde påvisas i båda de rökta produkterna men inte i den orökta kontrollgruppen. Korvarna som behandlats med rökarom uppvisade dessutom högre nivåer av ämnet än de traditionellt rökta korvarna. Resultaten tyder därmed på att valet av rökmetod kan påverka förekomsten av furan-2(5H)-one i den färdiga produkten.

Studien var en pilotstudie med ett begränsat antal prover och resultaten bör därför bekräftas i större undersökningar. Trots detta bidrar arbetet med ny kunskap inom ett område där jämförande data fortfarande är begränsade och där ytterligare forskning efterfrågas av både forskare och myndigheter.

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