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Heat-resistant moulds: Assessment, prevention and their consequences for food safety and public health

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Abstract: Heat-resistant moulds (HRMs) are the spoilage factors of thermally processed products such as pasteurised items and fruit products, which may cause financial losses and decrease food quality. Various variables may play a role in food contamination by HRMs, such as the processing environment, packaging, staff practices and air in the production site. Prevention of spoilage by HRMs for processed food products can be done through the reduction and decontamination of these microorganisms. This review aims to provide a perception of HRM and mycotoxin contamination, assessment, prevention and their consequences for food and human health.

Keywords: mycotoxins; fungi; food contamination; human health

Heat-resistant moulds (HRMs) are the spoilage factors of thermally processed products such as pasteurised items and fruit products, which may cause financial losses and harm to food quality. The heat resistance of these moulds depends on their ability to produce heat-resistant structures such as ascospores, which are similar to bacterial spores. These moulds can endure heat procedures of food with their ascospores and are regularly found to be related to fresh fruits. Moreover, they may pose a health risk due to their metabolites and mycotoxins (Aydin et al. 2005; Gumus et al. 2010; Ishara and Gunasena 2021). These types of moulds are broadly dispersed in vineyards, plantations and areas in which fruits are grown, where they can live for quite a while and can therefore contaminate raw substances coming in touch with the soil. Once the foods are contaminated, it is difficult to inactivate the ascospores of HRMs. Thus, when contamination occurs, an obvious development of mycelium will be seen, and if mould spores are found at high concentrations, it is a terrific financial loss for industries (Tranquillini et al. 2017). HRMs are characterised by the generation of ascospores or comparable structures with heat resistance. This allows them to endure the thermal processes. Germination of ascospores may cause visible growth of mycelia on fruits (Beuchat and Pitt 2001). Ascospores produced by HRMs grow after some time of non-active development that can be broken with a sublethal heat treatment that will allow their germination and development under ideal conditions. This procedure is known as heat activation (Samapundo et al. 2018). After activation and if the conditions are approved, the ascospores can germinate and develop, causing food deterioration during storage at proper temperatures (Rico-Munoz 2017). Heat activation is required to begin the spore germination cycle and, in this way, it can also be an element in the development and recovery of HRMs (Enigl et al. 1993). HRMs are additionally ready to create a few mycotoxins that pose a hazard to human wellbeing (Rico-Munoz 2017). The occurrences of HRMs are generally in fruit products, and their capacity to resist sanitisation and pasteurisation

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Table 1. Tolerance of heat-resistant moulds (HRMs) isolated from foods

Mould	Heat-resistant structure	Heating medium	Heat resistance
Byssochlamys fulva	ascospores	glucose-tartaric acid, pH 3.6	90 °C, 51 min, 1 000-fold
		grape juice, 26 °Brix	85 °C, 150 min, 100-fold
Byssochlamys nivea	ascospores	grape juice	88 °C, survived 60 min
		apple juice	99 °C, survived in juice
Eupenicillium lapidosum	ascospores	blueberry juice	81 °C, 10 min, survival; 81 °C, 15 min, death, z = 10.3 °F
	cleistothecia	blueberry juice	93.3 °C, 9 min, growth; 93.3 °C, 10 min, death z = 10.6 °F
Eupenicillium brefeldianum	ascopores	apple juice	90 °C, 1 min, death z = 7.2 °C
	cleistothecia	apple juice	90 °C, 220 min, death z = 11.7 °C
Talaromyces macrosporus	ascospores	apple juice	90 °C, 2 min, death z = 7.8 °C
		fruit-based fillings	D91 °C = 2.9 min to 5.4 min, z = 9.4 °F to 23.3 °F
		apple juice	D90.6 °C = 1.4 min, z = 9.5 °F
		apple juice	D90.6 °C = 2.2 min, z =5.2 °C
	cleistothecia	apple juice	90 °C, 80 min, death z = 11.7 °C
Monascus purpureus	whole culture	grape juice	survival several min at 100 °C
Humicola fuscoatra	chlamydospores	water	80 °C, 101 min, 10-fold inactivation
Phialophora sp.	chlamydospores	apple juice	80 °C, 2.3 min, 10-fold inactivation
Neosartorya fischeri	ascospores	water	100 °C, 60 min, survival
		fruit-based fillings	D88 °C = 4.2 min – 16.2 min, z = 5.4 min = 11 °F
		apple juice	87.8 °C, 1.4 min, z = 5.4 min = 11 °F
Neosartorya fischeri var. glaber	ascospores	water	90 °C, 60 min, survival
		grape juice	85 °C, 10 min, 10% survival
Thermoascus auranthiacum	whole culture	grape juice	88 °C, 60 min, survival

D – decimal point reduction value which is the time or dose required for a one-log cycle (90% reduction) in the microbial population under lethal conditions; z – number of degrees (temperature) or dosage units required for a one-log reduction in the D value

Source: Adopted from Rico-Munoz et al. (2015)

is still a difficult issue for fruit manufacturing. However, additionally because of their capacity to develop under low pH (pH < 4.0) and constrained headspace oxygen ranges, the ascospores of numerous species are detected after a sublethal temperature or pressure (dos Santos et al. 2018). Several factors contribute to HRMs spoilage in finished items. For instance, the increasing requirement of consumers for minimally processed foods and fewer additives. The rising prevalence of plastic packages that do not give a true hermetic seal and even limit to apply maximum temperature ranges in heat processing may increase the risk associated with HRMs spoilage (Biango-Daniels et al. 2019). The approved procedure used commercially is a treatment for 2 s at 90 °C (USFDA 2004). Rico-Munoz et al. (2015) rewieved heat tolerance of HRMs as we presented in Table 1.

CONTAMINATION SOURCES OF HRMS IN FOOD PRODUCTS

The quantity of ascospores on fruits or related components is commonly less than 1 per each 100 g, so it is difficult to detect. The tolerable degree of contamination will rely upon whether the item is a major or minor ingredient and what sort of processing it will be exposed to (Rico-Munoz 2017). Machines or manipulation tools and air are a source of mould contamination. Tacker et al. (1999) reported that exposure to ambient air is an important contamination pathway. An investigation was carried out for the contamination of *Penicillium* and *Aspergillus* spores in polystyrene containers. Eighteen per cent of the cups were polluted after production. At the point when the cups were ex-

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posed to air for 1 h, contamination was 100%. HMRs demonstrated good development under a wide pH interim or at refrigeration temperatures (Tacker et al. 1999). Another study assumed higher resistance to a sanitising chemical like hydrogen peroxide (H_2O_2) from the ascospores of *Talaromyces* or *Aspergillus*. More investigations could be done for HRMs, as for practical purposes they appeared to be generally appropriate as target microorganisms rather than vegetative microorganisms, for example, *Aspergillus niger*, prompting for more performing results because of their utilisation during biological validations of sanitising processes on machinery used for refrigerated items (pH > 4.5) or non-refrigerated acid items (pH ≤ 4.5) (Scaramuzza et al. 2020). Staff practices are indicators of food quality and have been related to the presence of HRMs in raw agriculture. Great manufacturing practices for worker hygiene and health may impact the microbial quality of the product (Snyder and Worobo 2018).

COMMONLY ISOLATED HRMS

Eupenicillium, Byssochlamys, Talaromyces and *Neosartorya* are classified as HRMs. Despite the fact that heat resistances of HRM ascospores are different from strain to strain, HRMs commonly live for more than 30 min at 75 °C. After HRMs are activated by heat, germination develops to ultimately cause early deterioration (Samapundo et al. 2018). *Byssochlamys* spp. are known to produce a few significant mycotoxins including byssochlamic acid, byssotoxin and patulin. Experiments verified that *Byssochlamys nivea* synthesised pectic enzymes separated the structure and contributed to disintegration in the organoleptic nature of processed strawberries. Additionally, patulin is considered a neurotoxic, immunotoxic metabolite and carcinogenic, it can also cause some other diseases such as haemorrhage, ulceration and gastrointestinal tract distension (Samapundo et al. 2018). Ascospores produced by *Byssochlamys fulva* are an issue with canned fruit products due to their uncommon thermal resistance in acid products. *B. fulva* disintegration has occurred in a few foods and fruit drinks. *Penicillium* separated from foods may be the reason for spoilage. Moulds ordinarily separated from pulsed electric field-treated fruit smoothies and juices were *Penicillium bialowiezense, Penicillium expansum,* and *Penicillium buchwaldii* (Groot et al. 2019). *Neosartorya fischeri* is the main source of deterioration in heat-processed foods. Ascospores of *N. fischeri* may spoil heat-processed foods by their germination and development under a micro-aerobic state. The toxigenicity of these moulds poses a risk to general well-being. Spoilage of agricultural raw materials is frequently a consequence of their exposure to the soil (Panek et al. 2016). Heat processing is a typical technique for pasteurisation used in food production. However, in a study done it was indicated that heat procedures at 75 °C for 30 min did not inactivate *N. fischeri* ascospores. The mix of high pressure and thermal treatment (HPTP) or power ultrasound [thermosonication (TS)] decreases the treatment temperatures and additionally the processing time for mould spores and enzyme inactivation. This thus will raise the process efficiency and item quality. Temperatures up to 90 °C have been used to inactivate *N. fischeri* ascospores (Silva 2017).

METHOD OF HRM ANALYSIS

The standard of HRM test methods is an activation of ascospores. Due to the low concentration of ascospores in samples, enormous samples must be investigated for their effective detection. Filtration or centrifugation might be used to concentrate the ascospores (Rico-Munoz 2017). For HRM testing, cultural methods were described by Beuchat and Pitt (2001). As enumeration principles, different strains within similar species may need different temperatures and treatment times to accomplish maximum activation. Ascospores might be stressed by the heat procedure, highly acidic media are not recommended, but for low acid foods that are intensely contaminated, bacterial spore acidification or the adding of chloramphenicol to the plating medium might be required to inhibit the bacteria for better isolation of the moulds. With the Petri dish method, aerial contamination during plating may be a problem alternatively; the direct incubation method can be used. It can take from 7 to 14 days for the germination of ascospores. For a faster way of analysis, polymerase chain reaction (PCR) can be employed uniquely to assess the affinity of fungi (Rico-Munoz 2017). PCR based on the isocitrate dehydrogenase (IDH) gene method was developed to effectively detect patulin-producing moulds in food. It shows high sensitivity, detecting 10^2–10^3 conidia g^{-1} in foods (Luque et al. 2011). PCR strategies for the discovery of *Paecilomyces, Talaromyces, Hamigera, Thermoascus,* and *Aspergillus* (with *Neosartorya* transform) species are quick and helpful techniques for distinguishing food items (Rico-Munoz 2017).

Quantitative microbial risk assessment (QMRA) is a methodology which is generally used as an instrument to decide and deal with the risks of foodborne path-

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ogens; it has lately been applied to evaluate the spoilage hazard of food by fungi (dos Santos et al. 2018). With the utilisation of risk assessment, theoretically, the impact of all intercessions on the last risk can be resolved, which can help to plan the proper controls in the food safety management system. In practice, the evaluation has understandably enormous uncertainty and variability (Zwietering 2015). The various sources of variability along the food supply chain that are connected with spoilage of foods, fungal development and the improvement of a risk assessment model are divided into three groups: *i*) those related to the environment such as storage conditions; *ii*) the fungus, for example, the physiology of fungal spores; and *iii*) the purchaser (Gougouli and Koutsoumanis 2017). To assess and evaluate the degree of contamination ahead of time or progressively, much work has been placed into the advancement of early notice frameworks like Rapid Alert System for Food and Feed (RASFF) and prediction models. Most of the prediction models are focused on factors influencing the occurrence of recognised mycotoxin risks and incorporate climate factors such as temperature and humidity (Kandhai et al. 2011). It is recommended to include complementary information in RASFF data, for an entire approach to early recognition of rising risks. This data includes risk management measures, safety evaluations, foundation information on hazards and surveillance patterns. Different kinds of microbiological hazards such as moulds and bacteria have been reported through the RASFF framework (Kleter et al. 2009).

FOOD GROUPS UNDER THE RISK OF HRM CONTAMINATION

HRM ascospores are generally found in the soil, especially in which leafy foods and fruits are grown (for example beets, coconuts, sugar cane, maize, apples, mangoes, grapes, pineapples, papayas, passion fruit, blueberries, strawberries). Components like liquid sweeteners fabricated from beets, sugar cane, or maize, tea leaves, root powders, coconut water and other refreshments may contain HRM ascospores (Rico-Munoz 2017).

HRMs consistently spoil pasteurised items, for example, canned fruits and fruit juices made of items promptly polluted by soil (Dagnas and Membré 2013). *N. fischeri* moulds produce ascospores. They can likewise develop under microaerophilic conditions inside fruit packs and carbonated beverages, causing spoilage. The ascospores are regularly connected with the deterioration of pasteurised fruit items, jams, purees and canned fruits (Silva 2017). It is realised that the ascospores of *Byssochlamys* spp., which is a typical HRM, have been found in milk and milk products. Besides milk and milk products, HRMs are distributed in the soil and are connected with the deterioration of the item containing fruits such as ice cream and yoghurt (Aydin et al. 2005). *Penicillium nordicum* is frequent in dry-cured meats and dairy items (Lopes et al. 2018). *Penicillium glabrum* has been separated into an enormous diversity of items, including cheese, nuts, and bottled mineral water (Nevarez et al. 2010). *Talaromyces trachyspermus* has been separated from heat-processed cheeses, canned strawberries and tea-based refreshments (Yamashita et al. 2019). Ochratoxin A (OTA) mycotoxin is found in coffee beans, red wine, spices, cereals and nuts (Calado et al. 2014).

Ascospores can survive the heat process in canned fruit items and accordingly develop under reduced oxygen. The ascospores of certain moulds are particularly critical in food spoilage because they are heat resistant. Two examples of these moulds are *Penicillium* and *Byssochlamys*. *Penicillium* moulds produce mycotoxins and are heat resistant; they ordinarily spoil processed fruit products with blue and blue-green tinges. *Byssochlamys* moulds can cause deterioration because of their heat-resistant ascospores and pectinases, which are enzymes that they produce (Tucker 2008).

The mycotoxin content in food relies upon the fungal species, product composition, collecting, handling and storage conditions, temperature and mechanical damage, oxygen, moisture, substrate composition, insects, and spore load (Alsharif et al. 2019). There are many known types of mycotoxins and they can spoil a wide assortment of foods. The familiar ones are patulin, fusarin, OTA, and aflatoxin. They are practically all cytotoxic, causing disorders in different cell structures, like membranes, and intervene in important cell processes such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) union. The consumption of food spoiled by mycotoxins is connected with chronic and acute illnesses in people and animals (Tucker 2008). The harmful impact of mycotoxins on human health is known as mycotoxicosis, its seriousness relies upon the toxicity of the mycotoxins, age, the nutritional condition of the person and the degree of exposure (Peraica et al. 1999). OTA is one of the common food contaminating mycotoxins which has nephrotoxic effects (renal damage) in poultry and may promote tumours in humans (Hussein and Brasel 2001). Urinary OTA is a potential biomarker of OTA exposure. A study by Wafa et al. (1998) on end-stage renal disease showed remarkably high levels of OTA in urine compared to two reference groups (Wafa et al. 1998). Aflatoxin can cause severe conditions for animals

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and humans by causing different complications, for example, immunotoxicity, hepatotoxicity, and teratogenicity. Also, poisonings occur through respiratory and cutaneous pathways (Kumar et al. 2017). In Kenya between 2004 and 2005 more than 150 people passed away due to outbreaks of acute aflatoxicosis (Strosnider et al. 2006). Patulin is teratogenic, immunosuppressive, genotoxic, and neurotoxic (Calado et al. 2014). Also, patulin is recognised as a causative agent of gastrointestinal disorders with ulceration but as per the International Agency for Research on Cancer, patulin is not classifiable as carcinogenic to humans (Puel et al. 2010).

Mycotoxin contamination is considered one of the foodborne issues, that expands and increases in incidence as significant public health and economic issue (Piñeiro 2008). Impacts of animal feed adulteration with mycotoxins include decreased immune ability, expanded susceptibility to diseases, and increased application of antimicrobials in animal flocks. The resistance to antimicrobials expanded in animals and transferred the resistant organisms to humans which increased public health concerns (Fink-Gremmels 2008). Four public health strategies were identified for aflatoxin: *i*) evaluate the impact on human health and the severity of the disease after aflatoxin exposure; *ii*) assess the effectiveness and the extent of the result caused by continuous intervention strategies; *iii*) increase the disease reconnaissance, food checking, laboratory, and public health responses in affected districts; *iv*) in acute aflatoxicosis outbreak a response protocol should be available (Strosnider et al. 2006). Essential intervention strategies to decrease aflatoxin exposures in the post-harvest period can have a critical effect in high exposure populations but are unlikely to eliminate exposure. Moreover, these strategies cannot be targeted particularly at high-risk people, for example, people with chronic hepatitis B virus (HBV) infection. So, intervention approaches likewise include chemoprevention that interferes with the absorption of mycotoxins once ingested (Wild and Turner 2002). Multidisciplinary research such as assessment in epidemiological studies and studies of animal and human metabolism which develop chemoprevention has provided the scientific platform to help in decision making and decrease the risk in public health.

PREVENTION OF SPOILAGE BY HRMS IN PROCESSED FOOD PRODUCTS

Prevention of spoilage of thermally processed items by HRMs would require decreasing or decontamination of ascospores from the fresh ingredients and the processing environment (Rico-Munoz 2017). To control HRMs in fruit products, it is important to use high-quality products which have been suitably checked, washed and disinfected keeping up sterile conditions. A consumable water wash with confirmed products would physically eliminate the soil from raw materials, then the fruit should be treated with sanitisers. For example, acidified sodium chlorite or chlorine dioxide could be a powerful method to inactivate HRM ascospores if the correct concentration is used. Researches that were performed on raw fruits and vegetables have demonstrated that chlorine dioxide can be a beneficial sanitiser (Rico-Munoz 2017). There is not a lot of data on treatments to completely remove or get rid of HRMs ascospores from packaging. For aseptic packaging, Delgado et al. (2012) indicated that heat treatment (70 °C) and hydrogen peroxide (H_2O_2) (35%) for 6 s would ensure package sterility. If the counts of HMRs are more than 1 spore $(100\ cm)^{-2}$ or if moulds show a higher heat resistance, treatment with 40% hydrogen peroxide (H_2O_2) at 70 °C $(10\ s)^{-1}$ would give a chance of survival to one of every 104 packages. This would give a high spoilage rate because the beverage industry focuses on having less than one complaint about mould contamination per million bottles (Delgado et al. 2012).

It was shown that the impact of ethanol vapours on spore inactivation relied upon temperature. Raising the temperature from 10 °C to 30 °C was a higher priority than an increase from 5% to 10% (w/w) ethanol for clarifying the inactivation of *Penicillium digitatum* and *Penicillium italicum* (Dao and Dantigny 2011). The inhibitory impact of ethanol has been accounted for both in mould germination and multiplication; ethanol either stops development or postpones it by disturbing the cell membrane. In the manufacture of food items, ethanol is applied straightforwardly on the product for example in the packaging atmosphere or sprayed on the surface (Dagnas and Membré 2013). Adding ethanol increases the mould-free shelf-life and usability of bread when applied after baking and cooling at concentrations from 0.5% to 3.5% (wt/wt) portion weight. Food products, for example, cake treated with > 2% ethanol (wt/wt), were refused by buyers based on odour enhancement as well as flavour. However, it is not enough to prevent mould growth (Dao and Dantigny 2011).

The moulds which contaminated the food are strict aerobes but some of them such as *Fusarium* can develop at oxygen concentrations as low as 0.5% or 2%. Moulds

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can likewise utilise oxygen from the matrix, not only from the atmosphere. In this manner, depression in the packed food items must be related to the lethal impact of another compound, for example, carbon dioxide (CO_2) or nitrogen (N_2) (Dagnas and Membré 2013).

When contamination cannot be avoided, decontamination is required before such raw materials are used for human consumption as food. With this point of view, the mycotoxin must be inactivated and fungal spores ought to be destroyed. The physical characteristics of a commodity must not be changed remarkably and the expense of decontamination must be lower than the waste of contaminated raw materials (Halasz et al. 2009). There are different physical ways of decontamination of agricultural products such as taking away spoiled grains or a portion of the contaminated harvest, washing techniques, radiation, ultrasound and extraction with organic solvents that can be considered possible methods of decontamination when the spoilage spread is limited to a small area (Awad et al. 2010). Some specialists stated that the best way of decontamination is detoxification by biodegradation since it gives an opportunity to remove mycotoxins under mild conditions (not extremely harsh compared to other methods) and without using unsafe chemicals. According to progress in the investigation of metabolic procedures in microorganisms fit for degrading mycotoxins, particular enzymes engaged in the degradation procedure have been recognised. This reality with the improvements in genetic engineering opens new ways of decreasing mycotoxin spoilage (Halasz et al. 2009). For the decontamination method to be financially doable, it should be effective without making new toxins, presenting new lethal compounds, or modifying the nutritional value or other preferable parameters of food. Also, it must be simple, reasonable and make use of existing innovations (Awad et al. 2010).

CONCLUSION

The occurrence of HRMs in food products and their capacity to overcome sanitisation and pasteurisation are still a great challenge for the food industry. Mycotoxins from those microorganisms make HRMs more important in terms of public health and cause acute and chronic illnesses in humans. Machines, air and staff practices can impact the contamination level. Prevention of spoilage can be done through the reduction and decontamination of HRMs. However, more researches are required to develop assessments of HRM contamination and elimination.

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