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RESEARCH ARTICLE

Colchicine as a food contaminant: rare occurrence but persistent in stored honey and during yogurt fermentation

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Abstract

Colchicine, a plant toxin with aneugenic and potentially genotoxic properties, is predominately derived from the autumn crocus (*Colchicum autumnale*). Although it has been used as a drug since ancient times, severe poisoning or even death may occur in humans and animals when colchicine is ingested in larger amounts. If consumed by food-producing animals, the transfer of colchicine to animal-based food products, as observed with other plant toxins, seems likely, posing a potential health risk to consumers. In the late flowering period of *C. autumnale*, honey bees may have limited alternatives and may collect its nectar and pollen, potentially transferring colchicine to honey. A literature research on the relevance of colchicine as food contaminant was conducted, followed by experiments to examine its stability in stored honey and milk fermented into yoghurt. The literature review revealed that colchicine is rarely detected as a food contaminant. However, it has been shown to transfer into milk of ruminants following the ingestion of *C. autumnale*. Experiments with artificially contaminated samples demonstrated, for the first time, the persistence of colchicine in honey stored for 4 weeks under dark conditions and during the fermentation of milk into yoghurt. Although the overall risk to consumers currently appears low, further research is needed to determine whether the continued spread of *C. autumnale* poses a higher risk to livestock and consumers than previously assumed.

Keywords Colchicine · *Colchicum autumnale* · Food contamination · Honey safety · Milk fermentation · Plant toxins

1 Introduction

Colchicine is a tricyclic plant alkaloid belonging to the tropolone derivatives. Unlike other typical plant alkaloids, it has an exocyclic nitrogen atom outside its ring core structure (Fig. 1). In Europe, colchicine naturally occurs in autumn crocus (*Colchicum autumnale*, syn. meadow saffron), a plant predominantly found in meadows throughout Europe. All parts of the plant are toxic and contain high amounts of colchicine, up to 1.9% of dry mass. Besides colchicine, other minor derivatives, such as demecolcine, colchiceine, and colchicoside, have also been identified in *C. autumnale* (Alkadi et al. 2018).

Colchicine has been used as a drug since ancient times and is currently used to treat gout or Familial Mediterranean Fever (Alkadi et al. 2018). During the COVID-19 pandemic, it was discussed as potential treatment for cytokine storm, an exaggerated immune reaction triggered by SARS-CoV-2 infection (Kamel et al. 2022). As a veterinary drug, colchicine was used to combat papilloma virus in horses and cattle (EFSA 2013). However, colchicine has a narrow therapeutic range, meaning side effects or fatal overdoses can occur quickly. At the cellular level, it inhibits the formation of tubulin microtubules from α- and β-tubulin dimers. These microtubules are essential components of the mitotic spindle, which is involved in chromosome division in eukaryotic cells. By arresting mitosis during metaphase, colchicine acts as a strong spindle toxin (Finkelstein et al. 2010), posing a risk of fatal poisonings in humans and animals when overdosed or ingested in large quantities. Besides aneugenicity, colchicine was found to exhibit genotoxic effects both in vitro and in vivo. No acceptable daily intake (ADI)

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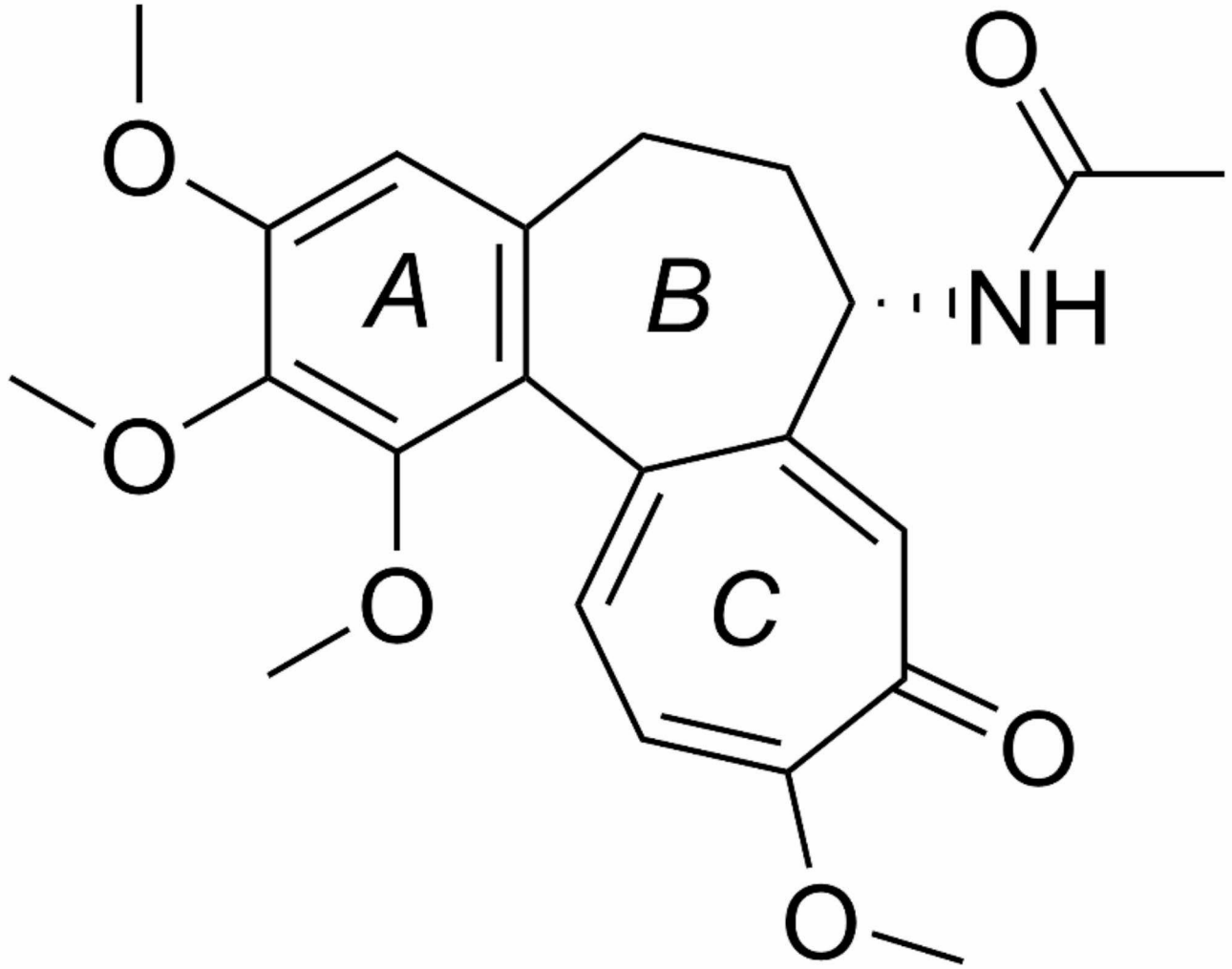

Fig. 1 Chemical structure of naturally occurring (*S*)-(–)-colchicine, consisting of of a tricyclic system, containing the trimethoxy phenyl A-ring, the saturated acetamido group-containing B-ring and the tropolone C-ring, both consisting of 7 carbon atoms

has been established (Committee for Veterinary Medicinal Products 1995). Consequently, colchicine has been included in Table 2 of European Commission Regulation (EU) No 37/2010, effectively banning its legal use in livestock farming. This regulation enforces a *de facto* zero-tolerance policy for colchicine in animal-derived foodstuffs, as no Reference Point for Action (RPA) is set until now.

In humans, severe colchicine intoxications so far have only been observed after *C. autumnale* was unintentionally ingested after being mistaken for an edible plant. Such cases have been reported in Italy and India (Giorgetti et al. 2019; Rao et al. 2016). Between 2010 and 2019, the German Joint Poison Information Centre documented 207 inquiries related to *C. autumnale*, including 4 fatal intoxications (Wendt et al. 2022). In animals, fatal poisonings have occurred in horses and food-producing livestock such as sheep, cattle and pigs in Austria, Germany, and Switzerland (Chizzola and Janda 2002; Kamphues and Meyer 1990; Kupper et al. 2010; Lohner and Gindele 1989; Schrader et al. 2001). These findings suggest that colchicine may pose a risk to consumers through its potential transfer from food-producing animals into certain food products.

The aim of this study was to identify possible entry routes of colchicine into the food chain, based on existing literature, and to assess whether colchicine could act as a contaminant in food.

Since certain plant toxins are known to be affected by food processing (Kaltner 2022), this study also investigated the persistence of colchicine in artificially contaminated honey during storage and in milk during its fermentation into yoghurt, to examine whether colchicine levels change during these processes.

2 Materials and methods

2.1 Chemicals and reagents

Acetonitrile and methanol (both LC-MS grade), formic acid (99–100%), ammonia solution (25%), and n-hexane (all of analytical grade) were purchased from Th. Geyer (Renningen, Germany). Glucono-δ-lactone (GDL, 98%) was obtained from J&K Scientific (Pforzheim, Germany). Ammonium hydrogen carbonate (LC-MS grade) as solvent additive and ultra-pure water from a Milli-Q™ system were obtained from Merck (Darmstadt, Germany). Colchicine and senecionine *N*-oxide were purchased from Phytolab (Vestenbergsgreuth, Germany), and colchicine-d6 (isotopic purity 99.1%) from TRC (Toronto, Canada). Stock solutions (1 mg/mL) and spike solutions (10 µg/mL) of the reference standard were prepared in methanol and stored at -20 °C.

2.2 Preparation of spiked food samples

Three floral honeys were purchased from a local supermarket and tested for their storage suitability in a one-week trial. Nine portions of 2.0 g honey in 50 mL tubes were spiked with senecionine *N*-oxide to 250 ng/g, stirred and stored at 20 °C at dark conditions, serving as a control experiment. Samples were analysed immediately (0 h), after 3 and 7 days in triplicate. Based on the results of this trial, one honey was chosen for the subsequent main experiment. Eighteen 2.0 g portions of the chosen floral honey were placed in 50 mL tubes, spiked with colchicine to 250 ng/g, stirred, and stored similarly. Colchicine levels were analysed at 0 h and after 1, 3, 7, 13 and 28 days in triplicates.

Ultra Heat Treatment (UHT) milk (3.5% fat) purchased from a local supermarket was warmed to 43 °C using a drying cabinet. To produce yoghurt, 3 portions of 100 g warmed milk were placed in glass beakers and each was spiked with colchicine to 100 ng/g and inoculated with 0.10 g Lyofast SYAB 1 mix culture (Sacco, Cadorago, Italy), consisting of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus* and probiotic strains of *Lactobacillus acidophilus* and *Bifidobacterium animalis* spp. *lactis*.

To generate non-fermented yoghurt-like milk gels, 1.10 g of GDL were added to 3 portions of 100 g warmed milk. Then, each portion was homogenised by using separate glass sticks. All beakers were covered with a watch glass and kept at 43 °C. The pH was monitored after 0, 2, 4 and 7 h using a daily-calibrated pH electrode. Yoghurt and milk gel samples were taken at 0, 2, 4 and 7 h in triplicates and stored at -20 °C until analysis.

2.3 Sample extraction

Extraction of colchicine from yoghurt and milk samples was conducted based on the procedure of Mulder et al. (2020) to extract pyrrolizidine alkaloids from milk samples. Samples (3.0 g) in a 50 mL tube were spiked with colchicine-d6 solution (10 µL, i.e., 33.3 ng/g milk or yoghurt), serving as an internal standard, and shaken. After adding 15 mL formic acid (0.2%) and 5 mL n-hexane, tubes were overhead-shaken (30 min, 1 rpm) and centrifuged (15 min, 3500 *g*, 20 °C). The aqueous bottom phase was transferred, adjusted to pH 9 to 10 with 150 to 200 µL ammonia (25%), checked with pH strips and centrifuged again similarly. The supernatant was loaded onto solid phase extraction (SPE) cartridges (Strata-X™ 33 µm, 6 mL, 200 mg) from Phenomenex (Aschaffenburg, Germany) preconditioned with each 6 mL of methanol and water. Cartridges were washed using each 6 mL of formic acid (1%, aq.) and ammonia (1%, aq.), dried under vacuum (1 min), and eluted with 6 mL methanol. Eluates were dried at 50 °C under nitrogen, reconstituted in 1.0 mL methanol/water (10/90, v/v) and filtered with a 0.2 µm PVDF syringe filter (Macherey-Nagel, Dueren, Germany) into a glass vial. Finally, 100 µL of this extract were diluted with 900 µL of methanol/water (10/90, v/v).

For honey samples, 2.0 g were spiked with colchicine-d6 solution (50 µL, i.e., 250 ng/g honey) and 20 mL formic acid (0.2%) was added. The subsequent procedure was identical to that for milk and yoghurt, except that the aqueous phase was not transferred to another tube.

2.4 Instrumentation, software and parameter of analysis

A Dionex Ultimate 3000 RS (Thermo Scientific, Dreieich, Germany) coupled to an API 3200 triple quadrupole mass spectrometer (MS) from Sciex (Darmstadt, Germany) was used for analysis. Analytical method parameters were used according to Klein et al. (2022). In brief, chromatographic separation was performed on a Kinetex™ Evo C18 column (100 × 2.1 mm, 1.7 µm) with SecurityGuard™ Ultra Evo C18 2.1 mm pre-columns (both Phenomenex, Aschaffenburg, Germany). 10 µL were injected at a flow rate of 0.3 mL/min and 30 °C oven temperature. Eluents were ammonium hydrogen carbonate (10 mmol/L at pH 9.0, A) and acetonitrile (B). The liquid chromatography (LC) gradient was 0.0 min 0% B, 0.2 min 5% B, 6.0 min 10% B, 19.0 min 28.6% B, 21.5 min 70% B, 21.6 min 95% B, 23.1 min 95% B, 23.2 min 0% B and 30.0 min 0% B. Colchicine levels were calculated by cross-multiplying the known concentration and peak area of the internal standard colchicine-d6 with the colchicine peak area in a sample.

MS data were acquired with Analyst 1.6 (Sciex, Darmstadt, Germany) using scheduled multiple reaction monitoring (MRM) mode. Transitions for colchicine were m/z 400.1 → 358.4 (quantifier) and m/z 400.1 → 152.3 (qualifier); for colchicine-d6 m/z 406.2 → 362.3 (quantifier) and m/z 406.2 → 344.3 (qualifier). Data processing and peak integration were done using MultiQuant 3.0 from Sciex (Darmstadt, Germany). Further analysis was performed with Excel 2019 (Microsoft, Redmond, WA, United States). Chemical structures were drawn with ChemDraw 20.1. (PerkinElmer, Waltham, MA, United States).

2.5 Statistical analysis

Comparison of data for colchicine levels was performed using OriginPro 2021b software (Origin Lab, Northampton, MA, United States). The levels at each timepoint of sampling during honey storing or yoghurt fermentation were compared to the respective levels at t_0 using a paired sample Student's t-test for means at significance level 0.05.

3 Results and discussion

Colchicine, a plant toxin produced by *C. autumnale*, is listed in Table 2 of European Commission Regulation No. 37/2010 due to its classification as genotoxic compound, resulting in a *de facto* zero-tolerance for its presence in animal feed. Similar to other plant toxins, a carry-over of colchicine from contaminated feed to food seems possible. The non-systematic literature research using Web of Science, Scopus, and Google Scholar to identify studies addressing colchicine as food contaminant revealed that no studies reported its presence in plant-based foods, and one study detected colchicine at trace levels below the method's limit of quantification (LOQ) of 0.1 µg/kg in a single sample of cooked ham (Izzo et al. 2023).

Our literature review highlighted that currently little is known about colchicine as a food contaminant. One possible reason is that monitoring programs barely have focused on this compound. Colchicine appears more likely to occur in animal-based foods due to its potential transfer from contaminated feed to secretions or tissues, such as milk or meat. Although no studies have reported colchicine in retail milk, evidence indicated that the toxin can transfer into the milk of grazing animals after ingesting *C. autumnale*, even after a single dose (Hamscher et al. 2005; Panariti 1996). Interestingly, colchicine has been demonstrated to inhibit milk yield in lactating animals, likely due to its binding to tubulin in mammary tissue, causing interference of cellular movement due to disruption of microtubules (Oliver and Smith 1982).

In addition to milk, colchicine was found to transfer into bone marrow, with smaller amounts found in heart and muscle tissues following a single oral dose administration in sheep (Panariti 1996). Therefore, relevant concentrations of colchicine in meat intended for human consumption seem unlikely. While grazing animals typically avoid feed containing bitter-tasting poisonous plants, amounts of *C. autumnale* of up to 2% dry weight in feed were still ingested by 5 out of 6 horses in a study of Mueller et al. (2021). The likelihood of colchicine uptake increases when young spring leaves or autumn flowers of *C. autumnale* are ingested in pastures or when the plant contaminates feed such as hay or silage. Consequently, the uptake of colchicine by grazing animals cannot be ruled out if *C. autumnale* is present in their feed. This is supported by findings that colchicine can withstand storage, drying of feed (Cortinovis and Caloni 2015), and ensiling (Chizzola et al. 2015). In certain Central European grasslands, extensive land use has led to critical population densities of *C. autumnale*, potentially increasing the risk of colchicine contamination in food and poisoning in grazing livestock (Jung et al. 2012; Winter et al. 2014). Thus, in Germany it is recommended that feed from extensively managed farmland is tested for plant toxins, including colchicine, as part of the national Control Programme for Feed 2022 to 2026 (German Federal Ministry of Food and Agriculture 2021).

Honey, an animal-based food, is susceptible to contamination by plant toxins, such as pyrrolizidine alkaloids (Edgar et al. 2002). Toxins in pollen and nectar foraged by honey bees can transfer into honey. However, previous studies have not reported colchicine contamination in honey. This absence is likely because colchicine is not included in analytical methods or because the late flowering period of *C. autumnale* (August to October) occurs after most beekeepers have already harvested their honey. Moreover, if administered to honey bees in very high amounts of up to 5 mg/g feed, colchicine significantly decreased their survival rate (Forkpah et al. 2014). Therefore, the likelihood of colchicine contamination in floral honey seems low. On the other hand, during that period of the year honey bees might hardly find alternative floral sources of pollen and nectar and then harvest *C. autumnale* pollen and nectar (von der Ohe 2015). Forest honeys, commonly harvested in the late summer could, therefore, have a higher risk of colchicine contamination.

In conclusion, although colchicine contamination in food is rarely reported, its potential transfer into animal-based food has been demonstrated in previous studies. Contamination of animal feed with *C. autumnale* and subsequent transfer of colchicine to tissue and excretions of food-producing animals seems the most likely pathway for colchicine to enter the food chain. As the European Food Safety

Authority (EFSA) assumed colchicine not to be included in monitoring programmes in many countries (2013), the evidence of this toxin in food-producing animals and their food products could be more relevant than currently assumed. Although it was highlighted that the occurrence of colchicine in food currently may be underestimated, the overall risk of food contamination still seems low. Nevertheless, there is currently a lack of studies to put this statement on a broader data basis.

3.1 Persistence of colchicine in yoghurt and honey

Milk is a food likely to contain colchicine if contaminated feed is ingested by dairy animals. Honey may also be contaminated under certain conditions, e.g., during late flowering period of *C. autumnale*, when honey bees might forage on its nectar and pollen. Previous studies have shown that some plant toxins are susceptible to food processing methods, such as baking, which may lead to transformation products (Kaltner 2022). Therefore, the current study experimentally investigated for the first time the persistence of colchicine during yoghurt production and honey storage.

The analytical method validation showed good analytical performance. For colchicine in honey, a limit of detection of 0.3 µg/kg, a recovery of 93.8 ± 2.4% and a repeatability, expressed as relative standard deviation, of 5.6% was achieved. For milk and yoghurt, the limit of detection was 0.2 µg/kg, recovery was 99.8 ± 0.6%, and repeatability was 4.1%. These results were in line with other MS-based methods that analysed colchicine in milk (Hamscher et al. 2005) and cooked ham (Izzo et al. 2023). In the current study, yoghurt was produced using a commercial yoghurt mix culture, while GDL was used to create a yoghurt-like product (milk gel) to mimic the acidic conditions of yoghurt production without fermentative microorganisms. No significant reduction in colchicine levels was observed in either yoghurt or milk gel during the fermentation period (Fig. 2). The pH decreased as expected, from 6.8 to 4.1 in yoghurt and from 6.3 to 4.6 in milk gel. These results demonstrated that colchicine can withstand fermentative microorganisms such as various *Lactobacillus* species, as well as acidic conditions typically present during yoghurt production. This finding aligned with previous research showing colchicine's persistence during ensiling, where these microorganisms also play a key role (Chizzola et al. 2015).

A preliminary one-week trial was conducted with 3 retail honeys spiked with senecionine *N*-oxide, a pyrrolizidine alkaloid known to decrease significantly in stored honey (Kaltner et al. 2018). After one week, senecionine *N*-oxide levels in the 3 honeys decreased notably by 18%, 23%, and 30%, respectively (data not shown). The honey with the largest reduction was selected for the main colchicine honey experiment, which lasted 4 weeks. Interestingly, colchicine levels in spiked floral honey did not significantly change during this period (Fig. 2), demonstrating its persistence in honey. Since floral honey is typically harvested in spring and summer, colchicine contamination seems unlikely due to the late flowering period of *C. autumnale*. However, forest honeys, which may be harvested in late summer and early autumn, could be more susceptible to colchicine contamination due to the limited floral alternatives for honey bees (von der Ohe 2015).

Therefore, future studies should investigate colchicine occurrence and persistence in forest honey. Moreover, these studies should also test colchicine behaviour at lower levels. The concentrations tested in this study (i.e., 100 µg/kg in milk or yoghurt; 250 µg/kg in honey) were selected to ensure that effects on colchicine persistence can be detected. In honey, these levels are not unrealistic regarding plant toxin contamination. Nevertheless, trace concentrations may be more prone to degradation or transformation, thus these levels should also be assessed in the future research.

4 Conclusions

In this study, we demonstrated that colchicine, the predominant plant toxin in *C. autumnale*, remained stable in artificially contaminated honey during storage and in milk fermented into yoghurt. The results showed that once colchicine enters the food chain, it persists in these foods. Although colchicine has been rarely reported in food–typically in single samples and only at trace levels– its relevance for livestock feed is significant, as fatal intoxications, e.g., in horses, still occur. This risk may increase in future due to the anticipated expansion of agricultural areas for grazing, which could facilitate the extensive spread of *C. autumnale*.

In more toxin-tolerant animals, such as ruminants, the plant may be consumed unnoticed, potentially leading to the transfer of colchicine into animal-derived food. Thus, although colchicine is banned from veterinary use, it could still enter the food chain under natural conditions. This issue should be adequately addressed in future monitoring and research efforts.

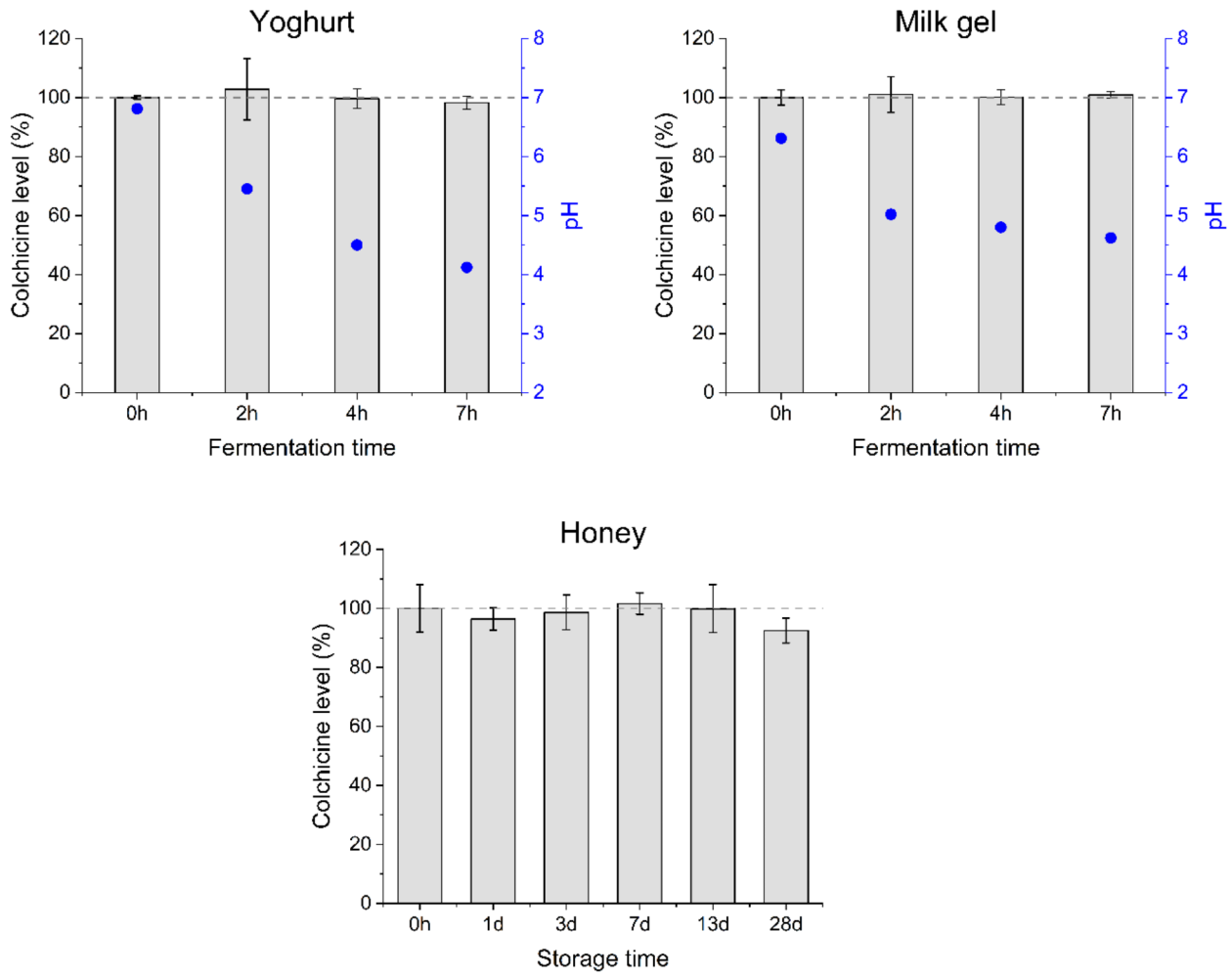

Fig. 2 Colchicine levels in yoghurt ($n=3$) or milk gel ($n=3$) fermented for 7 h, and in honey ($n=3$) stored for a period of 28 days. All levels were normalised to the colchicine levels at 0 h (t_0), which were set at 100% in each experiment (milk: 99.8 µg/kg; honey: 234.5 µg/kg); the error bars represent the standard deviation. To ensure a comparable acidic environment, the pH values of yoghurt and milk gel samples were also determined at each sampling time point. The significance of the results compared to the respective t_0 levels was tested using Student's *t*-test ($p \leq 0.05$); no significant differences were detected

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Data availability The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request.

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