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Occurrence of aflatoxin M_1 in goat's milk and effect of heat treatment on its stability

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Abstract

Aflatoxin M_1 (AFM_1) is often associated with hepatic cancer and can be traced in animal and human milk. This carcinogen is derived from aflatoxin B_1 (AFB_1) as a by-product of hydroxylation process, heat-stable, and difficult to be mitigated from milk. Numerous studies were done on AFM_1 treatments, however the research in Malaysia is limited. Therefore, this study was carried out to determine the occurrence of AFM_1 in raw and powdered formulations of goat's milk in Selangor, Malaysia and to examine the potency of AFM_1 via heat treatments such as boiling and pasteurization. A total of eight milk samples obtained from local farms and markets were subjected to immunoaffinity column (IAC) and analysed using High Performance Liquid Chromatography-Fluorescence Detector (HPLC-FD). Aflatoxin M_1-positive samples were further subjected to boiling and pasteurization at 100°C for 3 min and 63°C for 30 min, respectively. Results showed that three out of eight samples (37.5%) were positive for AFM_1 in the range of 0.772±0.185 to 4.265±0.371 µg/L. AFM_1 concentrations in raw, boiled, and pasteurized samples were not significant (p>0.05). In conclusion, AFM_1 is heat-stable and usual heat treatment employed in domestic or industries did not mitigate the toxin significantly. Goat's milk manufacturers or farmers must be informed of the toxin hazard and perform regular monitoring on the feed and farm facilities to avoid AFM_1 contamination at the early stage, thus producing safe and wholesome milk for public consumption.

1. Introduction

Aflatoxins are toxic secondary metabolites produced by *Aspergillus* species, mainly by *A. parasiticus and A. flavus* (Groopman *et al.,* 1988). There are many different types of aflatoxins; the naturally occurring aflatoxins are aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), and aflatoxin G_2 (AFG_2), which are normally found in feeds (Iqbal *et al*., 2015). AFM_1 is a mono-hydroxylated derivative of AFB_1, which contaminates milk from dairy ruminants (cow, goat, sheep, buffalo) fed with AFB_1-contaminated diet. Upon consumption of AFB_1-contaminated feeds, approximately 0.3 - 6.2% of the ingested AFB_1 undergoes biotransformation and converted to the monohydroxy derivative AFM_1 in the liver of lactating animals, by the action of cytochrome P450, and is secreted in milk (Vaz *et al.,* 2020). AFM_1 represents 95% of aflatoxins detected in milk, while other metabolites such as aflatoxin M_2 (AFM_2), aflatoxicol (AFL), M_4 (AFM_4), and Q_1 (AFQ_1) are detected in trace amounts, thus considered of less significance for public health (Giovati *et al.,* 2015).

Aflatoxins are classified as Group 1: Carcinogen to humans by the International Agency for Research on Cancer (IARC, 2012), with AFM_1 identified as hepatotoxic. AFM_1 demonstrated a direct metabolic inactivation in human cell lines (MCL5), and direct cytotoxicity in human intestinal enterocytes (Caco-2) (Giovati *et al.,* 2015). Considering the health risks associated with AFM_1, many countries have established

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the maximum residue levels (MRL) for AFM_1 in milk and dairy products. The European Commission (EC) (2010) established the AFM_1 maximum permissible level in milk at 50 ng/L in raw milk, the US Food and Drug Administration (1996) at 200 ng/L, while the Malaysian Food Regulation 1985 regulated that maximum limit for AFM_1 in milk and dairy products to be less than 500 ng/L. To avoid a carry-over, the legal limit of AFB_1 in dairy cow feedstuffs and in feed material has been set at 5 and 20 μg AFB_1/kg of feed by the EU and USA, respectively (Food and Agriculture Organization, 2004). The monitoring of AFM_1 contamination in milk represents a worldwide concern as minute amount of AFM_1 may be important and hazardous for high consumption group of population such as children and infant.

As a heat-stable toxin, AFM_1 does not easily affected by high temperature due to its high decomposition temperature ranging from 237-306°C (Rustom, 1997; Da -Wen, 2012). Reports abound which support the heat stability of AFM_1 in raw and processed milk products during pasteurization and processing (Iha *et al.,* 2011; Al -Delaimy and Mahmoud, 2015). Omeiza *et al.* (2018) applied several heat treatments to cow milk and compared the AFM_1 concentration before and after the heat treatments. Sterilization at 121°C showed a significant reduction (p<0.05) of AFM_1 up to 58.8%, while pasteurization conditions of 61°C for 15 - 20 min showed no significant AFM_1 reduction (p>0.05) in cow milk samples as low as 7.1-10.6%. However, the level of AFM_1 reduction was found to be inconsistent where only 8.9% AFM_1 was reduced at 100°C. Choudhary *et al.* (1998) reported that sterilization of milk at 121°C for 15 min reduced the AFM_1 by 12.21% and boiling by 14.50%.

Kiermeier and Mashaley (1977) reported that heating caused AFM_1 reduction between 12-35% in butter made from naturally contaminated cream. A study in Kenya noted that the processed milk had insignificant level of AFM_1 contamination with maximum value of 0.69 μg/L where raw milk had significant AFM_1 contamination with maximum value of 2.93 μg/L (Langat *et al*., 2016). Different heat treatments were widely integrated in effort to reduce the AFM_1 concentration in dairy products, yet the incorporation of other substances or living organisms may provide a great help to the problem. In Ethiopia, the AFM_1 concentration in raw milk samples were reduced when fermented into *ergo* (Shigute and Washe, 2018). Ergo is defined as traditional Ethiopian fermented milk produced spontaneously in a non-hygienic condition. In the process, fermentation was done naturally where the AFM_1 was reduced by 57.33% within 5 days. During fermentation, the pH of the milk samples was gradually decreased indicating that the removal of AFM_1 was linked to the microbiological works as microbes would consumes the nutrients and released organic acids.

In other study, heat treatments on milk supplemented with hydrogen peroxide (H_2O_2) at different concentration assisted in mitigating the AFM_1 concentration (Motawee *et al*., 2006). On the study, the AFM_1 degradation was the lowest when heat-treated at 75°C for 40 s and 90°C for 10 min at the presence of 0.1% H_2O_2. The highest AFM_1 degradation was recorded when the milk was boiled for 5 min and pasteurized at 63°C for 30 min, in the presence of 0.1% H_2O_2. However, a complete degradation was observed when the milk was sterilized at 121°C for 5 min with the presence of 0.1% H_2O_2 on the third day and remained constant until the fifth day. Overall, the results exhibited a positive reduction of AFM_1 with the increase of H_2O_2 concentration. This study was parallel to the findings by Aman (1992), where the AFM_1 was greatly degraded when the milk samples were supplemented with 1% H_2O_2 followed by heat treatment at 36°C for 30 min and 75°C for 15 s and boiling for 5 min, respectively. The study also reported no changes in the AFM_1 concentration in the absence and presence of H_2O_2, with or without heat-treated (63°C for 30 min and 75°C for 15 s). Minimal reduction of AFM_1 (4.3%) also was noted when the milk sample was boiled for 5 min with absence of H_2O_2. Based on the study, H_2O_2 improved the AFM_1 reduction in combination with heat treatments particularly, boiling.

Heat also affected the AFM_1 content based on a study in Serbia (Tomašević *et al*., 2015), which was performed in two consecutive years involving seasonal variations. Based on the study, AFM_1 in raw milk was found at 0.358 and 0.375 μg/kg, in winter and spring, respectively, while in summer and autumn, the AFM_1 were reduced at 0.039 and 0.103 μg/kg, respectively. This shows climate conditions during sample collection in temperate countries have significant effect on the AFM1 concentrations. This trend was also seen in another study where AFM_1 in raw milk during winter season was higher than the summer with mean values of 311.8 and 207.0 ng/L, respectively (Ibrahim *et al*., 2016). This showed that heat, even not directly imposed, helps reduced the AFM_1 content in milk.

Other than heat treatments, there are several mitigation strategies reported to minimize the AFM_1 contamination. The novel processing techniques involving a microwave, UV, pulsed light, electrolyzed water, cold plasma, ozone, electron beam and gamma (γ) irradiation treatment have the potential to mitigate aflatoxin contamination (Mahato *et al.,* 2019). Nevertheless, it is noteworthy to acknowledge that the most crucial part is the implementation of advanced agricultural technologies, good agricultural practices

(GAPs), good manufacturing practices (GMPs) and good storage practices (GSPs) along the supply chain of milk and milk products (Kamle *et al*., 2019).

The occurrence of AFM_1 in Malaysia have been reported in milk and milk products such as liquid milk, powdered milk, cheese, yogurt, and other milk-based products. Farah-Nadira *et al.* (2017) reported the occurrence of AFM_1 in several milk and milk products in Terengganu, while Shuib *et al.* (2017) reported the occurrence of AFM_1 in fresh cow milk in Penang. The contamination levels for positive samples were below limit allowable in the Malaysian Food Regulation 1985. In the similar study, Shuib *et al.* (2017) also reported that AFM_1 contamination in the human milk samples were absent. In another human biomarker study, Sulaiman *et al.* (2018) reported that high association between urinary AFM_1 and the dietary intake among adults in Selangor was contributed by cereal products, followed by eggs, and dairy products.

The availability of information for AFM_1 in goat's milk in Malaysia were scarce. Goat's milk industry in Malaysia has been growing at a significant rate in the last decade and is considered an important agricultural commodity despite the lack of officially recorded data regarding goat milk production and consumption (Sithambaram and Hassan, 2013; Shahudin *et al.,* 2018). The significant growth in demand for goat's milk among the public is due to its health benefits contributed by the unique features of goat's milk as compared to milk from other ruminants (Mohsin *et al.,* 2019). Therefore, this study aims to determine the occurrence of AFM_1 in raw and powdered goat milk from local farms and retail stores in Selangor, Malaysia. The stability of AFM_1 in milk samples were also studied through heat treatments, which were boiling and pasteurization. Both heat treatments were selected to represent the home practice of consumer after receiving local milk supply, with boiling shown to reduce the AFM_1 in milk in short time, while pasteurization condition is widely applied in dairy industries to market milk products (Aman, 1992).

2. Materials and methods

2.1 Materials and chemicals

A standard solution of AFM_1 (1 mL, 10 µg/mL in acetonitrile) was purchased from Supelco (USA). Methanol and acetonitrile (HPLC grade) were purchased from Merck (Darmstadt, Germany). Ultrapure water 18.2 MΩ-cm from ELGA LabWater (UK) was used for experiment and preparation of mobile phase. AflaStar M1 R immunoaffinity column (IAC) was purchased from Romer Labs (USA). A reversed-phase column (Hypersil Gold, C18, 100 × 3 mm, 3 µm) was obtained from Thermo Fisher Scientific (USA) and used for HPLC separation. Filter paper (glass microfiber, 934AH, GF/B circles 90 mm) from Whatman (UK) was used to filter the milk samples after centrifugation.

2.2 Sample collection

Samples of goat milk were acquired from goat farmers for raw milk and retail stores for powdered milk in Selangor, Malaysia. A total of six liquid milk were gathered from dairy goat farms in different regions such as Dengkil (A), Serdang (B), Sabak Bernam (C), Kajang (D), Bangi (E), and Klang (F), while powdered milk were purchased from two different manufacturers in Seri Kembangan (G) and Bangi (H), Selangor. The samples were stored in 250-mL polyethylene terephthalate (PET) bottles and kept in an ice box during collection, handling, and stored in freezer (-18°C) until further analysis. The powdered milk was reconstituted in water on the day of extraction.

2.3 Extraction of aflatoxin M_1 using immunoaffinity column

Extraction of AFM_1 in goat's milk was done following the method of AOAC INTERNATIONAL Official Method 2000.08-2004. Frozen milk samples were thawed at 37°C in water bath and 20 mL of each liquid milk sample was centrifuged at 5000×*g* for 20 min at 4°C to remove the lipid layer. After centrifugation, the lipid layer was discarded using a clean spatula and immediately filtered using filter paper to obtain skimmed milk. Next, the skimmed milk (50 mL) was loaded into IAC column for extraction and purification phase. The sample was allowed to pass through the column at a rate of 1-3 mL/min. The IAC was washed twice with 10 mL phosphate-buffered saline (PBS, pH 7.4) to remove the extraneous non-specific materials. Liquid residue was removed by applying pressure on the top of the column. To elute the AFM_1, collection tube was placed under the column and elution was done using 3 × 0.5 mL of water-free methanol. Before elution, the methanol was allowed to sit for 1 min in the column. The flow through was dried with a nitrogen stream at 50°C and suspended with 1 mL of mobile phase. The suspension was then filtered on a 0.22 µm membrane filter and subjected to HPLC analysis.

2.4 Quantification of aflatoxin M_1 using HPLC

Identification and quantification of AFM_1 was achieved using an HPLC chromatography system which composed of HPLC (Waters 600, USA) with fluorescent detector (Waters 2475, USA) (excitation: 365 nm and emission: 435 nm) and an autosampler (Waters 717 plus Autosampler, USA). A photochemical reactor enhanced detection (PHRED) derivatization cell (Aura Industries,

USA) was used to enhance the AFM_1 detection. The separation was performed on a reversed phase column, Hypersil Gold, C18 (100 × 3 mm, 3 mm) and kept in a column heater (Waters 8875, USA) at 40°C. Empower 2 Chromatography software (Waters, USA) was used for data acquisition, processing, and reporting.

Isocratic elution using water: methanol: acetonitrile mixture (70:25:5, v/v/v) was used throughout the analysis. The mobile phase was prepared freshly by filtering the mixture on (0.22 mm, 47 mm) nylon membrane filter and was degassed by sonication (Power Sonic 420, South Korea) for 30 min. Injection volume of 10 µL was subjected into HPLC, and the flow rate was maintained at 0.7 mL/min for 10 min running time. AFM_1 standard curve was prepared using blank goat milk spiked with AFM_1 standard at 0.5, 1.0, 2.5 and 5.0 mg/L.

2.4 Heat treatments on aflatoxin M_1-positive milk sample

Milk samples detected with AFM_1 were further treated with heat via pasteurization and boiling methods. For pasteurization, 100 mL of each AFM_1-positive sample was heated at 63°C for 30 min in a metal bowl using water bath. For boiling method, 100 mL of AFM_1-positive sample was placed in metal pot and heated on a stove until reached the boiling point (100°C) for 3 min. Time was recorded upon boiling. The heat-treated sample was then cooled at room temperature before analysis.

3. Results and discussion

3.1 Aflatoxin M_1 calibration curve

Liquid goat milk sample pre-analysed for absence of AFM_1 was used as blank sample for construction of calibration curve. The calibration curve of AFM_1 showed a linear regression with coefficient, R^2 = 0.998, which was in the acceptable range (R^2>0.995). High R^2 indicates an excellent and reliable linear regression for the determination of analyte in the samples. Figure 1 shows the curve's linear regression of AFM_1 from the fortified blank goat's milk. The instrument limit of detection (LOD) for the method developed for determination of AFM_1 in milk was 0.5 mg/L, therefore it was used as the lowest point in the standard curve (Norlia *et al.,* 2018; John *et al.,* 2019). Although lower LOD is much desirable, the level is considered acceptable as the maximum limit allowed in Malaysian Food Regulation (1985) is 0.5 mg/L in milk and milk products. Hence, surveillance and monitoring of AFM_1 can be performed using this method.

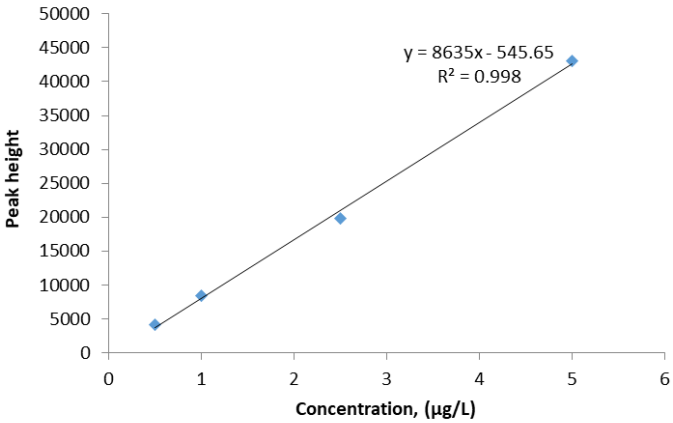

Figure 1. Standard curve of aflatoxin M_1 from the fortified blank goat's milk (R^2 = 0.998).

3.2 Aflatoxin M_1 extraction and detection

Brand A, B, C, D, E, and F represent liquid goat milk while brand G and H represent powdered goat's milk. From Table 1, three out of eight samples of goat's milk were found to be positive for AFM_1, which were Brand B, C, and D (liquid milk). Mean concentration of AFM_1 for brand D was the highest with 4.265±0.371 µg/L, followed by brand B at 0.789±0.219 µg/L and the brand C, 0.772±0.185 µg/L. The AFM_1 concentration in these three samples exceeded the AFM_1 regulatory limit of 0.50 µg/L according to the Malaysian Food Regulations 1985.

Table 1. Occurrence of aflatoxin M_1 in eight different brands of liquid and powdered goat's milk collected from local farms and retailers in Selangor, Malaysia.

Brand	Samples	AFM_1 (µg/L)
A	Liquid milk	nd
B	Liquid milk	0.789±0.219
C	Liquid milk	0.772±0.185
D	Liquid milk	4.265±0.371
E	Liquid milk	nd
F	Liquid milk	nd
G	Powdered milk	nd
H	Powdered milk	nd

Values are presented as mean±SD of triplicate (n = 3). nd: Not detected (< LOD).

Based on previous findings, the contamination of AFM_1 in goat's milk was mainly contributed by the goat's feed contaminated with AFB_1 (Applebaum *et al*., 1982; Ruangwises and Ruangwises, 2009; Wang *et al*., 2012). The substrate or the ingredient that comprises an animal feed is the most important factor in the fungal growth and mycotoxin production mainly due to its nutritional composition (Alvarado *et al.,* 2017). From the information provided by the farm's manufacturer, the goats were fed with pellet, Napier grass, soy hull, maize, and soybean. Grains such as maize and soybean are the common source of aflatoxins. Serious contamination of

maize and soybean with AFB_1 for animal feeds have been reported mainly in tropical and subtropical countries (Streit *et al.,* 2013; De Rijk *et al.,* 2015; Gruber -Dorninger *et al.,* 2019) due to the climate condition.

Climate change has been significantly associated with the increased risk of crop contamination with *aflatoxin*-producing fungi. It influenced the expression of certain AF biosynthetic genes such as *aflD* (structural gene), *aflS,* and *aflR* (main regulatory gene) by the interaction between the temperature and water activity (a_w) condition (Battilani *et al.,* 2016; Assunção *et al.,* 2018). The optimum temperature for growth and aflatoxin production by *A. parasiticus* and *A. flavus* is 25 - 35°C and 28 - 30°C, respectively (Bhat *et al.,* 2010). As that optimum temperature is provided in warm-temperature zones, aflatoxins are a far greater problem in tropical regions such as Malaysia (Afsah-Hejri *et al.,* 2013). They can be contaminated with aflatoxins via an initial phase during crop development and a second phase during crop maturation (Mahato *et al.,* 2019). Hence, the origin of the feed ingredients and the length of storage during the transportation must be considered to make a conclusion about mycotoxin contamination in a region.

The fungal growth in animal feeds after harvest during storage is influenced by the temperature, humidity, water activity (a_w), the integrity of the grain, insect damage, and the quantity and type of the mycobiota (Alvarado *et al.,* 2017). Favourable growth conditions for causal fungi include substrate moisture content (15 - 30%), ambient temperature (25 - 30°C), and relative humidity (above 86%), which could be found commonly in all tropical climates (John *et al.,* 2019). A weak standard operating procedure (SOP) of the feeding management promotes the growth of AFM_1-producing fungi. For example, feed directly placed on floor without any protection layer will allow absorption of moisture to the feed and promote growth of aflatoxin-fungi producer. Unsystematic design of feed store with poor ventilation system would trap moisture especially during heavy rain and increase the incidence of fungal growth on the feed (Sivakumar *et al*., 2014; Shahudin *et al.,* 2018). John *et al.* (2019) suggested the used of polyethylene-laminated aluminium (PELA) as packaging, stored at room temperature (25°C) yielded the lowest concentration of AFB_1 in peanuts. Therefore, proper ventilation, insect infestation, and proper temperature control are the most important factors to minimize mycotoxin contamination at the storage level. By implementing of good storage management techniques, mycotoxin contamination can be minimized.

3.3 Heat treatments on aflatoxin M_1-positive milk sample

There were no significant differences (p>0.05) in the AFM_1 concentration for all three samples after boiling and pasteurization processes, as shown in Figure 2. The observation is explained by the heat-stable characteristic of AFM_1 with decomposition temperature ranging from 237-306°C (Rustom, 1997; Kelly *et al.*, 2005). Some other reports also corroborate that aflatoxins were stable during heat treatments such as pasteurization and sterilization (Van Egmond *et al.,* 1977; Wiseman and Marth, 1983; Govaris *et al.,* 2002). Little or no reduction is expected to occur during cooking processes such as boiling and pasteurization at temperatures of conventional food processing (80-121°C). Similar observations were reported by previous studies. Pallarés *et al.* (2021) reported that AFB_1 was not reduced after thermal treatment at 90°C for 21 s in milk beverages observing concentrations close to 100 µg/L. Govaris *et al.* (2002) observed no significant differences in the AFM_1 contents in spiked milk at 0.050 and 0.10 g/L after heating at 92°C for 3 min, suggesting that AFM_1 is relatively stable during pasteurization and sterilization processes. In contrast, other studies reported significant reductions of AFM_1 in milk after heat treatments. Deveci (2007) achieved AFM_1 reductions between 12 and 9% in artificially contaminated milk (1.5 and 3.5 µg/L) after pasteurization treatment under 72°C for 2 min, while 7.62% of the AFM_1 level in milk is reduced by pasteurization, as reported by Bakirci (2001). Choudhary *et al.* (1998) concluded that destruction of AFM depends on the time and temperature combination of the heat treatment applied. Different types of analytical methods used to determine the AFM_1 in milk samples may also contribute to the difference in observation.

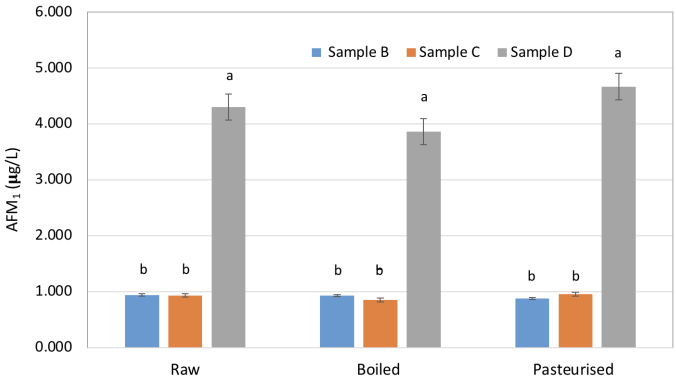

Figure 2. Changes of aflatoxin M_1 concentration in milk samples after boiling and pasteurisation. Results are compared to raw milk before heat treatment. Data are presented as mean±SD of triplicate (n = 3). Bars with different notations are statistically significantly different (p<0.05).

For industrial applications, pasteurization is used as a standard method as a mean to kill pathogens to ensure milk is safe for consumption and extend shelf life of milk

and milk products. There are different regimes of pasteurization; 'High Temperature Short Time' (HTST), 'Low Temperature Short Time' (LTLT), and 'Ultra High Temperature' (UHT) (Kelly *et al.,* 2005). HTST involves heating milk to 71.7°C for 15 - 25 s. Once the milk has been heated, it is then cooled very quickly to less than 3°C, which makes it suitable for continuous pasteurization system. LTLT utilized low heat (63°C) and low pressure and held for 30 min. The prolonged holding period alters the structure of the milk proteins, making it suitable for making cheese and batch pasteurization. UHT is a closed pasteurization system that involves heating between 135 - 150°C for 1 - 2 s (Kelly *et al.,* 2005).

In many studies, the roles of microbes to improve the degradation of AFM_1 in milk, particularly lactic acid bacteria (LAB) were incorporated as these strains were excellent in binding toxins. Bacteriocin released from the heat-killed *Lactobacillus rhamnosus* and *Lactobacillus acidophilus* improved the detoxification of artificially spiked AFM_1 in UHT skimmed milk (Nassar *et al*., 2018). The bacteriocins from both strains exhibited a significant AFM_1 reduction ranging from 33% to 77%. The heat-killed *L. acidophilus* significantly had better capability to bind AFM_1 (mean percentage: 0%, 27.3±2.9% and 39.07 ±10.33%) compared to *L. rhamnosus* with mean percentage of 0%, 12.3±2.6% and 14.3±3.48%. Another study also exhibited significant reductions of AFM_1 by *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum* and *Lactobacillus acidophilus* either in viable, heat-treated or acid-treated stages with *L. plantarum* being the remarkable strain in detoxifying AFM1 in yogurt (Elsanhoty *et al*., 2014).

Besides biological approach, the ability of kaolin and calcium bentonite (Ca-bentonite) to degrade AFM_1 in milk were also evaluated (Moussa *et al*., 2020). Both clays detoxified the AFM_1 significantly as compared to the untreated milk. In the study, kaolin reduced AFM_1 content from 86.1% to 93.3% while Ca-bentonite from 93.7% to 97.7% without harming the nutritional contents of the milk. It was also revealed that the increased in Ca-bentonite would reduce the AFM_1 comprehensively. The ability of sequestering agents was further adopted using activated charcoal, sodium bentonite, Ca-bentonite, and esterified glucomannan which all the agents bound AFB_1 more than 95% despite of any pH ranging from 95% to 99% (Diaz *et al*., 2003). The capability of the agents to remove AFB_1 would further reduce the AFM_1 contamination in milk.

Apart from heat treatment method, the application of high voltage atmospheric cold plasma (HVACP) in detoxifying AFM_1 was also studied. Nguyen, Palmer, Phan *et al*. (2022) reported that the HVACP method reduce the AFM_1 in skimmed milk efficiently with increasing treatment times (1-20 min), using gas containing 65% oxygen (MA65: 65% O_2, 30% CO_2, 5% N_2) rather than air, increasing voltage (60-80 kV) and reducing sample volume (30 to 10 mL). Besides, the study also reported that direct treatment on the milk samples provide effective reduction compared to indirect treatment. MA65 degraded the AFM_1 in milk by 78.9% followed by air with 65% with no change in milk colour. The cold plasma technology was also proven effective in reducing the AFM_1 contamination in milk in another study by Nikmaram and Keener (2022). In the study, one -minute treatment of 90 kV using modified air (MA65) effectively reduced the AFM_1 level in skimmed and whole milk with 41.9% and 37.8% respectively. Normally, the skimmed milk would enhance better removal of AFM_1 since the removal of milk's cholesterol helps better removal of AFM_1 without any substantial effects on the milk (Šimko and Kolarič, 2022). Nevertheless, greater reductions were noticed when milk samples were treated at a short time following post-treatment storage where 87% reduction achieved when the samples were treated using HVACP for 3 min following 4 h of post treatment.

Irradiation was one of the potential methods in reducing the AFM_1 contamination. Hassanpour *et al*. (2019) reported that 51.5% of AFM_1 was reduced in pasteurized milk after 4 days and 99% reduced after 8 days in comparison to the control. The milk samples were irradiated using radioactive granite at an acceptable dose rate, 0.39 mGy daily. Based on the International Atomic Energy Agency (IAEA) report, the dose rate applied does not affecting the sensory and chemical qualities of the milk but improved the shelf life of the milk. Thus, making radioactive granite as one of the potential and safer methods to detoxify AFM_1. The reduction of AFM_1 using ultraviolet (UV) light at 254 nm was also performed. From the study, short-wave UVC radiation degraded AFM_1 in milk up to 50% after 20 min of exposure despite of the initial AFM_1 level. Other factors such as samples' depth, time of treatment and stirring were proven to significantly reduced the AFM_1 without affecting the milk colour (Nguyen, Palmer, Loo *et al.,* 2022).

4. Conclusion

This study reported on the occurrence of AFM_1 in liquid and powdered goat's milk in Selangor, Malaysia. Effects of heat treatments on the stability of AFM_1 were also determined. The results showed that 37.5% of the collected milk samples were detected with AFM_1

ranging from 0.772±0.185 to 4.265±0.371 µg/L, while AFM_1 concentration in raw, boiled, and pasteurized samples were insignificant (p>0.05). Albeit having a small number of samples analysed in this study, the data are crucial as they represents one of the first reports on the occurrence of AFM_1 in liquid and powdered goat's milk at consumer level and its stability after heat treatments. This study highlights the risk of hazard from aflatoxin contamination upon consuming and processing of goat's milk. It contributes to the body of knowledge and increase awareness for goat's milk producers and product manufacturers to maximize mitigation strategies and to general consumer concerning food safety issues. A larger sample population along the food chain supported by a risk assessment study are warranted for data reliability and validity, hence a more accurate food safety risk can be concluded. Based on the data in the study, boiling and pasteurization were insufficient to reduce the contamination of AFM_1 in the goat's milk. At a farm level, a guided feed-processing procedure and farm SOP must be followed by farm owners or managers to minimize the risk of feed contamination by mycotoxins, therefore AFM_1 level in animal milk can be further abated.

Conflicts of interest

The authors declare no conflict of interest.

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