Advanced Risk Analysis for Biological Hazards in Greek PDO Food Products

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Abstract
Food safety is increasingly concern both consumers and public health authorities. Food safety management systems have been implemented in food industry in order to protect consumer's health. Moreover, risk analysis strategies are usually included in food safety management plans. The aim of this study was to present and suggest an integrated system for risk analysis in food industry. Risk assessment combined with Next Generation Sequencing technology were performed. Greek food products which hold a PDO or PGI quality scheme (Avgotaracho Mesolonghiou and Vostizza currant) were selected as case studies because of their uniqueness and economic impact. From the results obtained, existing strategies were adequate in terms of Greek regulation for pathogens in foodstuff. However, NGS technique opens up possibilities to detect total microbiota of food products and minimize possible foodborne incidents. NGS analysis of Greek PDO products identified bacterial species (*Bacillus cereus* and *Acitenobacter Venetianus)* that can provide managers extra information about potential consumers' exposure to biological hazards. In conclusion, by advanced risk analysis, existing limitations regarding foodborne pathogens could be addressed. Therefore, this integrated risk analysis plan should be implemented in food industry in terms of food safety.
Keywords: biological hazards, food industry, NGS, risk assessment, risk factors
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1. Introduction

The relationship between food quality and safety had become a debate, due to the fact that these issues are not clearly differentiated(Freitas et al., 2020). From consumer's point of view, a high quality food product is considered to be safe(Sadílek, 2018). Therefore, since food safety has become mandatory for food quality, public authorities are insisting that comprehensive quality management systems should be developed and utilized in food industry (Röhr et al., 2005). In parallel, researchers have developed a variety of improved analytical methods for detection of contaminants in a food product, even in a complex matrix(Chiou et al., 2015). Next Generation Sequencing technology, microfluidic devices or biosensors have been proposed as promising tools in order to assess food safety (Alahmad et al., 2021; Jagadeesan et al., 2019; Mehrotra, 2016).

Food safety management systems, including risk management activities are vital for food industry(Panghal et al., 2018). Managers, through a risk management plan, can define a goal for industry, identify potential hazards, analyze consequences, evaluate threats and finally make decisions for risk treatment (Aven, 2016). Risks associated with food products should be constantly evaluated due to the fact that, production of unsafe food products can affect public health. Moreover, the economic consequences for the food industry could be devastating(Jahangoshai Rezaee et al., 2018). Therefore, there is an urgent need for incorporation of risk assessment strategies in food sector in terms of food safety. Risk assessments plans can be quantitative or qualitative and target to risk treatment and monitoring(Fan & Stevenson, 2018). Through a completed risk assessment plan, managers are able to access information and answer some critical questions(Workshop et al., 2014). What are the hazards? Who be might be harmed and how? What are you doing to control the risks? (Hse, 2008).

Hence, this study focus on advanced risk analysis applied in the production of two Greek PDO products in terms of Public Health regarding foodborne pathogens. Moreover, this study aimed to analyze potential biological hazards and contamination points for risk assessment applied to production stages of two Greek PDO food products: Avgotaracho Mesolonghiou and Vostizza currant. Next Generation sequencing analysis of final food products was also performed in order to obtain information about the total bacterial population and to associate the results with risk assessment strategies.

2. Risk identification and assessment

Risks in food industry can be defined by two parts, A: the likelihood that a hazard will affect us and B: if it does, the severity of its consequences(Food and Drug Administration, 2021). Hence, in order to conduct a risk assessment in food industry, it is essential need to have details about the flow diagram of company.

Then, to identify and rank potential biological hazards, analysis of each production stage with potential contamination points and associated risk factors should be examined. Two independent reviewers with adequate reliability and independently performed the quality and quantity appraisal of each case study included.

Additionally, Next Generation Sequencing approaches were conducted in order to highlight dominant microorganisms in food products. Therefore, evaluation of existing risk management activities can be achieved. In parallel, additional measurements for food safety reassurance could be proposed.

3. Results and Discussion

3.1 Case study: "Avgotaracho Mesolonghiou"

Avgotracho Mesolonghiou" (fish eggs from *Mugil Cephalus*), a popular Greek PDO product, is examined as a case study of seafood, for this risk analysis. Figure 1. provides details about the flow diagram of "Avgotaracho Mesolonghiou" production while Table.1 provides information about risk assessment for its production regarding microbiological hazards(Dimitriou et al., 2016). Finally, Next Generation sequencing approach was performed for 16srDNA of bacterial population isolated from Avgotaracho Mesolonghiou samples.

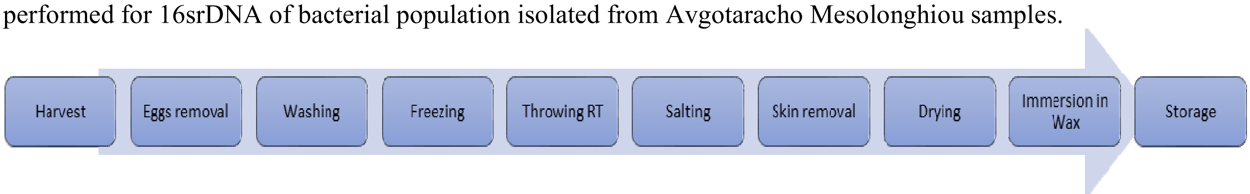

Figure 1. Flow diagram of "Avgotaracho Mesolonghiou" production

Regarding aquaculture industry, location and environment of fisheries area are considered to be as high risk for potential contamination source. Moreover, process, package and storage procedures are also reported in several publications as production stages with significant biological hazards (Chintagari et al., 2017; Dumen et al., 2020; Huss et al., 2000).

More in detail, environmental conditions, poor hygiene practices, contaminated feed or run off waters from human sewage are potential microbiological hazards in aquaculture(Amagliani et al., 2012). Additionally, scientists pointed out as potential risks , seafood or seafood contact surfaces for biofilms formation(Mizan et al., 2015).

Fishing practices, salt concentration, sanitizing of processing surfaces, personal hygiene and storage temperature are indicated as potential risk factors for seafood(Sikorski & Kolodziejska, 2002). Exposure to referred risk factors is possible in "Avgotaracho Mesolonghiou" production, as well. Interestingly, until now, seafood is considered to be a main carrier of a variety of illnesses transmission to humans(Afreen & Ucak, 2021).

Table 1. Risk assessment of microbiological hazards for "Avgotaracho Mesolonghiou" production

Production Stage	Contamination Point	Risk Factors	Average Risk
Harvest	Environment	Temperature	12,5
		Acidification	3,5
	Location	Clean Water	12,5
		Physico-Chemical parameters of water	8
		Fishing Practices	9
	Storage	Temperature	9
Eggs Removal	Handling	Hygiene practices	12
		Surface	12,5
Washing	Equipment	Cleaning - equipment maintenance	10,5
	Handling	Hygiene practices	7
		Clean water	7
Freezing 4-6C	Conditions	Temperature	11
	Equipment	Cleaning - equipment maintenance	9
Throwing RT	Handling	Hygiene practices	8
		Surface	9
	Conditions	Temperature	10,5
Salting	Handling	Training of employees	14,5
	Seawater	Quality	9
		Cleaning - equipment maintenance	8
		Duration	11
Skin removal	Handling	Hygiene practices	13,5
		Training of employees	4,5
Drying	Equipment	Cleaning - equipment maintenance	9
	Handling	Hygiene practices	8,5
		Surface	11
		Air quality	11
		Duration	7
Immersion in Wax	Equipment	Cleaning - equipment maintenance	5
	Wax	Quality	3,5
		Hygiene practices	5,5
Storage	Handling	Hygiene practices	12
	Conditions	Temperature	6,5
		Cleaning - equipment maintenance	13

From Next Generation Sequencing analysis of fish eggs from Mesolonghi, the dominant bacterial species are *Acitenobacter Venetianus.* According to a study conducted in 2016, authors identified microbial species in seawaters, including *Acitenobacter Venetianus*, that are associated with arsenic affected locations(Goswami et al., 2015). This bacterial pathogen has related with mass mortalities in aquaculture(Huang et al., 2020). *Macrococcus Micrococcus Luteus* followed by NGS results. According to recent bibliography, this bacterium is considered to be part of normal microflora of seafood(Akayli et al., 2020). Additionally, these bacterial species can usually be used as probiotics in fisheries, although under specific conditions can be pathogenic(AKAYLI et al., 2016).

Overall, by the combination of next generation sequencing results with risk assessment conducted, it is evidenced that in production area, cleaning in facilities, hygiene requirements and seafood handling are adequate for managing potential biological hazards. However, total quality managements and good hygiene practices should be applied constantly in all production stages in order to minimize and control microbiological risks in terms of public health.

3.2 Case study: "Vostizza currant"

As risk analysis case study for agricultural products, Vostizza currant (*Vitis Vinifera var. apyrena*) was selected. Vostizza currant is a valuable Greek PDO product with high nutritive components and its production take place in Aighio (Papadaki et al., 2021). Figure 2. depicts flow diagram of Vostizza currant production. Table 2. Provides details about risk assessment for production of Vostizza currants. Finally, NGS technology was performed to identify total bacterial population extracted from currants.

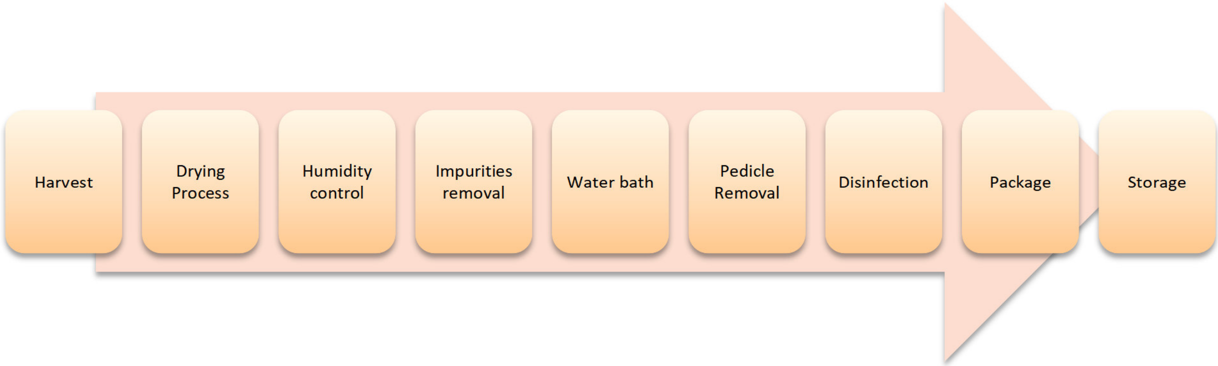

Figure 2. Flow diagram of "Vostizza currant" production

In agriculture industry , most common risk factors reported are irrigation water, washing water, processing practices and storage conditions (Balali et al., 2020; Nguyen-The, 2012; Park et al., 2012). Exposure to a variety of risk factors is possible in Vostizza currant production as well. Regarding agricultural practices, irrigation water and composts or manure have been defined as major risk factors for Vostizza currants. Soil amendments such as manure, composts and irrigation water have been pointed out as risk factors in a variety of agricultural products such as leafy crops and tomatoes (Allende & Monaghan, 2015; Bezanson et al., 2014; Pagadala et al., 2015). Hence, water is considered to be an important contamination source for fruits and vegetables. Therefore, it is essential need to implement preventive control measures for minimizing possible risk regarding water. Use of high quality water, sanitization on regular basis of water reservoirs and water treatments such as filtration or disinfection are highly recommended strategies, which should be included in existing in existing measures(Uyttendaele et al., 2015). Additionally, it is evidenced that transmission of foodborne pathogens to fruits and vegetables could be supported by contaminated soils amendments. Although, manures and composts are a sustainable solution for agriculture, proper handling is required to reduce the prevalence of foodborne pathogens(Sharma & Reynnells, 2018).

Drying process is a crucial procedure due to the fact that can affect food products' quality. In terms of microbial safety of dry fruits, a variety of alternative drying technologies have been developed (Bourdoux et al., 2016). Regardless applied drying technology, parameters such as drying degree or temperature can increase likelihood of microbial contamination(Radojčin et al., 2021).

Furthermore, disinfection practices and storage conditions have been already indicated as risk factors in dry fruits production(Zare & Jalili, 2020). In order to maintain microbial quality and safety of dry fruits, optimal disinfection treatments and storage conditions for each food type should be examined. According to a study conducted in 2021, *L. monocytogenes* survived on raisins and strawberries for 336 days at 4ºC storage temperature(Cuzzi et al., 2021). However, future studies should investigate growth and fate of foodborne pathogens on Vostizza currants.

Table 2. Risk assessment of microbiological hazards for "Vostizza currant" production

Production Stage	Contamination Point	Risk Factors	Average Risk
Harvest	Agricultural Practices	Irrigation Water	12
		Soil	9
		Manure	10
	Handling	Hygiene practices	10,5
		Training of employees	7,5
Drying Process	Equipment	Cleaning - equipment maintenance	8,5
	Handling	Hygiene practices	10,5
		Surface	8,5
		Drying Degree	13
Humidity control	Conditions	Humidity	9
	Equipment	Cleaning - equipment maintenance	4,5
Impurities Removal	Equipment	Cleaning - equipment maintenance	6
	Handling	Hygiene practices	10
		Training of employees	4,5
Water bath	Equipment	Cleaning - equipment maintenance	11
	Handling	Clean water	10
		Hygiene practices	11,5
Pedicle Removal	Handling	Hygiene practices	10
		Training of employees	4,5
	Equipment	Cleaning - equipment maintenance	10,5
Disinfection	Handling	Hygiene practices	8
		Training of employees	8,5
	Equipment	Cleaning - equipment maintenance	10,5
Package	Handling	Hygiene practices	9,5
	Conditions	Cleaning - equipment maintenance	9
Storage	Conditions	Temperature	9
	Handling	Training of employees	7

From Next Generation Sequencing analysis of Vostizza currants, dominant bacterial species were Bacillus *cereus. Bacillus cereus* bacteria have been isolated from both animal and plant origin food products (Berthold-Pluta et al., 2019). More specific, strains of Bacillus cereus can occur in a variety of food products such as rice, pasta, dry fruits, candies, chocolates, eggs, meat, fish, juices and soft drinks(Messelhäusser et al., 2014). This spore forming bacterium can survive in stress conditions such as heat treatment due to its resistant endospores(Rosenquist et al., 2005). Moreover, it is referred in literature as hygiene indicator during production, handling, storage and distribution(Kumari & Sarkar, 2014). Hence, contamination sources could be many points in production line.

Overall, as a result, existing safety management system in production should be upgraded. Therefore, advanced risk analysis is proposed which include risk assessment strategies and NGS approaches, as updated and integrated food safety management system.

4. Conclusion

This study aimed to analyze possible risk factors in production of two different Greek PDO delicacies and suggest advanced risk analysis by combination of risk assessment plans with NGS approaches. Incidents of foodborne illnesses are constantly reported and therefore foodborne pathogens are a global public health threat. Although, existing food safety management plans aimed to address these issues, there is still no guarantee for safe products in food sector. Advanced risk analysis is proposed as integrated food safety management system, in which risk assessment strategies with NGS tools are combined. This upgraded risk analysis strategy could be the key to manage and minimize possible hazards in any production line in food industry. Further studies should be performed in order to investigate contamination sources throughout the supply chain. Moreover, NGS technologies should be integrated with predictive microbiology in the whole process of risk-based decision making throughout the supply chain. Finally, a future challenge will be replacing existing conventional methods by utilizing omics technologies in food industry.

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