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REVIEW

A bibliometric analysis of research trends on the microbiological safety of low-moisture foods

[1]Onyeaka, H., [1]Anumudu, C., [1]Miri, T. and [2,3,4,*]Ahmad, N.H.

[1]*School of Chemical Engineering, University of Birmingham, Birmingham, B15 2TT, United Kingdom*
[2]*Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[3]*Halal Products Research Institute, Putra Infoport, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[4]*Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

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Abstract

Research has shown that some pathogens, including *Salmonella* and other spore-formers, survive and remain viable in low-moisture foods (LMF). This review highlighted trends in publication and outlook of the research field, using articles published in Scopus between 2001 to 2020. Systematic article retrieval, data analysis, and trend visualization were conducted using Scopus analytical tools, Excel software, and VOSviewer, respectively. A total of 267 articles were published on the microbial safety of LMF, with a rapid rise in the number of publications per year. The U.S. has the highest research output (143 publications), followed by China (35 publications). Washington State University Pullman, USA, has the highest number of publications from a single institution, while Professor Juming Tang is the author with the highest number of publications. Evaluation of trends in LMF research highlighted an initial focus on the detection of possible microbial contaminants in the foods and exploring the relationship between water activity and microbial survival. *Salmonella* was identified as the leading cause of foodborne diseases associated with the consumption of LMF. Newer research is focusing on the molecular characterization of microbial contaminants of LMF and approaches to eliminate these pathogens and spoilage agents from LMF. In recent years, the trend has shifted towards the use of novel technologies for microbial and toxin inactivation to mitigate and minimize the risks due to the presence of these microbial agents and their metabolites in LMF.

1. Introduction

Low-moisture foods (LMF) are defined based on their water activity ($a_w \leq 0.85$) (Codex Alimentarius, 2015). LMF is vastly consumed as ready-to-eat snacks (nuts and nuts spread, dried fruits, cereals), seasonings (spices and herbs), instant-mixed beverages (creamers, milk, protein powders), or used as baking ingredients (cocoa powder, wheat flour). Although LMF is not perceived as a health threat by consumers, food manufacturers, and food regulators, there have been some episodes of LMF foodborne outbreaks and product recalls, causing numbers of hospitalizations and even deaths. The most common etiologic agent linked to LMF is *Salmonella* spp. (Centers for Disease Control and Prevention (CDC), 2004; CDC, 2007; CDC, 2020; CDC, 2008a; C CDC, 2008b; CDC, 2009; CDC, 2010; CDC, 2014; CDC, 2016a; CDC, 2016b; U.S. Food and Drug Administration, 2017; CDC, 2018; European Food Safety Authority, 2018; U.S. Food and Drug Adm istration, 2018b; U.S. Food and Drug Administration, 2018c; U.S. Food and Drug Administration, 2018d; Jones *et al.*, 2019; U.S. Food and Drug Administration, 2019a; Johansen *et al.*, 2021), although few cases linked to *Escherichia coli* (U.S. Food and Drug Administration, 2019b) and *Cronobacter* spp. have been reported (Hayman *et al.*, 2020). *Salmonella* is resilient in LMF due to its ability to produce extracellular polysaccharides which protects them from dehydration (Lehner *et al.*, 2005). Some high-profile LMF foodborne outbreaks include; infant formula powder in Spain (1994) contaminated by *Salmonella enterica* serovar Virchow (Usera *et al.*, 1996), raw almonds in Canada (2005)

*Corresponding author.
Email: *nurulhawa@upm.edu.my*

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contaminated by *Salmonella enterica* serovar Enteritidis (CDC, 2004), peanut butter in the U.S. (2009) contaminated by *Salmonella enterica* serovar Typhimurium (CDC, wheat flour in the U.S. (2019) contaminated by *E. coli* (U.S. Food and Drug Administration, 2019b), rice cakes in Japan (2011) contaminated by shiga-toxigenic *E. coli* (Nabae *et al.*, 2021) and caramel apples in the U.S. (2014) contaminated by *Listeria monocytogenes* (CDC, 2015).

Foodborne disease outbreaks from microbial contamination of low-moisture foods have far-reaching impacts on consumers' health, brand confidence, product recalls, and financial losses. Evaluation of the financial impact of 22 LMF food recalls within a 10-year period in the United States of America showed a Cumulative Abnormal Return (CAR) loss of $243 million. This loss is significant, considering that this was recorded within the United States of America alone. When the scale of global recalls in LMF is taken into consideration, the financial implications will be astronomical (Gomez and Marks, 2020). Similarly, the burden of diseases and deaths continues to rise due to microbial contaminants and their metabolites in LMF. A study by Van Doren *et al*. (2013) which evaluated the scope of foodborne ailments associated with the consumption of dried spices between 1773 and 2010 highlighted that microbial contamination of spices resulted in 1946 cases of human illnesses with 128 hospitalizations and 2 deaths.

Microbial contamination of LMF can be from various sources, including from the farm, during transportation, processing and post-processing handling. Cross-contamination from raw ingredients or the processing environment has been recognized as the leading source of microbial contamination events during the post-processing of LMF (Podolak *et al.*, 2010). A range of factors can affect the presence and survival of microorganisms in LMF. These include; bacterial strains and serotypes (Santillana, 2014), physiological state (Hildebrandt *et al.*, 2016), relative humidity (Jeong *et al.*, 2011), and water activity (Bianchini *et al.*, 2014; Smith and Marks, 2015; Channaiah *et al.*, 2016; Syamaladevi *et al.*, 2016). Furthermore, food composition and structure (Enache *et al.*, 2014; Li *et al.*, 2014; Limcharoenchat and Marks, 2018), inoculation method (Enache *et al.*, 2015; Limcharoenchat *et al.*, 2018), types of water-binding solutes (Mattick *et al.*, 2001; Pena-Melendez *et al.*, 2014), recovery and enumeration method, and temperature (Shachar and Yaron, 2006; Villa-Rojas *et al.*, 2013; Rachon *et al.*, 2016) have been shown to influence *Salmonella* survival and thermal inactivation kinetics in LMF. Although *Salmonella* is the leading pathogen responsible for LMF outbreaks, these critical factors can also be applied to assess the persistence of other foodborne pathogens linked to previous LMF outbreaks.

The inactivation of microorganisms in LMF can be achieved by thermal processes such as extrusion, steam pasteurization and radiofrequency, as well as non-thermal processes such as gaseous technology, high-pressure processing and plasma technology. Given that pathogen inactivation in LMF is influenced by many critical factors, the state-of-art of these pathogen inactivation kinetics should be based on updated scientific literature. Therefore, a bibliometric approach was utilized to collate the current research in LMF and microbial safety, visualizing the volume of the publications and citations thereof. The extent of networking and collaboration among active participating authors was evaluated to identify the authorities in the field and the common research subject within LMF studies, particularly for early researchers. Ultimately, this review aimed to establish the trends in the microbiological safety of LMF, technological advancements in the processing of LMF, publication and funding bodies, and regulatory advances in LMF. This will help researchers and food regulators prioritize significant issues related to LMF contamination and enable the rapid understanding and retrieval of resources.

2. Materials and methods

2.1 Database

The database utilized for this study was generated by searching the Scopus database on April 9th, 2021. In general, only original research and English articles were considered in this review (Table 1). Articles focusing on the survival/inactivation of foodborne pathogens or surrogate microorganisms in low-moisture foods, food model systems, and pet foods were included in this review. Articles focusing on the assessment of cell response against stresses including/excluding desiccated stress (LMF food can be present/absent) were also included. On the other hand, articles focusing on the survival/inactivation of foodborne pathogens in deli meats, animal feed, and food contact surfaces were excluded from this review. In addition, articles that do not contain microbiological analysis or only focus on detection/quantification methods were also excluded. Scopus database was utilized to retrieve published articles because of its broad coverage of journals and articles published in the English language and the natural sciences (Mongeon and Paul-Hus, 2016). In addition, Scopus provides tools for the extensive analysis of retrieved articles regarding the author's affiliation, institutions, and funding bodies, amongst others (Guerrero-bote *et al.*, 2021), thus allowing for the analysis of trends in publications directly.

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Table 1. Inclusion and exclusion criteria of published papers for this review.

Inclusion criteria	Exclusion criteria
• Investigation of the survival/inactivation of foodborne pathogen in low-moisture food model (solutes/humectants)	• Research on the survival/inactivation of foodborne pathogen on food contact surfaces
• Assessment of foodborne pathogen cell response against stresses including/excluding desiccated stress (LMF food can be present/absent)	• Assessment of physical/technical attributes of LMF only (without microbiological analysis)
• Study that assessed survival/inactivation of foodborne pathogen in pet food (direct contact to children)	• Assessment of survival/inactivation of foodborne pathogen in animal (i.e. ruminants) feed
• Utilization of surrogate microorganism(s) targeting foodborne pathogen commonly associated with LMF	• Assessment of growth/inactivation of lactic acid bacteria in LMF during fermentation
• Study that investigated the inactivation of dried foodborne pathogen without the presence of LMF	• Assessment of microbiological analysis in the agricultural setting
• Articles only	• Study that used LMF as antimicrobial compounds without the presence of foodborne pathogen
• English only	• Assessment of the detection/quantification method of foodborne pathogen not related to LMF
	• Study associated with deli meats
	• Review papers

2.2 Search strategy

Publications were retrieved by undertaking a focused search of the keywords ('Low moisture foods', 'Low water activity foods' or 'Dried foods') AND 'Foodborne pathogen') in the “Topic” tab to retrieve documents in which these keywords occurred in the title, abstracts and keywords. The search keywords were encased in the Boolean operator (" ") to ensure the retrieval of only publications that have one or more of the exact keywords utilized in the search without any alterations. Retrieved publications were filtered to select only articles published in the last 20 years (2001-2020). This year's range was chosen to obtain the most current research within the field. The range of 20 years allows for retrieving enough articles to obtain a comprehensive trend in changes and focus of publications within the field. Articles published before the year 2000 were excluded as these may have become obsolete or newer perspectives have emerged. Furthermore, before the year 2000, only a few articles were published on the topic of interest. Articles published within these 20 years were further filtered to select only original articles published in the English language, removing reviews and conference proceedings. Primary research was selected as these are considered direct contributions to the research field and can show improvements within the research field and newer perspectives. Furthermore, reviews tend to have higher citations, which may not fully represent the visibility of research conducted within the field (Miranda and Garcia-Carpintero, 2018). The authors vetted the filtered articles individually to ensure that the publications fit the evaluated scope and preliminarily classified them using Scopus analytical tools before being imported.

2.3 Data validation and bibliometric analysis

Filtered articles were imported to the Excel® format for categorization, analysis and evaluation to ensure no repetition. When duplicates were identified, they were removed manually. Excel® was used to classify the publications based on publication year, publishing institutions, publishing journals, country of origin, funding agencies and author affiliations. VOSviewer software (Jan and Ludo, 2010) was utilized to create a bibliographic map of the publications to analyze the research trends in the field holistically. VOSviewer Software was utilized for visualizations because of its ability to identify and link research based on several parameters, including country of origin, institutional affiliation of authors, co-citing and co-occurring publications [https://www.VOSviewer.com/publications]. This allows for the grouping of research into linked clusters based on the number of connections and the strengths of these publications. Maps generated by VOSviewer highlight the linkages between contributing authors, documents, institutions and funders, allowing for in-depth analysis. Figure 1 highlights the flowchart of processes and steps utilized in selecting and analyzing retrieved publications. Finally, the highest cited publications were categorized according to the publishing country to evaluate the topical issues within the research field across the top publishing countries.

3. Results and discussion

3.1 Trends in publication

The bibliometric search of publications on low-moisture foods' microbiological safety from 2001 to 2020 yielded a total of 414 publications which were filtered down to 267. Analysis of the retrieved database

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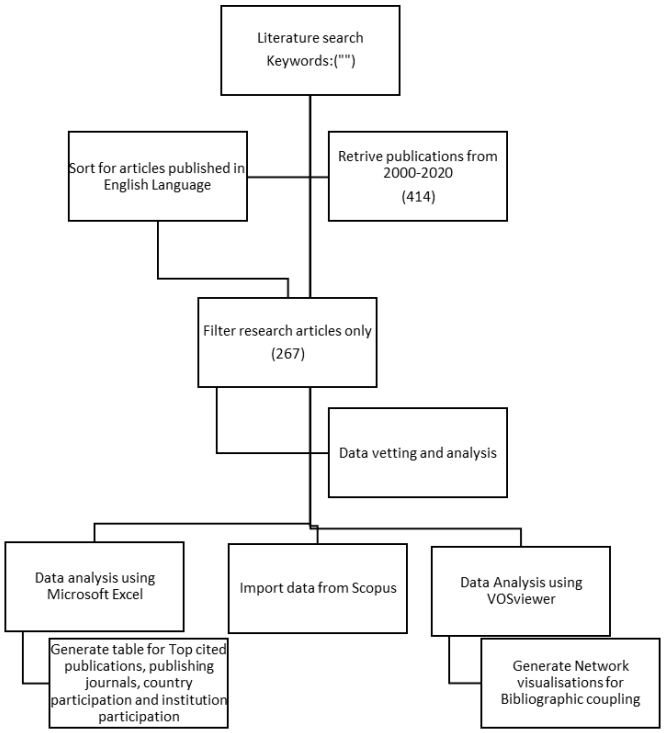

Figure 1. Data retrieval and analysis flowchart.

is based on the number of publications, top publishing journals, institutions, funding organizations, top publishing countries and network analysis. These indicators are chosen to highlight papers and institutions at the forefront of LMF safety, identify leading experts in the field, and explore networks of collaborations across these indicators.

Figure 2 shows the number of publications per year on LMF microbiological safety between 2001 and 2020. For the first 10 years (2001 to 2010), the number of publications per year was between 0 and 2. However, the number of publications sharply increased to 8 in 2011, and this continued to increase yearly, up to 66 papers in 2020. The strong increase in the number of published research in LMF from 2011, can be attributed to several possible factors such as food outbreaks related to LMF, tighter food safety regulations, increased funding for research and technological advances. For example, there were large outbreaks of *Salmonella* in LMF, with over 400 cases linked to black pepper outbreaks in 2010 (Centers for Disease Control and Prevention, 2010); over 700 cases linked to peanut butter and peanut butter-containing products between 2007 and 2009 (Centers for Disease Control and Prevention, 2007; Centers for Disease Control and Prevention, 2009). In addition, the Food Safety Modernization Act (FSMA) 2011 in the U.S. seems a key contributor as well (U.S. Food and Drug Administration, 2018a). The LMF research field is fast-growing with doubling of the research output within the years under review. This indicates interest in consumption as well as the safety of LMF. Furthermore, there is a vast aspect of LMF research that has not been fully explored and is spurring a renewed interest in the field. These include; processing technologies to improve the safety of LMF (Wason *et al.*, 2021), bacteria survival and growth kinetics on LMF (Finn *et al.*, 2013; Podolak *et al.*, 2017; Xie *et al.*, 2021) and investigations into the predictors of heat resistance of microorganisms in LMF (Garces-vega *et al.*, 2019). These research gaps have increased the yearly output of publications in LMF with a corresponding increase in citation and visibility of published literature.

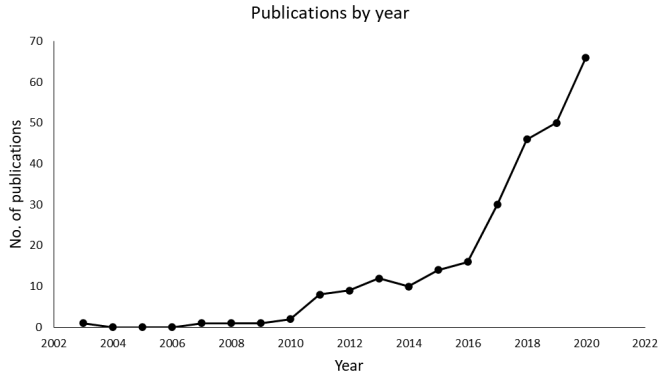

Figure 2. The total number of publications in LMF studies between 2001-2020.

The focus of research in LMF within the years evaluated was predominantly on

- Food microbiota
- Genomics of contaminants
- Inactivation mechanisms
- Growth kinetics
- Thermal resistance
- Process engineering
- Food outbreak investigations

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Earlier publications in LMF focused on the isolation and classification of microbial genera, toxins and metabolites from food products and advanced mechanisms for their inactivation. Publication focus morphed towards the molecular characterization of the microbial contaminants of LMF which persisted over the years (Lee *et al*., 2009; Jošić *et al.*, 2017). From the year 2011, studies focused more on the relationship between the food properties and survival of microbial agents within the foods, with emphasis on food product modifications for improved microbial safety (Velmourougane *et al.,* 2011a; Beuchat *et al*., 2013. The trend shifted towards the utilization of novel technologies for the pretreatment of foods and modelling of food processing methods (Lambertini *et al*., 2016; Farakos *et al*., 2016) for more effective inactivation of microbial toxins. Studies on novel food processing techniques investigated amongst others; high-pressure carbon dioxide (Chen *et al*., 2017), vacuum steam pasteurization (Shah *et al*., 2017), radio-frequency (Villa -Rojas *et al*., 2017), ultrasound and supercritical carbon dioxide (Michelino *et al*., 2018), gamma radiation (Odai *et al*., 2019). In more recent years, LMF studies have shifted to proteomics of LMF and contaminating microorganisms (Hu *et al*., 2020), new product development (Maia *et al*., 2020; Paz *et al*., 2020) and genetic survival mechanisms of LMF pathogens (Jayeola *et al*., 2020; Maerani *et al*., 2020).

3.2 Characteristics of low-moisture food research publications

A total of 58 journals contributed to LMF safety publications, with an average publication of 4.6 per journal. The top ten publishing journals are shown in Figure 3. The highest publication was by the Journal of Food Protection with 65 papers, followed by the International Journal of Food Microbiology (23 publications), while the Journal of Food Engineering with 6 papers is ranked 10th. The high contribution by the Journal of Food Protection and the International Journal of Food Microbiology is expected because the scope of these journals is on microbial food quality, prevention of food-related hazards, as well as risk assessments and mitigation. The top 10 active institutions on LMF safety are shown in Figure 4. Washington State University Pullman with 30 papers is the highest active, closely followed by the Food and Drug Administration and College of Agriculture with 27 and 26 papers, respectively. As most of the LMF-related outbreaks happened in North America (Harris and Yada, 2019; Yada and Harris, 2019), it is not surprising to see the top active LMF safety institutes from North America. This is in line with the finding of Figure 5, which shows the top 20 funding sources for LMF safety. It is clear that the majority of the top 20 funders are U.S. institutes. It is not surprising because the top funder, the National Institute of Food and Agriculture (NIFA), has allocated a budget of $1.3 billion in 2019, $1.4 billion in 2020, and $1.6 billion in 2021 to encourage agricultural-based research between research universities and federal agencies (U.S. Department of Agriculture, 2019; U.S. Department of Agriculture, 2020; U.S. Department of Agriculture, 2021). Compared to other countries such as the United Kingdom, between 2009 and 2019, the Food Standards Agency (FSA) suffered a budget cut of 51% (Food Safety Agency, 2019; Food Safety Agency, 2020; Food Safety News, 2020).

Similarly, in the African continent, most available funding is spent on ensuring food security, food production and processing and not on food food-based research, coupled with armed conflicts that destabilize the region (Sasson, 2012). However, these trends are changing, both for the United Kingdom and Africa at large, with the Research Councils UK (RCUK) instituting the Global Challenges Research Fund (GCRF), a £1.5 billion fund that aims at improving cutting-edge research within various areas in developing countries, including food safety research which is of interest to the United Kingdom (Global Challenge Research Fund, 2015). This fund was instituted in 2015, and with its disbursement, it is expected that research output with regard to food safety and other spheres will increase in the coming years.

In the U.S., recurrent multistate outbreaks associated with LMF highlight the critical need for food manufacturers to validate their current pathogen-kill step strategies through the incorporation of standard protocols and emerging novel techniques such as radio frequency (Jeong *et al.*, 2020) plasma technology (Choi *et al.*, 2020), isothermal treatment, pulsed light (Prasad *et al.*, 2019), gamma irradiation (Odai *et al.*, 2019), supercritical carbon dioxide and ultrasound (Morbiato *et al.*, 2019), freeze drying and other techniques. A total of 30 publications within the years under review focused on the intervention technologies that can be employed within the food industry to control or reduce the growth of microorganisms in LMF. The Food Safety Modernization Act (FSMA) 2011 is the most recent transformation of the U.S. food law and regulations, focusing on a preventive-oriented approach against foodborne illness. Food manufacturers from all sectors need to be FSMA compliant before 2024 (U.S. Food and Drug Administration, 2018a). A thorough review of FSMA requirements pertaining to LMF has been highlighted by Barach and Dunaif (2017). Nevertheless, one of the biggest hurdles in providing scientific-based evidence to mitigate foodborne pathogen contamination

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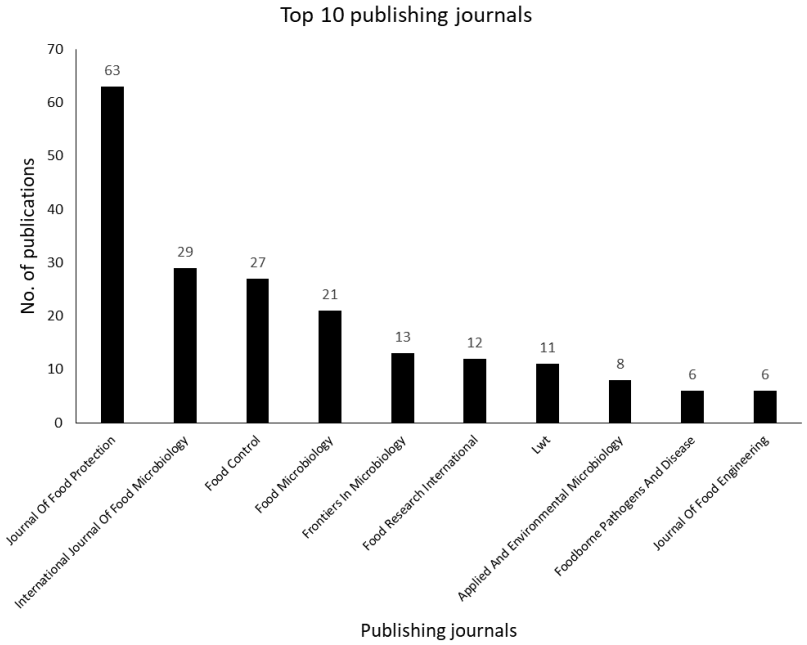

Figure 3 . Scopus selected publishing journals for LMF studies between 2001-2020.

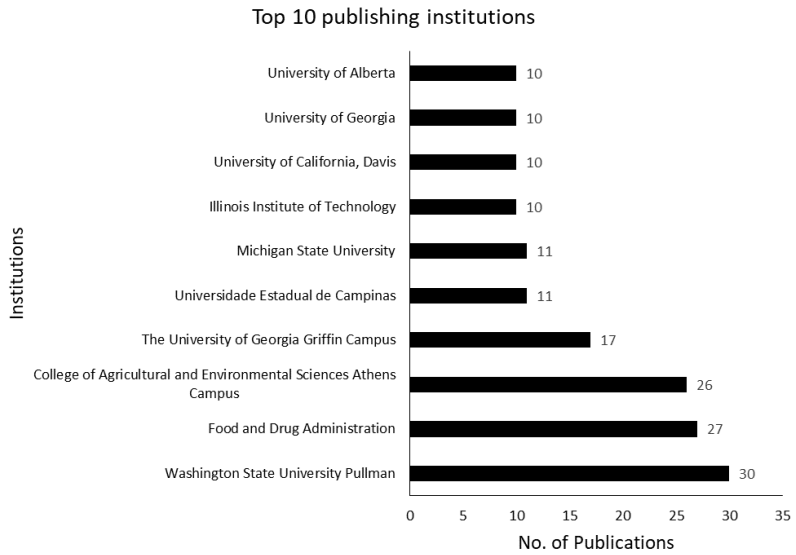

Figure 4. Top 10 institutions publishing in the LMF studies between 2001-2020.

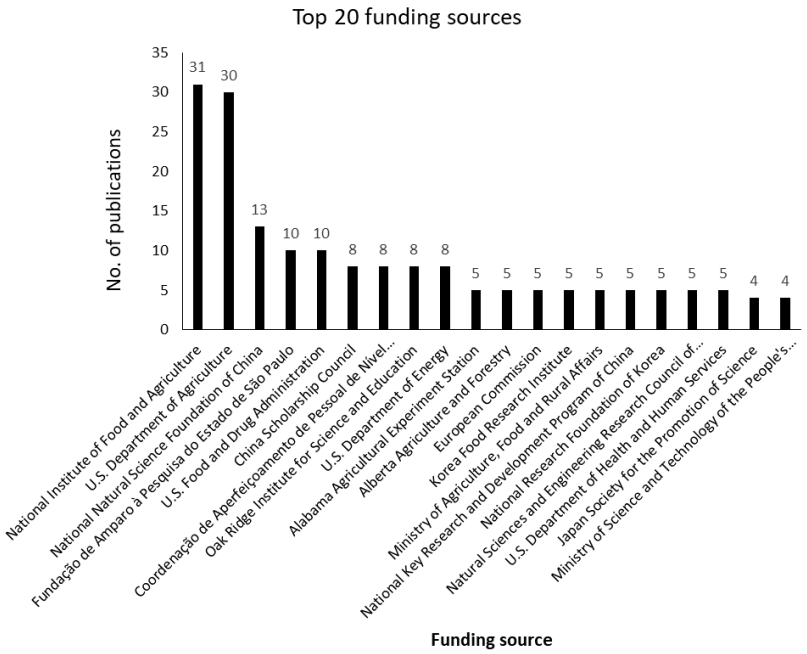

Figure 5 . Top 20 funding sources publishing in the LMF studies between 2001-2020.

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is limited knowledge on factors contributing to pathogen survival available regarding LMF processing. Therefore, major research grants through the National Institute of Food and Agriculture (NIFA) have supported researchers to address this need, thus yielding to most publication outputs in the U.S. (Figure 6).

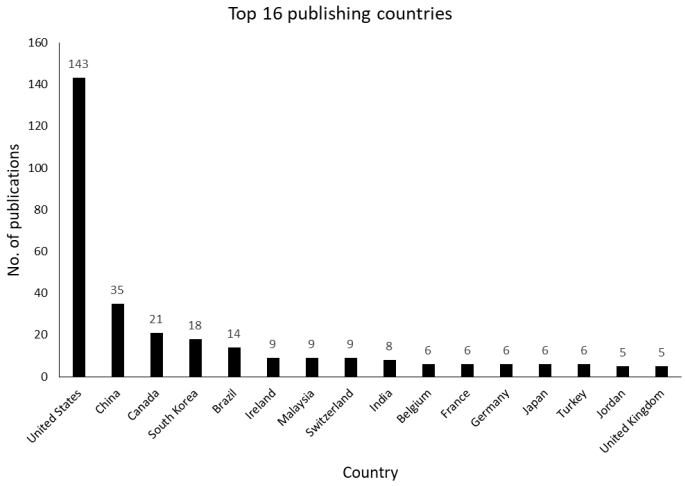

Figure 6. Top 16 publishing countries in the LMF studies between 2001-2020.

The geographic distribution of LMF publications can be partly attributed to the commodity of interest for the countries (Figure 7). For instance, the U.S. global producer of almonds with 90% of worldwide production is in California. Major *S. enterica* ser. Enteritidis outbreaks linked to raw almonds affected both the U.S. and Canada in 2004 (Centers for Disease Control and Prevention, 2004), causing 13 million pounds of almonds to be recalled. Later in 2011, the Almond Board of California established a guideline for validating the thermal process of raw almonds using a non-pathogenic bacterial strain, *Enterococcus faecium* NRRL B-2354, as a surrogate for *S. enterica* ser. Enteritidis PT30 because *E. faecium* NRRL B-2354 consistently exhibited higher thermal resistance than the target pathogen. These findings have led more researchers to utilize surrogate microorganisms as a pathogen-kill step validation tool in nuts and nut products (peanut butter, pistachio) and in wheat flour, spices, baked goods, dried fruits, and milk powders. In European countries such as Belgium, a study on detecting a low number of *Salmonella* in chocolate was published in 2011. This might be attributed to the multi-country *Salmonella enterica* serovar Oranienburg outbreak linked to chocolate in 2005, affecting another five countries, including Austria, Denmark, Finland, Netherlands, and Sweden (Werber *et al.*, 2005), as well as *Salmonella enterica* serovar Montevideo outbreak linked to chocolate affecting 42 persons in the U.K. (British Broadcasting Corporation, 2007). The implication of commodity-specific studies might explain why North America is the most active region involved in LMF studies.

3.3 Author contributions to low-moisture food research

Among 905 authors that contributed to research within this field, 94 authors met the criteria of being cited a minimum of 3 times (Figure 8). Most authors concentrated in Cluster 1, 2, 3, 4, and 6 come from different research groups across the U.S. and are active in LMF studies, especially during post-FMSA establishment. The highest authorship is by Juming Tang (21 documents, 550 citations and a total link strength of 44), followed by Meijun Zhu (12 documents, 243 citations and a total link strength of 29) and Larry Beuchat (10 documents, 183 citations and a total link strength of 7). Juming Tang's publications focused on the thermal inactivation of microorganisms, especially *Salmonella* in LMF and the impact of water activity at elevated temperatures (Villa-Rojas *et al.*, 2013; Syamaladevi *et al.*, 2016), nonthermal and emerging methods of microbial inactivation such as radiofrequency (Villa-Rojas *et al.*, 2017). Meijun Zhu's research interest revolved around the influence of thermal processing and water activity on microbial survival (Tsai *et al.*, 2019) and the potential of antimicrobial additives in retarding

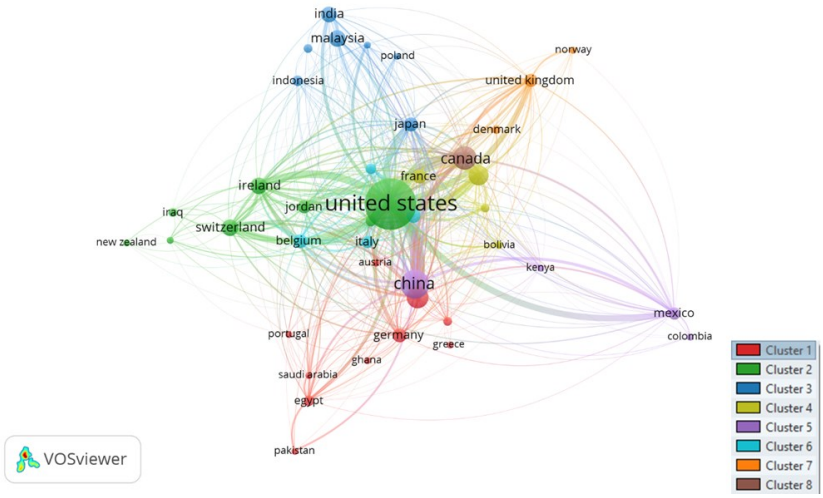

Figure 7. Network visualization of bibliographic coupling (collaborations) amongst all countries (colour).

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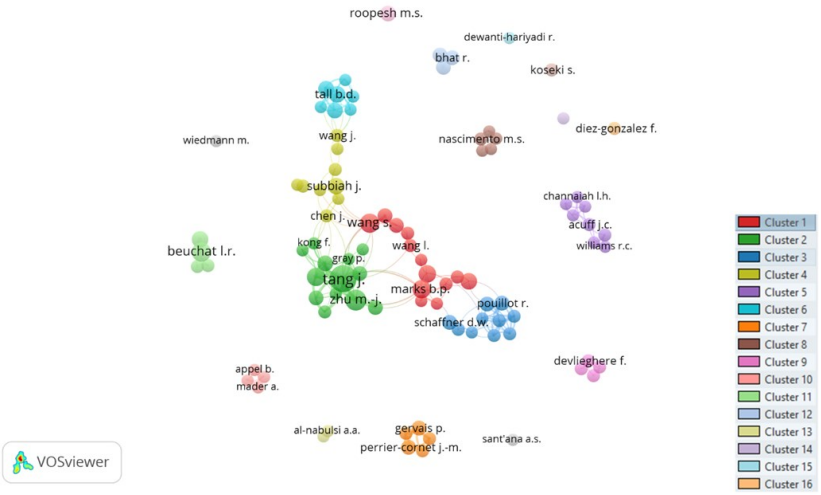

Figure 8. Co-authorship association of all authors with 3 publications and above (colour).

microbial growth and survival in LMF (Tsai *et al.*, 2017). Similarly, Larry Beuchat focused on the toxigenicity of LMF microbial contaminants (Kim *et al.*, 2014) and the inactivation of these contaminants using emerging techniques such as gaseous chlorine dioxide and sanitisers (Beuchat *et al.*, 2012; Beuchat, Mann and Alali, 2013; Lee *et al.*, 2018). The strongest co-authorship collaboration is between Tang and Zhu (11 collaborations) (Figure 8), and this could be attributed to the synergistic relationship they share within the same institution (Washington State University) as Juming Tang is a Biosystem Engineer whereas Meijun Zhu is a Food Scientist of the research group. The strongest co-authorship collaboration is between Tang and Zhu (11 collaborations) (Figure 8), and this could be attributed to the synergistic relationship they share within the same institution (Washington State University) as Juming Tang is a Biosystem Engineer whereas Meijun Zhu is a Food Scientist of the research group.

3.4 Analysis of keywords associated with low-moisture food research

Figure 9 shows the 80 keywords that had occurred a minimum of 20 times. The highest occurring keyword is "*Salmonella*" (134 occurrences and a total link strength of 728). This is followed by "food microbiology" (123 occurrence and total link strength of 807), and "food control" (102 occurrence and total link strength of 745). The strongest linkage is between "food microbiology" and "food control" (link strength 102), followed by "food microbiology" and "*Salmonella*" (link strength 85). In general, these keywords are strongly relevant as most LMF outbreaks have been linked to *Salmonella.* LMF studies on *Salmonella* focused on its survival mechanisms, the use of thermal and nonthermal technologies to inactivate foodborne pathogens, and predictive modelling to assess the risk of *Salmonella*. Therefore, the mixture of generic terms such as "low-moisture foods", "process validation", "predictive modelling" and specific terms including "*Salmonella* survival" and "*Salmonella* surrogate", can be utilized for upcoming LMF studies to help improve articles' visibility during literature search in online databases (Egan *et al.*, 2012). The scope of the problem posed by *Salmonella* in LMF is large. *Salmonella* was reported to cause food poisoning in 1,000 people in Germany and over 400 cases in the U.S. were recorded due to the consumption of contaminated paprika powdered potato chips (Lehmacher *et al.*, 1995) and black pepper (Centers

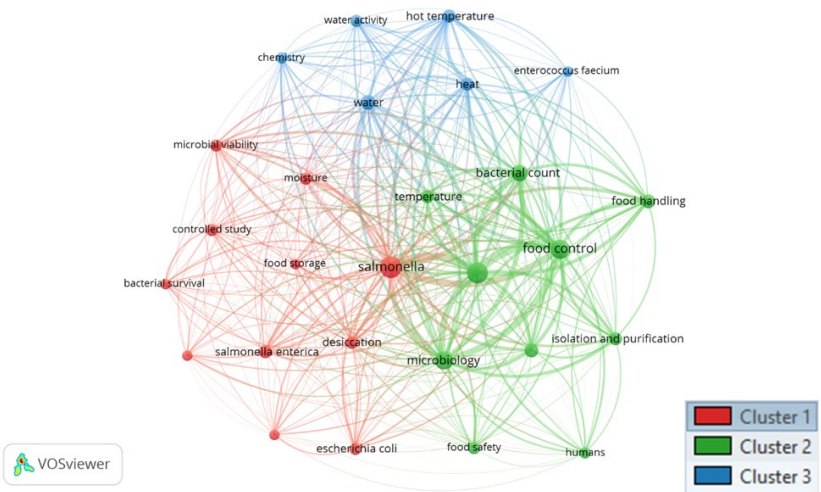

Figure 9. Co-occurrence of keywords that appeared a total of 20 times and above (colour).

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for Disease Control and Prevention, 2010), respectively. Similarly, more than 200 cases were linked to toasted oats cereal in the U.S. between April and June 1998 (Centers for Disease Control and Prevention, 1998); and over 400 cases were linked to peanut butter between August 2006, and May 2007 in the United States of America (Centers for Disease Control and Prevention, 2007). In peanut butter and peanut butter-containing products between 2008 and 2009 over 700 foodborne disease cases were recorded (Centers for Disease Control and Prevention, 2009). Spices typically receive minimal pathogen inactivation step, and previous studies have shown that *Salmonella* can survive up to 8 months in black peppers (Keller *et al.*, 2013). In high-fat products such as peanut butter, *Salmonella* resists thermal inactivation because fat content may provide a protective barrier for *Salmonella* (Enache *et al.*, 2014). From Table 2, other microorganisms that have been implicated with contaminating LMF are *Aspergillus* (Velmourougane *et al.*, 2011b), *Cronobacter* (Yan *et al.*, 2013; Koseki *et al.*, 2015), *Cronobacter* (Yan *et al.*, 2013; Koseki *et al.*, 2015; Lang *et al.*, 2017), *Bacillus* (Lee *et al.*, 2009; Frentzel *et al.*, 2018), and *Escherichia* (Koseki *et al.*, 2015; Lang *et al.*, 2017).

3.5 Highly cited low-moisture food publications

Out of the 267 selected documents, 116 met the criteria of being cited a minimum of 10 times and of these, 88 are co-cited with the other 113 (Figure 10). The highest cited publication is Villa-Rojas *et al.* (2013), with 94 citations. Given that the U.S. has been implicated in repeated *Salmonella* outbreaks associated with almond and almond products, validating the thermal processing of almonds is critically needed. Villa-Rojas *et al.* (2013) suggest that increasing a_w of almond flour up to ~0.95 prior to heating at 60°C for 12 mins can achieve a minimum of 4-log reduction, as recommended by the USDA. This finding supports the common practices in almond processing where washing or prewetting almonds prior to being subjected to heat treatment can be performed to achieve recommended *Salmonella* reduction while minimizing the impact on the quality of almonds. In addition to a_w, cell physiological state can be another factor for *Salmonella* persistence in LMF. According to Finn *et al.* (2013), *Salmonella* producing biofilm isolated from LMF processing environment is more resistant to sanitation than planktonic cells. All in all, a_w and cell physiological state are among the critical factors that have been consistently identified to influence *Salmonella* survival in LMF, as addressed in the recent validation guidelines for LMF control measures (Ceylan *et al.*, 2021). Meanwhile, the highest co-cited reference is Podolak *et al.* (2010) (23 citations and a total link strength of 134), followed by Beuchat, Mann and Alali (2013) (20 citations and a total link strength of 102). The strongest linkage was between Podolak *et al.* (2010) and Beuchat, Mann and Alali (2013) (6 links), Podolak *et al.* (2010) and Burnett *et al.* (2000) (6 links), Beuchat, Mann and Alali (2013) and Hiramatsu *et al.* (2005) (6 links) (Figure 11).

Table 2 shows information about the most cited papers on LMF studies from 16 countries with a minimum of 5 publications between 2001 - 2020. Of the 16 countries that have contributed to research within this field, the highest contributors are the U.S. (143 total publications), followed by China (35 Total publications). Although the U.S. is the highest contributor to the LMF research area, Table 2 suggests that Asian and European countries are at the forefront of research on LMF, out of the 16 countries, 12 countries (France, Germany, Ireland, Switzerland, Belgium, Turkey, India, China, Malaysia, Japan, Jordan and South Korea) are represented from these regions. The U.S. had the highest publication with 53.35% of the publications, followed by China with 13.05% of publications; Jordan and the United Kingdom have the lowest (1.86%) percentage of publications related to LMF within the list. In terms of the total number of citations, the U.S. (total citations of 2097) topped the table, followed by China (total citations 539) and Canada (total citations 180). The research output suggested that these countries are leading the research on LMF.

3.6 Outline of impactful articles in low-moisture food

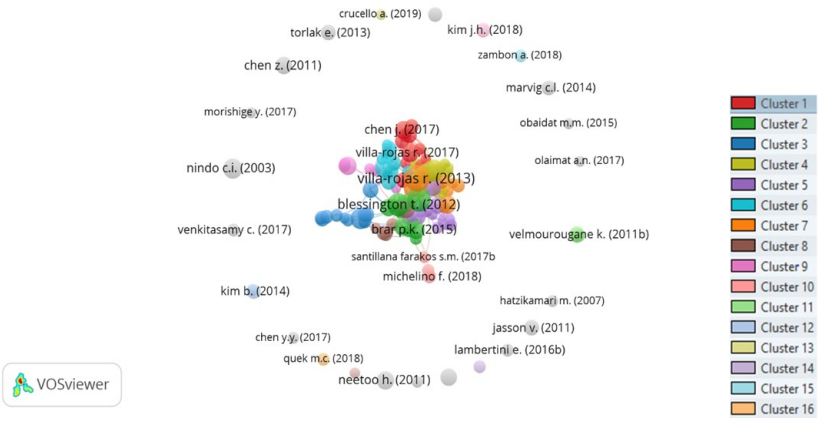

Figure 10. Citation relationship of all documents with 10 citations and above (colour).

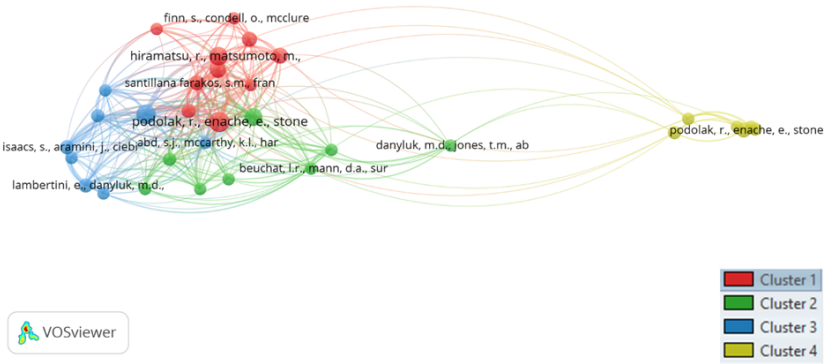

Figure 11. Co-citation pattern of all references with 7 or more citations (colour).

Table 2. Top 16 countries with 5 publications and above in the LMF studies between 2001-2020.

S/N	Country/ Territory	Total publication (TP)	Percentage of publication	Total citation TC)	Average citation (TC/TP)	Most-cited document	No. of citations
1	United States	143	53.35%	2097	14.66	Villa-Rojas *et al.* (2013)	94
2	China	35	13.05%	539	15.4		
3	South Korea	18	6.71%	257	14.27		
4	Canada	21	7.83%	180	8.57	Syamaladevi *et al.* (2016)	60
5	Brazil	14	5.22%	71	5.07	Crucello *et al.* (2019)	10
6	Ireland	9	3.35%	110	12.22	Yan *et al.* (2013)	25
7	Switzerland	9	3.35%	74	8.22	Izurieta and Komitopoulou (2012)	29
8	United Kingdom	5	1.86%	58	11.6		
9	Malaysia	9	3.35%	114	12.66	Lee *et al.* (2009)	31
10	India	8	2.98%	79	9.87	Velmourougane *et al.* (2011a)	26
11	Belgium	6	2.23%	65	10.83	Jasson *et al.* (2011)	24
12	France	6	2.23%	48	8.0	Lang *et al.* (2017)	20
13	Germany	6	2.23%	41	6.83	Frentzel *et al.* (2018)	13
14	Japan	6	2.23%	101	16.8	Koseki *et al.* (2015)	36
15	Turkey	6	2.23%	42	7.0	Torlak *et al.* (2013)	26
16	Jordan	5	1.86%	37	7.4	Olaimat *et al.* (2017)	11

research

Although citation impact is not the sole determinant to assess the research significance of individual researchers, research teams, or institutions, it is by far the most popular and simple tool for scientometric evaluation (Aksnes *et al.*, 2019). The most impactful paper is Villa-Rojas *et al.* (2013) with 92 citations. This study evaluated and modelled the heat inactivation of *S. enterica* ser. Enteritidis PT30 in a cultivar of almond kernel flour at different water activity values and temperatures. It was found that less time was required for the inactivation of microbial cells and reduction in population density at higher water activity. This is followed by (Syamaladevi *et al.*, 2016) authored in Canada with 60 citations. This study explored the relationship between temperature-induced changes in water activity and the thermal resistance of *Salmonella* in flour and peanut butter at high temperatures. It was found that increasing sample temperature from 20 to 80°C resulted in an increase in the water activity of all-purpose flour from 0.45 to 0.80, whereas there was a decrease in peanut butter from 0.45 to 0.04, giving new insight into how different compositions of low-moisture foods can affect temperature-induced changes in water activity. The article by Crucello *et al.* (2019), which was authored in Brazil and had ten citations was an exploratory study on the survival of *S. enterica* ser. Typhimurium. This study inoculated *S. enterica* ser. Typhimurium into four low-water activity foods (milk chocolate, powdered milk, black pepper, and dried pet food) and evaluated the transcriptome using RNA-Seq. The study found that gene regulation is influenced by food composition. Yan *et al.* (2013), with 25 citations (Ireland) report on the phenotypic characteristics and complete genome of *C. sakazakii* SP291, which has been implicated in human infections as a result of the consumption of powdered infant formula (PIF), whereas the article from Switzerland and the United Kingdom (Izurieta and Komitopoulou, 2012) (29 citations)

evaluated the heat resistance of *Salmonella* serovars (Enteritidis, Montevideo, Napoli, Oranienburg, Poona, Senftenberg and Typhimurium). It was found that the *S. enterica* ser. Oranienburg and *S. enterica* ser. Enteritidis PT30 were the most heat-resistant strains in cocoa and hazelnut shells. Other impactful research includes; Lee *et al.* (2009) (Malaysia, 31 citations), which evaluated the prevalence and enumerated *Bacillus* spp. and *B*. cereus in processed cereal products using the MPN-PCR method. In this research, it was found that 78% of processed cereal products destined for human consumption were contaminated by *B. cereus*, ranging from 30 MPN/g to more than 24,000 MPN/g. This is of relevance to food safety and highlights the importance of this method in addressing microbial food safety concerns by *B. cereus*. The study by Velmourougane *et al.* (2011a) evaluated the ability of commercial yeast (*Saccharomyces cerevisiae*) to inhibit Ochratoxin-A (OTA) formation by *Aspergillus niger* and *Aspergillus ochraceus* in coffee. The findings indicate that commercial yeast has an antagonistic and biocontrol potential to reduce contamination of coffee beans by ochratoxin producing mould and OTA contamination of coffee during on-farm processing. This is important in understanding the dynamics of mycotoxin formation in LMF. The highest publication (24 citations) from Belguim is Jasson *et al.* (2011), which studied two rapid detection methods [iQ-CheckTM *Salmonella* IIreal-time PCR (Bio-Rad) and VIDAS® Easy SLM (BioMérieux)] was employed to detect injured cells of *S. enterica* in chocolate. The research showed that the enrichment step is important for the correct enumeration of *S. enterica* cells in chocolate. Lang *et al.* (2017) (France, 20 citations) analyzed the effect of temperature and water activity on the decimal reduction time of *S. enterica* ser. Typhimurium, *S. enterica* ser. Senftenberg, *C. sakazakii* and *E. coli* using predictive modelling. The results showed that *S. enterica* ser. Typhimurium was the most resistant to temperature variations while the most resistant to water activity variations is *E. coli*. Similarly, Frentzel *et al.* (2018) (Germany, 13 citations) distinguished and quantified several groups of *B. cereus* in condiments using real-time PCR. The quantitative multiplex real-time PCR coupled with a DNA extraction method was an effective culture-independent approach for quantifying *B. cereus*. While Koseki *et al.* (2015) (Japan, 36 citations) evaluated the ability of four bacteria pathogens (*C. sakazakii*, *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes*) in powdered infant formula to survive desiccation conditions. The desiccation tolerance was in the range of *C. sakazakii* > *Salmonella* > *E. coli* O157:H7 > *L. monocytogenes*. This study further reveals through Monte Carlo simulation and the Weibull model that the storage temperature of powdered infant formula affected the survival of each of the analyzed bacterium. Torlak *et al.* (2013) (Turkey, 26 citations) evaluated the efficacy of gaseous ozone in reducing the microbial load of dried oregano at different concentrations. Ozonation at 5.3 mg/L for 90 mins resulted in over a 3.2 log reduction in the aerobic count of *Salmonella* serotypes and significantly more by 2.8 and 3.7 log after treatment with 2.8 and 5.3 mg/L of ozone for 120 mins. The final product obtained had an acceptable flavour, taste and appearance, highlighting that ozone treatment is a viable alternative for reducing microorganisms in dried oregano. Finally, Olaimat *et al.* (2017) (Jordan, 11 citations) evaluated the survival of *Staphylococcus aureus* in commercial undiluted tahini and highlighted that the organism can grow in the hydrated tahini. It further reveals that citric or acetic acid was able to reduce a load of *Staphylococcus aureus* in commercial tahini and to undetectable levels in the hydrated tahini.

3.7 Fungi and mycotoxins in low-moisture food

It is important to note that, dry and low moisture foods are notoriously prone to contamination by fungi due to their minimal water activity requirements. Fungi can grow and possibly produce toxins in foods with water activity as low as 0.60-0.70 (Pitt and Christian, 1968, Mugnier and Jung, 1985). The most implicated foods are stored grains, nuts and cereal products (Elfituri *et al.*, 2018; Ezekiel *et al.*, 2020). Contamination of LMF by one fungi species can result in a succession and can eventually lead to the growth of mycotoxigenic species such as *Aspergillus* and *Fusarium,* which are known to produce the mycotoxins aflatoxin and fumonisin, respectively. These mycotoxins have been classified as carcinogens (Daou *et al.*, 2021). The scale of contamination can be high, with one food product having several fungal species, metabolites and mycotoxins. A study that evaluated the fungal diversity and mycotoxin contamination in ready-to-eat low moisture foods in Nigeria recorded twenty-seven species of fungi across 12 genera, producing 21 uncommon metabolites and twenty -four mycotoxins (Ezekiel *et al.*, 2020). Additionally, fungi (yeast and moulds) can resist heat treatments that low moisture foods receive due to the nature of the fungi spores as well as the protective nature of some food components (Tournas, 1994; Dijksterhuis, 2007). The mycotoxins and other metabolites of fungi growing on low-moisture food are of public health significance as they make nutrients unavailable and have been linked to several disease conditions, including acute toxicosis, cancers, neural tube defect and reduced nutrient content (Gelineau-van *et al.*, 2009; Adam *et al.*, 2017; Liew and Mohd-Rizwan, 2018; Claeys *et al.*, 2020). From the database generated, 10 publications in LMF focused on fungi contamination and spoilage of LMF. Investigation

https://doi.org/10.26656/fr.2017.8(2).277

of fungal agents in LMF is an emerging area of interest. Current research focuses on toxigenic moulds, the use of genomics to sequence contaminating fungi, novel technologies for their elimination and the impacts of processing on mould development. Further areas of interest include modelling and the management of fungal and mycotoxin contamination in LMF. Table 3 further summarizes publications regarding fungi contamination and mycotoxins in LMF, showcasing contaminating fungi/mycotoxins, food products investigated and processing technologies utilized.

3.8 Microbial inactivation in low-moisture food, future perspectives

Microbial inactivation in LMF under isothermal and non-isothermal conditions is actively investigated to reduce foodborne outbreaks and product recalls associated with LMF. Nevertheless, there is yet no regulated minimum pathogen reduction level for LMF process validation. One of the possible reasons is the complexity of determining critical parameters influencing pathogen resistance in LMF as it is process- and product-dependent. In this regard, microbial inactivation models using parameter estimation can serve as a robust analytical tool for LMF process validation. The key strength of the parameter estimation approach is parameter errors are considered and the parameter has a physical meaning, which can increase parameter accuracy for predictive modelling (Dolan and Mishra, 2013). Given that the inactivation trend of microbes in LMF is not always linear, the use of linear models to fit nonlinear microbial inactivation data should be avoided to better predict the safe level of pathogen reduction in LMF processing. Development of user friendly web-based predictive modelling tools such as DMFit, GInaFiT, IPMP 2013/GraphPad Prism, and Microrisk Lab (Stavropoulou and Bezirtzoglou, 2019; Liu *et al.*, 2021) are great resources for researchers and the food industry for conducting in-house process validation for estimating microbiological risk assessment. Although caution must be taken to not overestimate the existing data, predictive modelling tools can narrow down the set of intrinsic and extrinsic variables pertaining to the product of interest, hence able to reduce experimental burden and cost. Altogether, the use of surrogate microorganisms in process validation, predictive modelling tools and free access databases can provide promising solutions to ensure the safety of LMF.

4. Conclusion

The survival of some microbial species in LMF is of concern because many LMF is consumed without further rigorous processing to eliminate microorganisms. Of paramount concern are milk powder, nuts and infant formulas, which pose a higher risk. The review of published literature within the past 20 years has highlighted a growing number of publications on the microbiological safety of LMF. This growth in recent years indicates a rising interest in microbial foodborne illnesses from low-moisture foods. This is due to rising reports of foodborne illnesses linked to the consumption of LMF and more stringent legislation regarding the safety of this group of food.

Research in LMF cuts across various fields, including food production, microbiology, agriculture, molecular biology, legislation and policy, with emphasis on novel food processing techniques for microbial inactivation in LMF and product stability. For the assurance of microbial food safety of LMF, control measures need to be harmonized at the food preparation/ canning level, as most contamination is due to a lack of adequate sanitation and post-processing contamination. Thus, efficient cleaning, food handling, and hygienic measures must be incorporated in LMF processing instead of extensively depending on low water activity to ensure microbial safety of the products. Further studies are required on the mechanism of microbial survival in LMF to design newer approaches for their control. The majority of publications are limited to bacteria; however, fungi are considered xerophilic groups, and thus, there is a higher probability of their survival in LMF. Therefore, more emphasis should be placed on research related to the fungal contamination of LMF and mycotoxins, which are of public health significance.

Conflict of interest

The authors declare no conflict of interest.

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Table 3. LMF publications with a focus on fungi and mycotoxins.

S/N	Title	Fungi/mycotoxin	Food product	Processing technology/ Method	Brief Summary	Reference
1	Fungal microflora in dried persimmon fruits	*Rhizopus* spp. *Penicillium* spp. *Aspergillus* spp.	Dried Persimmon Fruits		Dried persimmon fruit has a high fungi contamination, up to 95% of samples were contaminated with 17 different genera of moulds	Gündüz *et al.* (2020)
2	Fungal contamination of food commodities in Durban, South Africa	*Aspergillus* spp. *Penicillium* spp. *Fusarium* spp.	Bambara groundnut Maize and maize -derived products Rice spices		Ready to eat LMF products are heavily contaminated with fungi including mycotoxin producers which are of public health concern	Olagunju *et al.* (2018)
3	Analyses of fungal community by Illumina MiSeq platforms and characterization of *Eurotium* species on Liupao tea, a distinctive post-fermented tea from China	*Eurotium* spp. *Aspergillus* spp.	Liupao tea	Next generation (MiSeq) sequencing	A novel MiSeq (illumine-based) sequencing technique was utilized to evaluate fungi contamination of the Chinese Liupao dark tea. Taxonomic data showed contamination by 3 phyla, 6 families and 8 genera of fungi including *Eurotium* and the mycotoxin producer *Aspergillus.*	Mao *et al.* (2017)
4	Verification of radio frequency pasteurization treatment for controlling *Aspergillus parasiticus* on corn grains	*Aspergillus parasiticus*	Corn grains	Radio-frequency	Hot-air assisted radiofrequency heating was used in the pasteurization of corn samples while maintaining food quality. The treatment resulted in a 5-6 log reduction of *Aspergillus parasiticus,* indicating the applicability of this method in the control of fungal contaminants of LMFs.	Zheng *et al.* (2017)
5	Composting coffee wastes, a potential source of ochratoxigenic fungi and ochratoxin A contamination	*Aspergillus ochraceus* Ochratoxin A (OTA)	Coffee waste		This study investigated the growth of *Aspergillus ochraceus* and the production of the mycotoxin ochratoxin A by the fungi during the composting of coffee and coffee wastes. The study highlighted the persistence of ochratoxins in the LMF throughout the fermentation process, indicating the risk of mycotoxins associated with coffee.	Velmourougane *et al.* (2012)
6	Impact of delay in processing on mould development, ochratoxin-A and cup quality in arabica and robusta coffee	*Aspergillus ochraceus* Ochratoxin A (OTA)	Arabica and robusta coffee		This study investigated the impact delay between the harvesting of coffee berries and the onset of processing has on coffee quality, fungal growth and or mycotoxin production. It was found that although this delay affects the processing attributes such as drying times, rate and cup quality, there was an increased risk of ochratoxin	Velmourougane *et al.* (2011b)

Table 3 (Cont.). LMF publications with a focus on fungi and mycotoxins.

S/N	Title	Fungi/mycotoxin	Food product	Processing technology/ Method	Brief Summary	Reference
7	Modelling inactivation by aqueous chlorine dioxide of *Dothiorella gregaria* Sacc. and *Fusarium tricinctum* (Corda) Sacc. spores inoculated on fresh chestnut kernel	*Dothiorella gregaria* *Fusarium tricinctum*	Chestnut kernel	Aqueous chlorine dioxide inactivation	The study developed in vivo models for the inactivation of fungal spores, simulating commercial storage environment of chestnut kernels. Results obtained indicates that aqueous chlorine dioxide sanitizing techniques can retard fungal growth and can be employed in the prevention of chestnut kernel decay.	Chen and Zhu (2011)
8	Management of *Aspergillus ochraceus* and Ochratoxin-A contamination in coffee during on-farm processing through commercial yeast inoculation	*Aspergillus ochraceus* Ochratoxin A (OTA)	Coffee	Commercial yeast inoculation	This study attempted to reduce the contamination of coffee by *Aspergillus ochraceus* and the production of Ochratoxin by artificially inoculating *Saccharomyces cerevisiae* into the coffee during fermentation. This was found to reduce significantly the occurrence of mycotoxigenic moulds in the coffee beans, without negatively affecting the quality of the LMF. This study indicates that *Saccharomyces cerevisiae* can be successfully employed as a biocontrol agent that is antagonistic and reduces the incidence of ochratoxins in coffee.	Velmourougane *et al.* (2011a)
9	Impact of drying surface and raking frequencies on mould incidence, ochratoxin A contamination, and cup quality during preparation of arabica and robusta cherries at the farm level	*Aspergillus ochraceus* Ochratoxin A (OTA)	Arabica and robusta cherries		The impact of drying surfaces used in the processing of arabica and robusta coffee on the growth of toxigenic moulds and production of ochratoxin was investigated. This study showed that drying coffee beans on soil surfaces increases the risks of fungal contamination, while raking about 4 times a day reduces the incidence of fungi contamination and ochratoxin production and results in a better cup quality.	Velmourougane *et al.* (2010b)
10	Coffee berry borer (*Hypothenemus hampei*)—a vector for toxigenic moulds and ochratoxin A contamination in coffee beans	*Aspergillus ochraceus* Ochratoxin A (OTA)	Coffee bean		This study shows that coffee berry borer (*Hypothenemus hampei*) is a vector of mycotoxigenic fungi in coffee. It was observed that contamination of coffee with this vector resulted in a higher fungal level in the coffee and corresponding high levels of ochratoxin A.	Velmourougane *et al.* (2010a)

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