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Title: Sushi processing: microbiological hazards and the use of emerging technologies

Piotr KULAWIK¹, Dani DORDEVIĆ^{2,3}

Published in: Critical Reviews in Food Science and Nutrition

DOI: <https://doi.org/10.1080/10408398.2020.1840332>

¹ Department of Animal Products Technology, Faculty of Food Technology, University of Agriculture in Cracow, ul. Balicka 122, 30-149 Kraków, Poland

²Department of Vegetable Foodstuffs Hygiene and Technology, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences in Brno, Palackeho tr. 1946/1, 612 42 Brno, Czech Republic

³South Ural State University, Department of Technology and Organization of Public Catering, Lenin Prospect 76, Chelyabinsk 454080, Russia

***Correspondence should be addressed to:**

Piotr Kulawik (Ph.D.)
Department of Animal Products Technology,
Faculty of Food Technology,
University of Agriculture in Cracow,
Cracow, Poland
Phone: +48 509 377955
E-mail: kulawik.piotr@gmail.com

Abstract

Sushi meal has been adapting to different countries and traditions ever since it was invented. Recently there is a growing popularity of ready-to-eat sushi meals, with new sushi production plants emerging in many countries. This relatively new sushi industry is facing many challenges, one of which is the microbiological hazard related to sushi consumption. The aim of this review was to summarise the most significant aspects with regard to microbiological quality of sushi, reported cases of sushi-related poisoning, as well as the potential of modern innovative and emerging technologies to inhibit microbiological growth. Although there is a limited amount of studies in relation to sushi shelf-life extension, the existing data shows potential of using novel minimal processing technologies to improve the shelf-life and quality of sushi meals. Those technologies include the use of cold plasma, plasma activated water and electrolyzed water, as well as the use of innovative packaging and edible coatings. Based on the collected data, the possible microbiological hazards in the production process of sushi, with possible use of emerging technologies to reduce or eliminate those risks, are also emphasised.

Keywords: sushi, microbiological hazards, emerging technologies, food safety, shelf-life

Introduction

Sushi is a dish which probably originated around 1,600-1,800 years ago in China, as a fish meat wrapped in cooked rice and subjected to prolonged fermentation (De Silva and Yamao, 2006; Mouritsen, 2009). Sushi was introduced to Japan around the 7th century and from then on, began a slow transformation into the three most common sushi types known today: nigiri, maki and temaki. Nigiri-sushi is a type of sushi in which sushi rice is used to form the so called shari-dama, a sushi rice formed into a cuboid shape onto which rhomboid shaped ingredients, such as fish slices or roe, are placed. Maki-sushi, are formed by covering the sheet of dried nori seaweed with sushi rice onto which other ingredients, such as seafood, fruits or vegetables, are placed and the whole product is formed into a roll. Such prepared roll is usually cut into 6-8 pieces. Temaki is similar in composition to maki-sushi, however after placing the ingredients, the product is formed into conical shape (Hsin-I Feng, 2012). Although Japan is still the country with the highest sushi consumption (De Silva and Yamao, 2006), in recent years, a global increase of interest in sushi has been observed, with sushi restaurants present in almost all countries around the world (Hsin-I Feng, 2012). This is especially visible in European countries, where sushi has become a regular dish for many Europeans (Figure 1).

A trend that is becoming increasingly visible is the appearance of ready-to-eat (RTE) sushi meals available at retail chains worldwide (De Silva and Yamao, 2006; NSC, 2015, 2016a, 2016b). Such RTE sushi meals are stored in chilled conditions and although their shelf-life is usually not longer than a few days, they allow the potential customer more flexibility and convenience when it comes to time of consumption (Kulawik et al., 2018a). Although the production process of RTE sushi meals can generally vary from one sushi plant to another, in Figure 2, a diagram for general sushi production is presented. After raw material delivery and storage, the production process splits depending on the product. Rice is

70 firstly washed thoroughly and left to desiccate. Afterwards, the rice is mixed with an
71 appropriate amount of water and boiled until all the water evaporates, and then the rice is
72 mixed with a vinegar solution which usually contains rice vinegar, sucrose and salt (D.
73 Dordevic, Buchtová and Macharáčková, 2017; Kulawik et al., 2019c; Lorentzen et al., 2012).
74 Following, the rice is cooled down and used during sushi formation. Fish which is to be
75 consumed raw, should be frozen for a specific amount of time to reduce the risk of parasitic
76 infestation. The time depends on the recommendations and legislation bodies. For example,
77 the FDA requires maintaining a temperature no greater than -35 °C for 15 h or -20 °C for 7
78 days, while the European Union legislation requires maintaining a temperature of -35 °C for
79 15 h or -20 °C for 24 h (Podolska et al., 2019). After freezing, the fish and remaining frozen
80 components are thawed and subjected to preliminary treatment along with other ingredients,
81 such as fruits, vegetables, wasabi, etc. The preliminary treatment includes steps such as
82 washing, cutting, gutting, filleting, skinning, mixing and other processes necessary to prepare
83 all of the ingredients for product forming. The product forming process is a step in which
84 individual ingredients are combined to create the desired form of sushi. For example, in the
85 case of After preparation, the sushi is packed and stored at the appropriate temperature. In the
86 case of frozen RTE sushi sets, an additional freezing step is required.

87 Although RTE sushi meals are more popular in Japan, they are also becoming more
88 and more popular in other countries (Figure 1). For example, in a study by Altintzoglou et al.
89 (2016), comparing the sushi consumption patterns between consumers from Norway and
90 Japan, it was shown that respondents consumed an average of 1.43 and 3.24 sushi meals per
91 month, respectively. From those, 0.47 and 1.75 sushi meals per month were acquired from
92 Norwegian and Japanese supermarkets. The growing demand for RTE sushi meals has
93 resulted in the appearance of new processing companies, which specialise in sushi meal
94 production. However, this relatively new branch of the food industry is facing many

problems and challenges, which often result in very low shelf-life and high operating costs (Figure 3).

One of the most important of those challenges is spoilage related to microbiological growth. The aim of this review was to summarise the most significant aspects with regard to microbiological quality of sushi, reported cases of sushi-related poisoning, as well as the potential of modern innovative and emerging technologies to inhibit microbiological growth. Based on the collected data, the possible general microbiological hazards in the production process of sushi, with possible use of emerging technologies to reduce or eliminate those risks are emphasised in this review.

Microbiological quality of sushi

Sushi meals pose microbiological hazards mostly because they include raw fish and seafood. Microbiological quality of fish is influenced by many different factors, including: place of capture, water temperature, fish species and season of capture, but is also affected by post-capture manipulation and processing. For example, Keeratipibul, Techaruwichit and Chaturongkasumrit (2009) reported that cooked shrimp intended to be used as sushi ingredients had already been contaminated by coliform microorganisms during their processing. The reason for contamination was the prevalence of coliforms on devices used in cooked shrimp production and on workers' gloves (7.2%).

Although there are no specific critical limits of total bacterial contamination in sushi samples, the most frequently applied critical limit of 6 log of cfu/g established for RTE food products also seems to be applicable for sushi samples (ANZFA, 2001; Gilbert et al., 2000). This limit is further supported by Kulawik et al. (2018b), who studied total aerobic bacterial counts (TABC) of RTE sushi meals from 6 different sushi producers on the last day of their shelf-life and found that the microbiological contamination was usually between 5-6.5 log cfu/g. On the other hand, Sunniva Hoel et al. (2015) examined RTE sushi meals obtained from

Norwegian supermarkets and found that 48% of samples had a TABC of above 6 log cfu/g despite having still 1 one more day of shelf-life. The authors found a correlation between higher TABC contamination and the producer, which indicates that poor hygiene practises during production are probably the main factor influencing the bacterial loads of sushi meals. The main factor influencing microbiological growth in RTE sushi meals is the storage temperature. Based on the research by S. Hoel, Jakobsen and Vadstein (2017), the appropriate storage temperature for RTE sushi meals should not be higher than 4 °C with even relatively minor abuse of the temperature regime (8 °C) significantly influencing the growth rate of bacteria and reducing shelf-life.

Although it might seem that sushi prepared and served in restaurants should be fresher, due to lack of storage period of the produced meal, in research performed so far, this is indicated as untrue. The study comparing microbiological contamination of frozen sushi meals and sushi obtained freshly from restaurants conducted in Germany, found that while the mean TABC of frozen sushi samples was within the range of 2.4-3.0 log cfu/g, the mean TABC of restaurant sushi was 5.2-7.1 log cfu/g (Atanassova, Reich and Klein, 2008). In a different study carried out among 20 sushi restaurants in Copenhagen, Denmark, it was shown that the TABC critical limit of 6 log cfu/g was exceeded in 31 and 19 % of maki and nigiri samples (Leisner et al., 2014). Aside from TABC, sushi meals, both those in the form of stored RTE or acquired from restaurants, are often contaminated with various pathogens. In previous studies conducted during the three last decades, the presence of *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus* and mesophilic *Aeromonas* spp. have been reported among sushi samples in different parts of the world (A. A. Adams, Beeh and Wekell, 1990; A. M. Adams et al., 1994; Atanassova et al., 2008; Fang et al., 2003; Sunniva Hoel et al., 2015; Leisner et al., 2014; Miguéis et al., 2015; Pinto et al., 2012). The majority of the abovementioned pathogens are not typical microflora of sushi ingredients and

were most probably introduced in the product via cross-contamination resulting from poor hygiene practice during production. It is also worth mentioning that many of these studies were conducted in countries where it is mandatory to implement GHP/GMP and HACCP systems, like Germany, Portugal, Italy, Denmark or Norway, which indicates that in many sushi production establishments, those food safety systems do not work. Similar conclusions were reported by Bánáti and Lakner (2012), who raised concern that in many cases, the efficiency of HACCP systems is only partially utilised and the systems have been often diminished to accomplish administrative tasks.

Food-poisoning outbreaks related to sushi consumption

There have been several outbreaks of food poisoning related to sushi consumption (Table 1). In one of these outbreaks in Nevada, USA, 130 people reported illness, with enterotoxigenic *Escherichia coli* identified as being responsible, at least in part, of the outbreak. The contamination was probably due to lack of proper hygienic standards in the sushi restaurant, as observed by Jain et al. (2008). In another food poisoning incidence in Singapore, sushi rolls were connected with *Salmonella* intoxication. The outbreak investigation included environmental and food items (raw and cooked) examination, but all taken samples were culture negative for *Salmonella* species, although the investigators noticed certain hygienic issues such as inadequate conditions for hand washing and bare hand contact with ingredients. It is probable that *Salmonella* was present in the fish or other sushi ingredients because the outbreak included the consumption of food during an 18-day period (Barralet et al., 2004). In a survey of microbiological quality of sushi meals in Hong Kong, it was pointed out that 13.8% of sushi and 11.1% of sashimi samples investigated between 1997 and 1999 were of poor hygienic condition. Moreover, 0.26% of samples were contaminated with pathogens including *Vibrio parahaemolyticus*, *S. aureus*, *Salmonella* and *L. monocytogenes*.

During this period, exactly 142 people were contaminated due to sushi and sashimi consumption (CFS, 2000). In Japan, an outbreak of enterohemorrhagic *E. coli* O157:H7 poisoning was related to contaminated salmon roe (ikura) (Balfour et al., 2014).

Certain ingredients of sushi influence the microbiological quality of a meal, and can introduce certain barriers for pathogenic bacteria. One of such ingredients is vinegared rice. After the addition of vinegar solution to rice, its pH drops to the level of 3.9-4.6, inhibiting the possible growth of many food pathogens (A. A. Adams et al., 1990; A. M. Adams et al., 1994). This is supported by Skjerdal, Reitehaug and Eckner (2014), who observed that the growth of *Listeria monocytogenes* was slower in nigiri sushi samples than in sashimi. This delayed growth was explained by the lowered pH of fish samples due to contact with vinegared rice. The use of vinegared rice results in the reduction of the pH of the salmon by approx. 0.2-0.6 in case of analysing the pH of the fish alone and by 1 when analysing the whole nigiri sample (Kulawik et al., 2019b; Lorentzen et al., 2012). It was estimated that if nigiri samples were to be stored for 7 days at 4 °C, the initial contamination of fish fillet should not exceed 2 cfu/g of *L. monocytogenes* in order to adhere to the legislative critical limit of 100 cfu/g during the time of consumption (Skjerdal et al., 2014). Similarly, S. Hoel, Vadstein and Jakobsen (2018) observed that a combination of maintaining the appropriate storage temperature below 4 °C and the use of acidified rice can effectively prevent the growth of *Aeromonas salmonicida* in model salmon nigiri samples. Such growth inhibition was not observed when non-acidified samples were used or when the storage temperature was increased to 8 °C.

Sushi-related food-poisoning not only concerns bacterial contamination, but can be of viral origin as well. A sushi bar located in Niigata, Japan was the source of the hepatitis A virus (HAV) outbreak, which resulted in 9 people being infected. The city health authorities decided to close down the bar after confirmation that it was the actual cause of this outbreak

(Takeuchi et al., 2006). In yet another food poisoning outbreak documented by Tominaga et al. (2012), 23 cases of HAV were identified after consuming meals from a sushi bar. All infected persons visited a specific sushi bar, however, it was impossible to detect the exact reservoir or vehicle of HAV among the served meals due to fact that some of the infected persons ate only raw shellfish while others ate only cooked eel. Viruses can often survive harsh environmental conditions and prolonged storage periods. In 2017, there were three reported outbreaks of norovirus among school-children in Japan, affecting over 2,000 people in total (Kusumi et al., 2017). Although the outbreak was not directly related to sushi, the source of contamination was dried *Porphyra umbilicalis* seaweed, which is used in sushi production under the common name of nori (Garcimartín et al., 2015).

Innovative and emerging technologies for sushi decontamination

One of the main and most effective methods of lowering microbiological contamination is the use of thermal treatment. However, in case of sushi, thermal treatment of the whole product is often not possible since it contains raw or mildly processed fish and seafood. Moreover, since sushi belongs to the family of RTE food products, thermal treatment is not applied by the final consumer prior to consumption. Currently, most processors rely only on maintaining the proper hygiene regime combined with refrigeration to achieve a microbiologically stable product. Nonetheless, this is often not sufficient, since both the hygienic regime and cooling chain are often easily interrupted at various stages of production and distribution. The typical shelf-life of RTE sushi meals, without the use of any food additives or preservation technologies, is no longer than approx. 3 days (Steffen, Duerst and Rice, 2010). This causes many logistical problems and can result in high percentages of wasted product due to exceeding expiration dates. To extend this shelf-life, minimal processing technologies may be employed. Modified atmosphere packaging (MAP) is one of the most common and relatively

easy to apply method. Chen et al. (2003) studied the effects of MAP (30% CO₂, 65% N₂, 5% O₂) with and without the addition of 0.05% ethanol vapour on the microbiological quality of sushi stored for 72 h at 25 °C. They observed significant inhibition in the growth of TABC for MAP packed samples compared to the controls. Moreover, the addition of ethanol vapour further improved the microbiological stability of the product. Similar results were obtained by Mol, Uçok Alakavuk and Ulusoy (2014) who studied the effects of two MAP conditions (50% CO₂, 50% N₂ and 100% CO₂) on salmon hosomaki stored for 6 days at 4 °C. Both MAP packaging conditions significantly inhibited the growth of TABC and psychrotrophic bacteria. MAP packaging with 100% CO₂ also resulted in inhibiting the changes of colour parameters during storage. A different approach was used by Steffen et al. (2010) who described the use of the high-oxygen high-CO₂ MAP technique for RTE sushi meals by a sushi factory in Switzerland. High-oxygen MAP packaging has been reported to have similar antimicrobial effects as high CO₂ atmosphere (Jacxsens et al., 2001; Jayasingh et al., 2002). However, this method is not often employed due to significant increase in oxidation rate as well as related off-odours and off-flavours which may result in reduced shelf-life of the stored product (Jääskeläinen et al., 2016; Kim et al., 2010; Zakrys-Waliwander et al., 2012). To avoid these phenomena, Steffen et al. (2010) added 10% argon to the mixture, since it is heavier than oxygen, it drops and creates a barrier from oxygen around the product. The use of high oxygen MAP allowed the authors to combine MAP packaging with ozone decontamination. After packaging, the RTE sushi meals were subjected to ultraviolet radiation (UV) at a wavelength of 185 nm, which enabled the formation of ozone from the oxygen atmosphere sealed within the package, providing a second decontamination source.

The use of UV for the decontamination of food samples is a well-established preservation method. This method is based on the ability of shortwave UV radiation (UV-C) within the range of 200-280 nm to damage the DNA and RNA of the cell, resulting in

246 destruction of the microorganism (Fan, Huang and Chen, 2017). Aside from its advantages,
247 which include ease of use and low investment costs, the method additionally has a few
248 drawbacks which often limit its use within the food industry. Those limitations include low
249 surface penetration, low disinfection efficiency in the case of products with complex
250 structure, promotion of product oxidation and environmental hazard due to possible mercury
251 leak from mercury UV lamps (Keklik, Krishnamurthy and Demirci, 2012). Despite the
252 disadvantages of UV-C treatment, the method can be effectively used in hurdle with other
253 technologies, as shown in the previously mentioned case study by Steffen et al. (2010), who
254 combined the use of MAP, UV-C, ozone, electrolyzed water (EW) and ultrasounds, which
255 enabled him to improve the shelf-life of RTE sushi meals to at least 7 days. A recent study by
256 Mikš-Krajnik et al. (2017) also showed that UV treatment was more effective in reducing the
257 *L. monocytogenes* loads in raw salmon fillets, when combined with ultrasound and acidified
258 EW. It should be noted however, that when the same hurdle technology was used to measure
259 the reduction in TABC, the reduction was below 1 log cfu/g, which indicates that not all
260 microorganisms are similarly susceptible to the treatment. The UV treatment efficiency can
261 be improved by the use of pulsed light technology. This includes the use a series of short
262 bursts of light with high intensity. Although the method involves the use of wavelengths
263 within the range of 200-1100 nm, it usually engages the use of UV-C wavelengths (Mandal et
264 al., 2020). The implementation of pulsed UV (PUV) allows to deliver bursts with higher
265 energy, which increases penetration depth and reduces treatment time while increasing
266 treatment efficiency (Kulawik, 2019). To date, there are no reports on applying PUV to
267 improve the shelf-life of sushi. On the other hand, the method has been successfully used to
268 reduce the microbiological loads of *L. monocytogenes* on raw salmon, flatfish fillets and
269 shrimps and the *Pseudomonas fluorescence* counts in raw salmon fillets (Cheigh, Hwang and
270 Chung, 2013; Holck et al., 2018; Pedrós-Garrido et al., 2018). Nonetheless it should be noted,

that reported microbiological reductions, when using PUV, usually do not exceed 2 log of cfu/g, so if higher log reduction is needed, the method should be used in hurdle with other minimal processing technologies. Moreover, the use of PUV on sushi products can be further limited due to an increase in the surface temperature of the treated food products, which may affect the sensory properties, such as color or texture of the treated food product, as was the case of tuna carpaccio (Hierro et al., 2012).

The use of cold plasma as a non-thermal decontamination technique has recently gained much attention. Plasma has been successfully used to decontaminate fruits, vegetables, nuts, meat and seafood (Kulawik and Kumar Tiwari, 2019; Misra and Jo, 2017; Pignata et al., 2017). Although cold plasma has the potential to be used in sushi since it does not lead to an increase in the temperature of the treated product (Fridman et al., 2007), its decontamination effect on sushi seems limited. Kulawik et al. (2018a) studied the effect of cold plasma treatment with 70 and 80 kV for 5 min on salmon nigiri and hosomaki samples. Although they observed an initial reduction of up to 1 log of cfu/g in TABC in nigiri samples, the microbial counts of the treated samples increased more rapidly during storage without any significant differences observed between the treated and control nigiri samples. More pronounced decontamination effects have been observed for hosomaki samples with microbiological count reductions reaching up to 1.5 log cfu/g after 11 days of storage at 4°C, extending the shelf-life of stored hosomaki by at least 4 additional days. On the other hand, the authors reported that the cold plasma treatment increased the oxidation rate of both nigiri and hosomaki samples measured by the TBA index.

The limited decontamination effect of cold plasma could possibly be improved by combining it with MAP packaging, since currently used devices for non-thermal plasma generation enable the generation of plasma inside the sealed package. However, the mechanism and efficiency of plasma depends on the surroundings with different reactive

species formed in different atmospheres (Kulawik and Kumar Tiwari, 2019), therefore, further research is needed to establish the best modified atmosphere content as well as cold plasma generation parameters to achieve the highest decontamination effect.

Cold plasma can be also used to create plasma activated water (PAW). When water is subjected to plasma treatment, it results in, among others, the formation of various reactive species, reduced pH due to the presence of strong acids and increased oxidation-reduction potential. All of this causes high disinfection properties of PAW and effectiveness in inactivation of various foodborne pathogens (Thirumdas et al., 2018). Although PAW cannot be used directly on prepared sushi, it could be applied to decontaminate some raw materials before sushi formation. PAW has been effectively used to extend the shelf-life of various foods such as meat, seafood, fruits, vegetables or fermented products such as kimchi (Choi et al., 2019; Khan and Kim, 2019; Liao et al., 2018; Ma et al., 2015; Royintarat et al., 2020; Zhao et al., 2020). Many of those food products are common sushi ingredients. PAW treatment is usually performed by dipping the product in the solution, however, Liao et al. (2018) have also proposed the use of ice made of PAW to store shrimp. The PAW ice showed high electrical conductivity as well as high concentrations of ozone, nitrate and hydrogen peroxide. When compared to shrimp stored on normal ice, those stored on PAW ice demonstrated to have inhibited microbiological growth and total volatile base-nitrogen (TVB-N) formation. Surprisingly, the use of PAW ice also resulted in lower thiobarbituric acid reactive substances (TBARS) formation, despite the presence of many oxidising agents in PAW ice. This was attributed to inhibited psychrotrophic bacteria growth, which can produce lipoxigenase and peroxidase enzymes.

Similar in application to PAW, EW can also be used to improve the quality of sushi ingredients prior to formation, and this preservation method is actually already commonly used by companies in the sushi industry (Rahman, Khan and Oh, 2016; Steffen et al., 2010).

One of the main advantages of using EW is the simplicity of its production (Dewi et al., 2017). The detailed production process of EW has been described by Rahman et al. (2016). In brief, the diluted saline is introduced into the electrolysis chamber which contains a cathode, anode and membrane. NaCl dissociates when an electrical current is applied to electrodes and reacts with hydroxide and hydrogen ions which are also formed. The charged ions move towards the cathode or anode (depending on the ion charge), resulting in the formation of two solutions: acidic EW, formed near the anode, which contains HCl, O₂, Cl₂ or HOCl and basic EW, formed near the cathode and containing NaOH and H₂. Aside from simplicity of production, other advantages of EW implementation include no adverse effect on humans or the environment as well as its cost-effectiveness (Dewi et al., 2017). As in the case of PAW, EW can be applied to fish products by dipping them into the solution of EW or by application of ice made from EW (Jung et al., 2018). On the other hand, in a recent meta-analysis performed by Afari and Hung (2018), it was shown that the effectiveness of EW strongly depends on the surface smoothness of the disinfected food product, with the highest efficacy observed for smooth products such as tomatoes or eggs. When considering products with irregular surfaces, mainly meat and fish products, the antimicrobiological usefulness is greatly reduced.

Another possible method that may be used for sushi preservation is the application of edible coatings. Dehghani, Hosseini and Regenstein (2018) define edible coatings as any type of consumable thin material layer which enwraps food or food products to extend their shelf-life. Edible coatings usually consist of natural ingredients, mainly polysaccharides, lipids, proteins or their combination. They can be applied onto a food product through wrapping, brushing, rolling, dipping, spraying or electrospinning (Galus and Kadzińska, 2015; Kulawik, Jamróz and Özogul, 2019a). In the case of formed sushi products such as nigiri or maki, some of those application methods, i.e. dipping, brushing or rolling may prove to be difficult or

even impossible without destroying the product and its shape, nonetheless, the application through spraying, electrospinning and wrapping could be effectively used. Kulawik et al. (2019b) have used spraying technique to coat salmon nigiri with furcellaran-gelatin films incorporated with green tea extracts. The use of the coating significantly reduced oxidation progress during 8 days of storage at 4 °C, while not causing any deterioration to the sensory scores. Despite this, the coating did not extend the shelf-life of the product since it did not affect the microbiological growth of the bacteria, despite the promising *in vitro* antibacterial potential of the coatings. Although no other research has been performed in which the spraying technique was used to coat prepared sushi products, in this study, the potential of using edible coatings for shelf-life extension of RTE sushi sets is demonstrated. The spraying technique can be further enhanced by the use of electrospinning or electrospraying technology. These methods allow to create electrospun nanofibres or nanoparticles which can be applied onto the surface of a food product, resulting in a thin but tight coating. This can cause the formation of barrier properties and also, if appropriate active ingredients are included, the coatings may assume antioxidant or antimicrobiological functional properties. Moreover, appropriately performed electrocoating does not affect the sensory scores of coated food products (Alp Erbay et al., 2017; Anu Bhushani and Anandharamakrishnan, 2014; Cakmak, Kumcuoglu and Tavman, 2019). This technique has been successfully applied to fish products and strawberries (Alp Erbay et al., 2017; Ceylan et al., 2018; Peretto et al., 2017), however, no such application to sushi has been performed to date.

The use of antimicrobial active films is another possibility to improve the shelf-life of prepared sushi meals. The wrapping technique of active films has been reported to effectively prolong the shelf-life of various food products (Umaraw et al., 2020). However, the implementation of this method to the industry is difficult, since not all products can be wrapped in the films, and without direct contact between the active film and the product, the

shelf-life promoting efficiency of active packaging is greatly impaired (Barbosa-Pereira et al., 2014). On the other hand, the sushi industry is already using machines which simultaneously produce sushi pieces and wrap them in plastic film. Examples of this are the nigiri wrapping robot - Suzumo SGP-SNB, or the maki and temaki continuous production line - Suzumo SVR-SAE (Suzumo, 2020). Different type of active packaging employing the RFID technology has been presented by Ngai, Suk and Lo (2008). The authors used RFID timestamps on the sushi dishes at a sushi restaurant with a conveyor belt system to monitor the time the sushi dish spent on the conveyor. The system allowed to easily identify the products which spend too much time on the conveyor belt or had expired. This, in turn, resulted in strengthened food safety management.

High Pressure Processing (HPP) is another minimal processing method, which could possibly be used by the sushi industry. HPP is an innovative technology, allowing to reduce the microbiological growth and inactivate various enzymes related to quality degradation of stored products (Campus, 2010; Huang et al., 2014). Despite this, it should be noted that HPP can cause discolouration and changes in texture of fresh food products, which is not always desirable (Basak and Ramaswamy, 1998). When HPP is used on muscle products. it can result in denaturation of myoglobin and increased lightness while being applied to fish species such as salmon (Gudbjornsdottir et al., 2010), although this effect should not be visible in case of white flesh colour fish species (Campus, 2010). The undesirable changes in colour/texture can be avoided by a reduction in pressure. For example, in a review conducted by Truong et al. (2015), it was concluded that the denaturation of fish protein resulting in discolouration occurs at a pressure of 150 MPa or higher. On the other hand, in the same review, it was stated that to achieve significant and substantial inactivation of microorganisms, the applied pressure should be above 400 MPa if HPP is used alone. This

disadvantage, together with the additional drawback of high investment cost of the HPP system (Huang et al., 2017), limit the possible use of HPP in the sushi processing industry.

Aside from many emerging and innovative technologies for food technology currently being developed, there is a very limited number of studies focusing on the effect of minimal processing technologies regarding sushi. Although some results obtained for different food products such as fish or fruits and vegetables can be extrapolated to sushi, it should be noted that a prepared sushi piece has completely different matrices for each of its ingredients when studied alone. For example, the addition of vinegar affects not only the pH of rice but of the fish and other ingredients as well, resulting in an unparalleled sensory effect and texture of the final product. Moreover, sushi products have various shapes and sizes, resulting in different surface to mass exposure ratios of individual products, e.g. the oxidation changes or microbiological spoilage in rhomboid shape salmon pieces which are used for nigiri production can occur at a different rate than would be obtained for the whole fillet.

A summary of the possible direct and indirect usages of various innovative and emerging technologies within the sushi industry, along with their advantages and disadvantages, is provided in Table 2.

Microbiological hazards in the sushi production process

A recent review by Lehel et al. (2020) contains a brief list of good practices that should be implemented during sushi production. These include the use of approved raw material sources/suppliers, freezing of raw fish prior to processing, the use of appropriately acidified rice and maintaining a storage temperature of 5 °C or below. In this review, the potential microbiological hazards during sushi production are further investigated. To do so, potential general microbiological hazards are identified in the presented flow diagram (Figure 2) along with possible preventive measures, including the use of innovative and emerging

technologies that can be used to further ensure proper microbiological quality of sushi (Table 3). Hazard analysis was based on the collected data, existing hazard analysis and proposed guidelines (Codex Alimentarius Commission, 2014; Tzouros and Arvanitoyannis, 2000). The suggested microbiological hazards are general in their description and should be treated as general guidelines, since detailed microbiological hazard analysis is only possible when more details regarding the type of raw materials, equipment or the location of the production areas are known.

As can be seen, many microbiological hazards can potentially be reduced or eliminated if the emerging technologies were applied. This, aside from safety for the final consumer, could improve the shelf-life of RTE sushi, which, in turn, causes lower food loss and improved economic sustainability for the production plant (Gutierrez, Meleddu and Piga, 2017).

Concluding remarks

Microbiological contamination is one of the greatest issues faced by the sushi industry, with new information about sushi-related poisoning outbreaks appearing regularly. The emergence of novel, non-thermal technologies could revolutionise this branch of the food industry, however, there is still a limited amount research that would study the effects of those methods, alone or collectively, on the shelf-life and quality of various sushi products. The existing research, suggest that sushi is a difficult matrix in terms of reducing microbiological growth, and usually obtaining an increase in shelf-life by a few days is considered a success. The increased popularity of sushi and RTE sushi meals will probably continue to grow, leading to the opportunity for collaboration between the sushi industry and the scientific community.

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757 **Tables**

758 Table 1. Food poisoning outbreaks related to sushi

Country/product/year	Responsible pathogen	Outbreak range (cases)	Reasons	References
USA/ butterfly shrimp/2004	Enterotoxigenic <i>E. coli</i>	130	Poor food handling/hand hygiene	Jain et al. (2008)
Singapore/sushi/2004	<i>Salmonella</i> spp.	12	The source could be contaminated raw sushi ingredients. Inadequate hand washing conditions and bare hand contact with ingredients	Barralet et al. (2004)
Japan/ikura/1999	<i>E. coli</i> O157:H7	n/d	n/d	Balfour et al. (2014)
Hong Kong/sushi and sashimi/1997-1999	<i>Vibrio parahaemolyticus</i> , <i>Staphylococcus aureus</i> , <i>Salmonella</i> spp., <i>Listeria monocytogenes</i>	142, 0.26% of sushi samples contaminated	Poor hygienic conditions of sushi production	CFS (2000)
Japan/dried <i>Porphyra umbilicalis</i>	Norovirus	>2,000	Contaminated employer at production plant	Kusumi et al. (2017)
Japan/sushi bar/2011	Hepatitis A virus	27	It was not possible to detect exact reservoir or vehicle of hepatitis A virus	Tominaga et al., 2012
Japan/sushi bar/2006	Hepatitis A virus	9	A sushi bar was found to be the source of the infection	Takeuchi et al., 2006

759 n/d – no data

761 Table 2. Summary of possible direct and indirect uses of various innovative and emerging technologies within the sushi industry along with their
 762 advantages and disadvantages

Method	Direct / Indirect*	Examples of possible usage	Advantages of the method	Disadvantages of the method	Results obtained through treatment of sushi	References of studies which investigated the treatment on sushi
MAP	Direct and indirect	<ul style="list-style-type: none"> - Packed final product - Storage of perishable ingredients 	<ul style="list-style-type: none"> - Well-established method in the industry - Accepted by consumers - Does not reflect negatively on final product quality - Inexpensive in operation - Prevents both microbiological spoilage as well as oxidation - Non-thermal method 	<ul style="list-style-type: none"> - Requires additional investment costs (packaging machine) - Requires use of special packages - Packages prone to physical damage which can cause a hazard to consumer due to reduced shelf-life 	<ul style="list-style-type: none"> - Significant inhibition by up to 3 log cfu/g of TABC and psychrotropic bacteria counts in stored sushi 	<p>Chen et al. (2003)</p> <p>Mol et al. (2014)</p>
Cold plasma	Direct and indirect	<ul style="list-style-type: none"> - Packed final products - Unpacked final products - Sushi ingredients 	<ul style="list-style-type: none"> - Antimicrobial effect due to various mechanisms increasing effectiveness - Inexpensive in operation - No additional information on product 	<ul style="list-style-type: none"> - Not functioning in industry on a broader scale (hard to find industry-scale machines) - Can promote oxidation - Can be harmful to 	<ul style="list-style-type: none"> - Reduction of TABC of stored hosomaki by up to 1.5 log cfu/g resulting in 4 days shelf-life extension - No shelf-life 	<p>Kulawik et al. (2018a)</p>

		<ul style="list-style-type: none"> - Packages - Air sterilisation 	<ul style="list-style-type: none"> labels required - Non-thermal method 	<ul style="list-style-type: none"> humans if exposed - Antimicrobiological effect obtained only on surface of product 	<ul style="list-style-type: none"> extension of salmon nigiri samples - Plasma treatment increased the oxidation rate of sushi samples 	
UV and pulsed UV	Direct and indirect	<ul style="list-style-type: none"> - Packed final products (ozone generation when appropriate wavelengths are used) - Unpacked final products - Sushi ingredients - Packages - Air sterilisation 	<ul style="list-style-type: none"> - Well-established method in the industry - Various effects depending on used wavelength - Increased efficiency when using pulsed UV/light - No additional information on product labels required - Low investment and operating costs - Non-thermal method (with exceptions) 	<ul style="list-style-type: none"> - Can promote oxidation - Antimicrobiological effect obtained only on surface of product - Often limited decontamination effect - Can increase the temperature of the treated product if the process is carried too long - Lack of research related to direct use of UV/light on sushi 	<ul style="list-style-type: none"> - Shelf-life extension of sushi meals from 3 to 7 days when using in hurdle: UV, EW, MAP, ozone and ultrasounds - UV has been used to created ozone within the MAP packed samples - To date no studies of PUV performed on sushi 	Steffen et al. (2010)
Edible coatings	Direct and indirect	<ul style="list-style-type: none"> - Unpacked final products - Sushi ingredients 	<ul style="list-style-type: none"> - Low investment costs - Various additional effects, aside from antimicrobiological, can be obtained if appropriate ingredients of coatings are 	<ul style="list-style-type: none"> - Can induce sensory changes to final product - Effectiveness dependent on used ingredients - May require additional information on label (list 	<ul style="list-style-type: none"> - Edible coatings with tea extracts did not affect microbiological growth, but inhibited oxidation rate 	Kulawik et al. (2019b)

			used	of ingredients)	
			<ul style="list-style-type: none"> - Active compounds can be released, which penetrate the surface of product providing antimicrobiological effects in deeper parts of product - Non-thermal method 	<ul style="list-style-type: none"> - Depending on used ingredients, operating costs may be high 	
Plasma activated water	Indirect	<ul style="list-style-type: none"> - Sushi ingredients - Packages 	<ul style="list-style-type: none"> - Low investment costs - Non-thermal method - No additional information on product labels required 	<ul style="list-style-type: none"> - Cannot be used on final product - Can affect sensory quality of ingredients - Can promote oxidation - Can be harmful to humans if exposed - Product has to be immersed in water or placed on ice 	To date no studies performed on sushi
Electrolysed water	Indirect	<ul style="list-style-type: none"> - Sushi ingredients - Packages 	<ul style="list-style-type: none"> - Low investment and operating costs - Commonly used in sushi industry - Non-thermal method - No additional information on product labels required 	<ul style="list-style-type: none"> - Cannot be used on final product - Limited antimicrobiological effect - Product has to be immersed in water or placed on ice 	<ul style="list-style-type: none"> - EW was used to decontaminate sushi ingredients in sushi production plant - Shelf-life extension of sushi meals from 3 to 7 days when using in hurdle: UV, EW, MAP, ozone and
					Steffen et al. (2010)

ultrasounds					
HPP	Indirect	- Sushi ingredients	<ul style="list-style-type: none"> - Non-thermal method - Can deactivate various compounds affecting product quality deterioration - No additional information on product labels required 	<ul style="list-style-type: none"> - Low efficiency if low pressure is used - Can induce colour and texture deterioration if higher pressure is used - High investment costs 	To date no studies performed on sushi
Active packaging	Direct	- Packed final products	<ul style="list-style-type: none"> - Various additional effects, aside from antimicrobiological influence, can be obtained if appropriate ingredients of package are used - Non-thermal method - No changes to sensory parameters of product 	<ul style="list-style-type: none"> - High operating costs - Lack of research related to direct use of active packages on sushi 	To date no studies performed on sushi

763 Direct – can be applied directly onto prepared and ready-to-eat sushi; Indirect – can be used in sushi industry, but not on prepared sushi due to
764 possible quality loss of final product

Process	Microbiological hazard	Preventative measures	Innovative and emerging technologies that can be used to eliminate/reduce hazard
Raw material delivery	1) Arrival of microbiologically contaminated raw materials	1) Purchase of raw materials from verified suppliers 2) Visual inspection of delivered raw materials 3) Temperature measurement of delivered raw materials requiring special storage temperature 4) Disinfection of raw materials before their transfer to storerooms	- UV/light treatment - Cold plasma
Raw material storage	1) Growth of microorganisms on stored raw materials 2) Cross-contamination between raw materials or from infected personnel	1) Maintaining appropriate storage conditions: temperature/humidity 2) Obeying hygiene standards by personnel 3) Obeying cleaning and disinfection procedures 4) Air disinfection 5) Storage of products on active ice	- UV/light treatment (air disinfection) - Cold plasma (air disinfection) - Ozone (air disinfection) - Plasma activated water (active ice) - Electrolysed water (active ice)
Freezing/Thawing	1) Growth of microorganisms due to prolonged freezing/thawing time or inadequate frozen storage temperature	1) Ensuring appropriate air circulation in freezing 2) Ensuring appropriate temperature of storage	1) Plasma activated water 2) Electrolysed water

Preliminary treatment of raw materials	1) Prevalence and growth of microorganisms on raw materials during preliminary treatment 2) Cross-contamination between raw materials or from infected personnel	3) Immersing product in active water prior to freezing 1) Maintaining appropriate temperature conditions in rooms where preliminary treatment takes place (for example 0-4°C in fish cutting room) 2) Obeying hygiene standards by personnel 3) Obeying cleaning and disinfection procedures 4) Air disinfection 5) Disinfection of raw materials	- UV/light treatment - Cold plasma - Plasma activated water - Electrolysed water - HPP
Rice washing	1) Cross contamination of rice from personnel or water	1) Obeying hygiene standards by personnel 2) Ensuring appropriate microbiological quality of water used in facility 3) Subsequent thermal treatment should reduce risk to acceptable level	None
Rice desiccation	No distinctive hazards	None	None
Rice boiling	No distinctive hazards	None	None
Mixing with vinegar solution	1) Cross contamination of rice from personnel or water	1) Obeying hygiene standards by personnel	None

	2) Too high pH of rice after mixture allowing growth of many spoilage bacteria	2) High temperature of rice during mixture should reduce risk of cross contamination 3) Monitoring pH of vinegar mixture/rice	
Rice cooling	1) Uncontrolled growth of microorganisms during cooling	1) Ensuring appropriate pH of rice after mixture with vinegar solution 2) Ensuring quick reduction in temperature of rice to approx. 40°C after which rice can be used 3) Storage of rice in chilled storerooms if rice is prepared in advance 4) During reheating of rice (in case of previous chilled storage), ensuring appropriate temperature of rice (for example 72°C)	- UV/light treatment (air disinfection) - Cold plasma (air disinfection) - Ozone (air disinfection)
Product forming and cutting	1) Cross-contamination between raw materials or from infected personnel 2) Uncontrolled growth of microorganisms due to prolonged time of product formation	1) Obeying hygiene standards by personnel 2) Maintaining reduced temperature conditions in production rooms (for example 0-4°C) to ensure cold chain continuity	- UV/light treatment (air disinfection and formed product) - Cold plasma (air disinfection and formed product) - Ozone (air disinfection and formed product) - Edible coatings (coating of formed product prior to packaging)
Packing	1) Cross-contamination between	1) Obeying hygiene standards by	- UV/light treatment (air

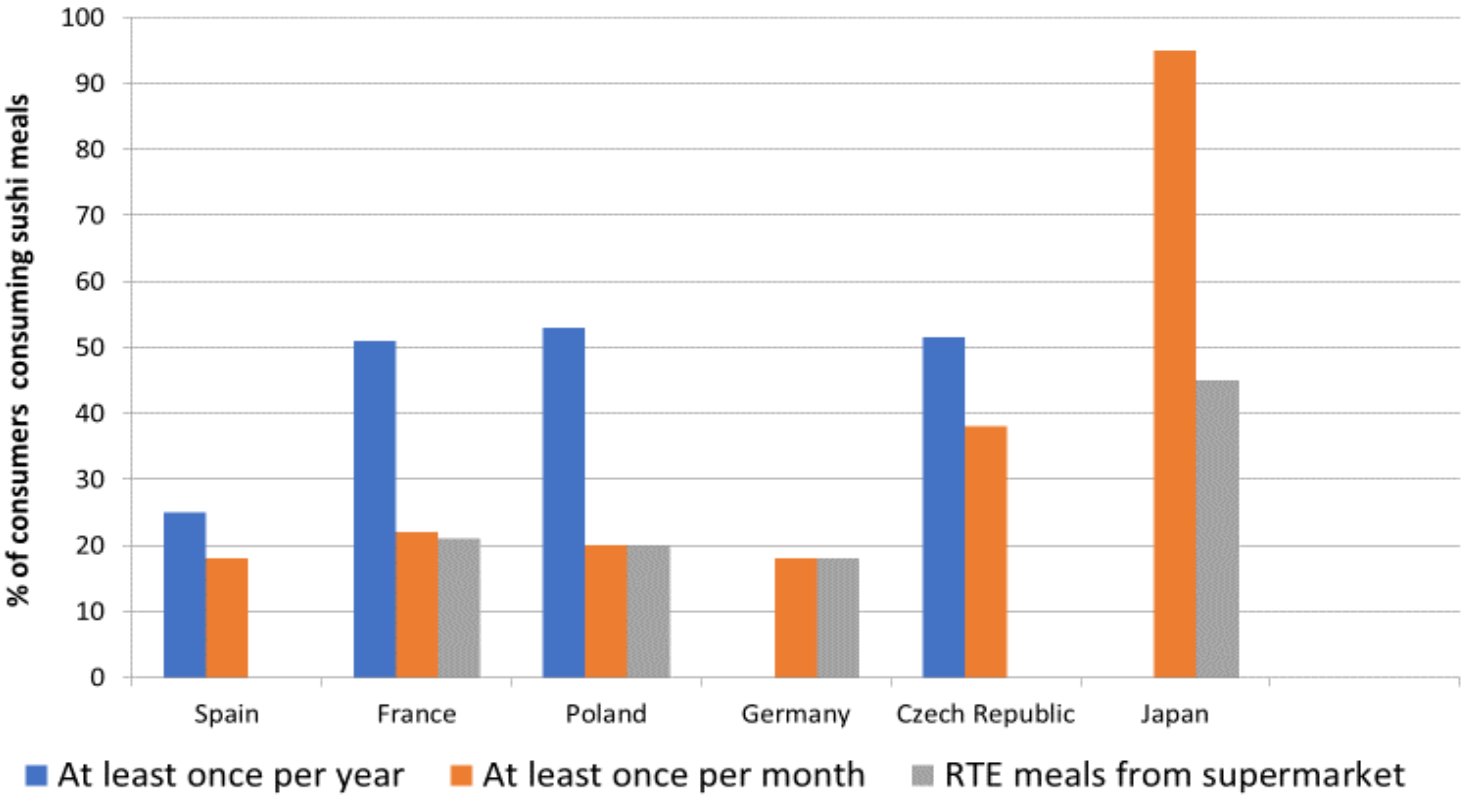
	raw materials or from infected personnel 2) Uncontrolled growth of microorganisms due to temperature increase	personnel 2) Maintaining reduced temperature conditions in packaging area (for example 0-4°C) to ensure cold chain continuity	disinfection, product on trays prior to sealing, in-package ozone generation) - Cold plasma (air disinfection and packed product) - Ozone (air disinfection, in-package ozone generation) - Active packaging - MAP
Final product storage	1) Uncontrolled growth of microorganisms due to temperature increase 2) Microbiological contamination due to damaged packages	1) Maintaining appropriate storage temperature 2) Obeying Good Manufacturing Practice rules during storage	None
Expedition	1) Uncontrolled growth of microorganisms due to temperature increase 2) Microbiological contamination due to damaged packages	1) Maintaining appropriate temperature of product during transport 2) Obeying Good Manufacturing Practice rules during transport	None

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769 **Figures**

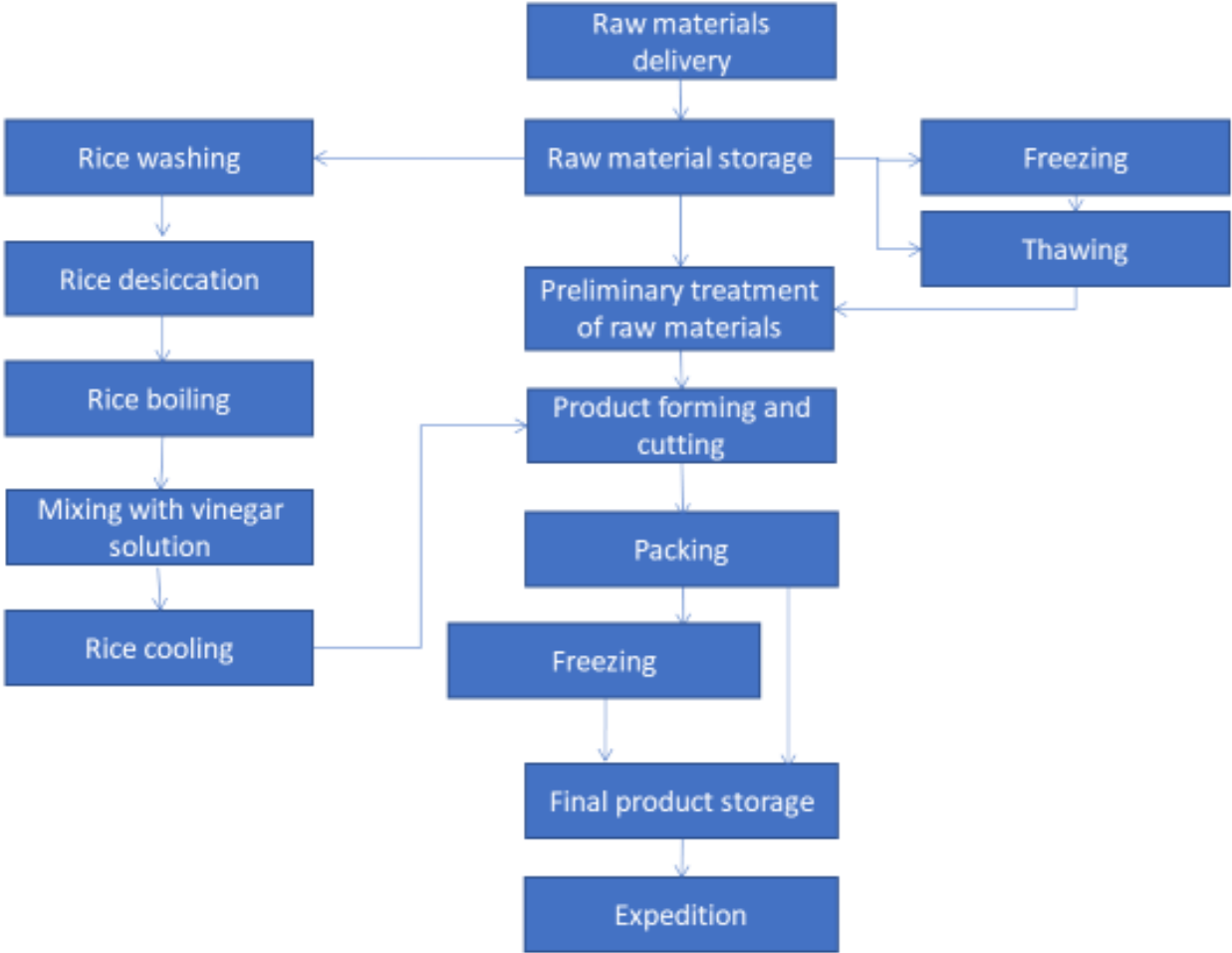
770 Figure 1. Percentage of consumers eating sushi meals in selected countries (De Silva and Yamao, 2006; D Dordevic and Buchtová, 2017; NSC,
771 2015, 2016a, 2016b, 2017).



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773 * No data available for the number of consumers eating sushi meals at least once per year in Germany and Japan; No data available for the number of
774 consumers eating RTE sushi meals from supermarkets for Spain and Czech Republic; RTE – ready-to-eat

775 Figure 2. Sushi production flow diagram



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778 Figure 3. Main challenges faced by the sushi industry

