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Occurrence of *Salmonella* and presumptive *Bacillus cereus* in sesame products from Swiss retail stores

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Abstract

Sesame products such as tahini (tahin) or halva (halwa or helva), originating from Arabic cuisine, are becoming increasingly popular in Switzerland. Pathogens, such as *Salmonella*, can contaminate sesame products, as evidenced by various product recalls. In this study, the occurrence of *Salmonella* and *Bacillus cereus* group members was investigated in 100 sesame products (25 sesame seeds, 16 halva, 19 different sesame pastes, 7 sesame bars, 25 hummus, and 8 other products containing sesame) collected from Swiss retail stores. None of the products were positive for *Salmonella*, whereas *B. cereus* group members could be detected with bacterial counts between 1×10² and 9×10² CFU/g in 11 out of 100 (11%) products. The 11 isolates identified by matrix-assisted laser desorption ionization-time of flight were whole-genome sequenced with Illumina technology to confirm the identity of the pathogen, determine its toxin gene profile, and perform *panC* typing. Most of the isolates harbored genes encoding the enterotoxins Nhe, Hbl, and CytK. The isolates were assigned to diverse *B. cereus* group members, including one identified as *B. cytotoxicus*. In addition, one of the isolates matched genetically with the Thurigiensis strain used in biopesticide products. In conclusion, none of the investigated sesame products contained significant levels of *Salmonella* or *B. cereus* group members. However, as *B. cereus* with pathogenic potential was detected in multiple samples, proper storage is crucial to prevent its growth and ensure consumer safety, especially for products with high water activity such as hummus.

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Introduction

Tahini is a paste made by grinding roasted or unroasted sesame seeds. It can be consumed as a “ready-to-eat” product or processed into halva, hummus, or baba ghanoush (Lake *et al*., 2010). Due to its low water activity (a_w), which is defined by the a_w-value, the product has a long shelf life of up to 2 years and can be stored at room temperature as a shelf-stable product (Unicomb *et al*., 2005). If liquid sugar syrup or honey and olive oil are added to tahini, then halva is created. This product can be further refined by adding chocolate, nuts, or fruits and is therefore often eaten as a dessert or sweet snack. Sesame seeds can be contaminated with pathogenic bacteria before, during, or after harvest, whereby biosecurity and hygiene play a major role. Irrigation systems, fertilizers, insecticides, pesticides, wild animals, transport containers and vehicles, human handling, and poor and incorrect storage can be the source of contamination (Chen *et al.,* 2009; Podolak *et al.,* 2010). Six production steps are required to produce tahini: soaking, hulling, draining and drying, heat treatment, grinding, and further processing (Lake *et al.,* 2010; Zhang *et al*., 2017). Three of these steps represent critical process stages concerning (re-)contamination: i) soaking; ii) drying; and iii) further processing.

During soaking, the seeds are soaked in salt or fresh water for 12-24 hours, which allows for the rapid growth of pathogens (Zhang *et al*., 2017). During the subsequent drying process, most pathogens die, but this usually takes place in open fields, where they are exposed to dust, aerosols, and various weather conditions

(Compaoré *et al*., 2020). During further processing, halva, hummus, or baba ghanoush is made, which can be recontaminated through the addition of chocolate, nuts, or fruit (Chen *et al*., 2009; Podolak *et al*., 2010). After this step, the a_w value is now high again, which means that bacterial growth cannot be ruled out.

As a possible decontamination measure, roasting and pasteurization of the end product can now be done. Roasting temperatures and times of 100°C for 60 minutes, 130°C for 50 minutes, or 150°C for 30 minutes are sufficient to bring the number of pathogens below the detection limit (Torlak *et al*., 2013; Zhang *et al.,* 2017). However, studies have shown that the roasting process is less effective if the sesame seeds have been dried first, as pathogens that survive the low a_w value are also heat-stable and are thus not eliminated (Zhang *et al*., 2017). The roasting process should therefore be carried out before moisture is removed (Zhang *et al*., 2017). *Salmonella* spp. are relevant hazards in these products due to their ability to survive for extended periods (Podolak *et al.,* 2010; Torlak *et al.,* 2013), and even small infectious doses can lead to illness (Finn *et al.,* 2013). It is described that the infectious dose is reduced from >10^5 CFU/g to less than 10-100 CFU/g for reasons that are still unclear (Finn *et al*., 2013). In addition to the food matrix (low a_w, high fat), the inhomogeneous distribution of *Salmonella* in the product itself is also discussed as a cause (Finn *et al.,* 2013). In addition, studies show a correlation between lower a_w and higher heat resistance, which makes inactivation by conventional thermal methods more difficult (Podolak *et al.,* 2010; Torlak *et al.,* 2013). Previous prevalence studies have described *Salmonella* in sesame products at varying frequencies, with prevalence ranging from 0% to 26.5% (Van Doren *et al*., 2013; Compaorè *et al*., 2020; Var *et al*., 2020; Coulombe and Tamber, 2022;). In the last 27 years, 20 outbreaks due to *Salmonella* in sesame products have been described worldwide. A total of 1662 cases have been documented, 88 of which had to be hospitalized. Among them was one death (Coulombe and Tamber, 2022).

The largest documented outbreak, with 802 cases occurring in 2007 in the USA, was due to a hummus product contaminated with *S.* Heidelberg. In Europe, there was an outbreak in 2019 linked to tahini products from Syria. Germany, Sweden, Norway, Denmark, and the Netherlands reported a total of 138 cases, with several serovars involved (*S.* Mbandaka, *S.* Havana, *S.* Orion, *S.* Amsterdam, and *S.* Senftenberg). This outbreak lasted for 3 years, which underlines the problematic aspects of these shelf-stable products (Coulombe and Tamber, 2022).

Bacillus cereus group members are spore-forming, gram-positive, ubiquitous bacteria that can be found in a wide variety of foods. The strains from the *B. cereus* group can have the ability to form toxins, which can lead to illness in humans. So far, there is no data in the literature on the occurrence of *B. cereus* group members in sesame products.

The aim of this cross-sectional study was to assess the occurrence and characteristics of *Salmonella* and *B. cereus* group members in sesame products collected at the retail level in Switzerland.

Materials and Methods

A first sampling was carried out in August 2023 and a second in January 2024. A total of 57 and 43 sesame products were collected from retail stores in Switzerland. The products included 25 sesame seeds, 16 halva, 19 different sesame pastes, 7 sesame bars, 25 hummus products, and eight other products containing sesame (*Supplementary Tables 1 and 2*).

The qualitative detection of *Salmonella* spp. was performed according to ISO 6579-1:2017 [without further adjustments (*e.g.*, for high-fat matrices)], whereas 25 g of the sample was diluted 1:10 in 225 mL of peptone water, homogenized, and incubated at 37°C for 24 h. The next day, 0.1 mL of this enrichment was added to 10 mL of Rappaport Vassiliadis (RV) broth, and 1 mL of the enrichment to 10 mL of Tetrathionate broth (TTB). The RV broth was incubated at 41.5°C and the TTB at 37°C for 24 h. These broths were then streaked onto Xylose-Lysine-Desoxycholate (XLD) and Rapid *Salmonella* (RSAL)-agar plates and incubated overnight at 37°C. Colonies with a black center on XLD agar and purple colonies on RSAL were considered suspicious.

For the quantitative detection of *B. cereus* group members*,* 10 g of the product was added to a saline solution (1:10) and 100 µL was spread onto Mossel agar plates (Cereus-Selective-Agar, Merck & Cie, Buchs, Switzerland; Egg Yolk Emulsion, Oxoid, Pratteln, Switzerland). The plates were incubated for 24-48 h at 30°C. Presumptive positive colonies were identified to species level by Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDITOF) (Bruker, MA, US), according to the manufacturer's instructions (Bruker) using the MBT Compass BDAL Library March 2023 from Bruker.

Isolates from presumptive *B. cereus* were sequenced using Illumina whole genome sequencing technology. Genomic DNA of the isolates was extracted from subcultures obtained from single colonies grown for 24 h at 37°C on sheep blood agar using the DNeasy Blood & Tissue Kit (Qiagen). Libraries were prepared using the Nextera DNA Flex Library Preparation Kit (Illumina) and sequenced on the Illumina MiniSeq platform (2×150 bp). Illumina read adapters and low-quality bases were trimmed with fastp (Chen, 2023) and draft genomes assembled using SPAdes 3.14.1 (Prjibelski *et al.,* 2020) implemented in shovill 1.1.0 (https://github.com/tseemann/shovill). Assembly quality was assessed using QUAST 5.0.2 (Gurevich *et al.,* 2013) and CheckM v1.1.3 (Parks *et al.,* 2015). Multi-locus sequence types, *panC* types, and virulence genes (including *ces*, *nhe*, *hbl*, *cytK*, *cry*, *cyt*, and *vip*) were identified using BTyper3 v3.2.0 (Carroll *et al*., 2020). The PubMLST *B. cereus* s.l. database (Jolley *et al.,* 2018) was used for multi-locus sequence typing. A potential biopesticidal origin of *B. cereus* isolates was investigated by determining their whole genome single nucleotide polymorphism (SNP) distance to 11 strains used in biopesticide products [ABTS-1857 (B401), ABTS-1857 (Xentari), ABTS-351, AM65-52, BMP144, EG2348, GC-91, NB-176, PB-54, SA-11, SA-12] using the CFSAN SNP pipeline (Davis *et al.,* 2015) as previously described (Biggel, Etter, *et al.,* 2022). All tools were run with default settings.

Additionally, for one product out of the five product groups (tahini, halva, sesame oil, sesame seeds, and further processed products (tofu and hummus)), the a_w value was measured using the Aqualab 3TE (Decagon Devices, Pullman, WA, USA) according to the manufacturer's instructions.

Results

Salmonella was not detected in any of the tested products. Eleven of the 100 products (11%) were positive for *B. cereus* group members (Table 1). The bacterial counts detected were between 1×10^2 and 9×10^2 CFU/g. MALDITOF identified 10 of 11 as *B. cereus* and one as *B. cytotoxicus*. Whole genome sequencing confirmed that all 11 isolates belonged to the *B. cereus* group. Based on the *panC*-gene, which is a housekeeping gene that enables classification into seven phylogenetic subtypes (Heini *et al.,* 2018), 4 of the 11 isolates belonged to *B. cereus panC* group III, five to *panC* group IV (*B. cereus sensu stricto*), one to the *panC* group II, and one to the *panC* group VII (*B. cytotoxicus*).

All *B. cereus* isolates harbored the non-hemolytic enterotoxin genes (*nheA, nheB, nheC*). Eight of the 11 isolates were positive for the hemolysin BL (*hblA, hblC, hblD*) and cytotoxin K (7 isolates for the *cytK-2* gene and one isolate for the *cytK-1* gene) (Table 2). The isolate carrying the *cytK-1* gene belonged to *panC* group VII (*B. cytotoxicus*) and showed a high average nucleotide identity (ANI) of 99.4% with the *B. cytotoxicus* medoid genome. Two isolates carried genes encoding insecticidal toxins. One of those differed by ≤2 SNPs from the biopesticide strains *Bacillus thuringiensis* subsp. kurstaki SA-11 and ABTS-351. None of the 11 isolates were positive for the *ces* gene encoding the emetic toxin cereulide. The a_w values from the different product groups represented through 8 products are listed in Table 3 and ranged from 0.11 (tahini) to 0.979 (hummus).

Table 1. Products tested positive for *Bacillus cereus* group members, their bacterial count and identification based on the *B. cereus* panC groups.

Sample	Product (brand)	Origin sesame	Bacterial count	Identification
SP1	Sesame seeds unpeeled (naturaplan)	Egypt	300 CFU/g	*B. cereus panC* group III
SP21	Sesame seeds (berem)	Turkey	600 CFU/g	*B. cereus panC* group IV [= *Bacillus cereus sensu (s.) stricto (s.)*]
SP49	Hummus basil (Anna's best)	N/A; Product produced in Switzerland	500 CFU/g	*B. cereus panC* group IV (= *Bacillus cereus s.s.*)
SP61	Tahina (al waldi al akhdar)	Lebanon	300 CFU/g	*B. cereus panC* group III
SP62	Hummus basil + almonds (naturaplan)	N/A; Product produced in Switzerland	100 CFU/g	*B. cereus panC* group IV (= *Bacillus cereus s.s.*)
SP63	Hummus garlic + pepperoncini (naturaplan)	N/A; Product produced in Switzerland	100 CFU/g	*B. cereus panC* group IV (= *Bacillus cereus s.s.*)
SP65	Sesame seeds unpeeled (alnatura)	Egypt	300 CFU/g	*B. cereus panC* group III
SP66	Sesame seeds peeled (MClassic)	China	900 CFU/g	*B. cereus panC* group IV (= *Bacillus cereus s.s.*)
SP81	Sesame seeds unpeeled (alnatura)	Egypt	100 CFU/g	*B. cereus panC* group II
SP94	Sesame seeds (Gökcehan)	N/A; Product produced in Turkey	100 CFU/g	*B. cereus panC* group VII (= *B. cytotoxicus*)
SP97	Tahin Helva, Halva vanilla (Koska)	N/A; Product produced in Turkey	100 CFU/g	*B. cereus panC* group III

N/A, not available.

Table 2. Characteristics of the isolated *Bacillus cereus* group members.

Isolate	Virulence factors				Sequence type	# *Bt* genes	Btyper3 taxa and biovar (ANI)
	Hemolysin BL	Non-hemolytic enterotoxin	Cytotoxin K	Cereulide			
SP1	*hblA, hblC, hblD*	*nheA, nheB, nheC*	-	-	Novel	1	*B. mosaicus* biovar thuringiensis; *B. thuringiensis* (93.7%)
SP21	*hblA, hblC, hblD*	*nheA, nheB, nheC*	-	-	1498	0	*B. cereus s.s.* (97.1%)
SP49	*hblA, hblC, hblD*	*nheA, nheB, nheC*	cytK-2	-	760	0	*B. cereus s.s.* (96.4%)
SP61	-	*nheA, nheB, nheC*	cytK-2	-	760	0	*B. cereus s.s.* (96.4%)
SP62	*hblA, hblC, hblD*	*nheA, nheB, nheC*	cytK-2	-	3315	0	*B. cereus s.s.* (97.1%)
SP63	*hblA, hblC, hblD*	*nheA, nheB, nheC*	cytK-2	-	8	9	*B. cereus s.s.* biovar thuringiensis; *B. thuringiensis* (98.7%)
SP65	*hblA, hblC, hblD*	*nheA, nheB, nheC*	cytK-2	-	novel	0	*B. mosaicus* (98.7%)
SP66	*hblA, hblC, hblD*	*nheA, nheB, nheC*	cytK-2	-	novel	0	*B. cereus s.s.* (97.3%)
SP81	*hblA, hblC, hblD*	*nheA, nheB, nheC*	-	-	1777	0	*B. mosaicus* (94.2%)
SP94	-	*nheA, nheB, nheC*	cytK-1	-	3316	0	*B. cytotoxicus* (99.4%)
SP97	-	*nheA, nheB, nheC*	cytK-2	-	novel	0	*B. mosaicus* (98%)

-, not present; *Bt, Bacillus thuringiensis*; ANI, average nucleotide identity.

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Table 3. Water activity values of selected products representing the corresponding product groups: tahini, halva, sesame oil, sesame seeds and processed products (tofu and hummus).

ID	Product	Brand	Origin	A_w value
SP60	Organic tahini	al waldi al akhdar	Lebanon	0.11
SP84	Tahin	Alnatura	Non-EU; product produced in the Netherlands	0.16
SP97	Tahin Helva, Halva (vanilla)	Koska	Product produced in Turkey	0.16
SP73	Sesame oil	Chop Stick	N/A; product produced in China	0.29
SP80	Furikake, sesame (roasted) + seaweed	Terra Sana	Non-EU; product produced in the Netherlands	0.30
SP90	Sesame	Zwicky	N/A	0.53
SP79	Smoked tofu (almond-sesame)	Taifun	Non-EU; product produced in Germany	0.95
SP88	Hummus (classic)	Père Olive	N/A; Mediterranean region; Distribution Belgium	0.98

Aw, water activity; N/A, not available; EU, European Union.

Discussion

Salmonella in sesame products is becoming increasingly important, as several outbreaks in Europe have already been attributed to this hazard (Meinen *et al.*, 2019; European Food Safety Authority, 2021). However, the occurrence of *B. cereus* group members is also described in the literature in low amounts, although this has been less investigated (Fay *et al.*, 2021; Anyogu *et al.*, 2024). Data on the occurrence of these pathogens in sesame products in Switzerland is still lacking. This study serves as a baseline data collection on the occurrence of *Salmonella* and *B. cereus* group members in sesame products.

Our screening of 100 sesame products identified *B. cereus* in 11 samples, whereas *Salmonella* was absent. Most *B. cereus* isolates carried non-hemolytic enterotoxin genes (*nheA, nheB, nheC*) and hemolysin BL genes (*hblA, hblC, hblD*), which have been associated with the diarrheal syndrome. One of the *B. cereus* isolates matched with commercially used biopesticide strains, suggesting agricultural use as a likely source of contamination. Given the presence of enterotoxins in these strains, consumer safety has been raised as a concern (Biggel, Jessberger, *et al.*, 2022). Additionally, one isolate from sesame seeds was identified as *B. cytotoxicus*, a *B. cereus* group member previously associated with dehydrated potato products and insect-based foods (Etter *et al.*, 2024).

Based on the measured a_w values, the products can be classified into three categories: category 1 with a_w values of <0.2 (sesame pastes, tahini, halva); category 2, a_w values between 0.3 and 0.5 (sesame seeds and sesame oil); category 3, a_w values >0.94 (processed products such as tofu or hummus with sesame).

Sesame products should be long-lasting and safe due to their low a_w values. Our study shows that products of category 1 (a_w value <0.2) and category 2 (a_w value between 0.3-0.5) in particular do not pose a health risk concerning *B. cereus* group members, as the bacterial counts detected (100-900 CFU/g) are too low and the pathogen cannot grow. *Salmonella* that survive the low a_w value, however, may lead to illness even in the smallest amounts (10-100 CFU/g). Although no *Salmonella* could be detected in our study, it cannot be assumed that all sesame products are *Salmonella*-free. The inhomogeneity of *Salmonella* in sesame products has already been described (Coulombe and Tamber, 2022). However, category 3 products (a_w value >0.94) should always be stored in the refrigerator, as the pathogen growth of both *Salmonella* and *B. cereus* group members cannot be ruled out.

In addition to cooling, the contamination or growth of pathogens can also be reduced through product manufacturing or product handling. Hygiene and proper roasting or thermal treatment (*e.g.,* pasteurization) play a key role here. In Switzerland, there are no official guidelines on these processes yet, but there are studies that have already carried out experiments and shown which methods are effective under which conditions (Torlak *et al.*, 2013; Zhang *et al.*, 2017). There is also a Hazard Analysis and Critical Control Points model published by the World Health Organization considering traditional foods for the Eastern Mediterranean Region (World Health Organization, 2008).

Due to the long shelf life, outbreaks can be prolonged and product recalls can be missed. It is therefore advisable to subscribe to public warnings issued by the federal government.

Conclusions

Future studies should examine a larger sample size to consider the inhomogeneity of *Salmonella* in sesame products. Random testing of products from a batch from manufacturing companies would minimize the risk of missing *Salmonella* contamination. The creation of nationwide product guidelines or import regulations would also minimize a potential risk.

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Online supplementary material:
Supplementary Table 1. Sesame products sampled in August 2023 from Swiss retail stores.
Supplementary Table 2. Sesame products sampled in January 2024 from Swiss retail stores.