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RADIOFREQUENCY TREATMENT TO CONTROL MICROBIAL FOOD SPOILAGE ISSUES - A CRITICAL REVIEW

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Abstract

Food spoilage may be defined as a process or few changes that could lead to an undesirable or unacceptable product for consumption. Food stability may be hampered by the contamination of a large diversity of microbial spoilers, from prokaryotes (Gram-negative and Gram-positive bacteria) to unicellular (yeasts) and multicellular eukaryotes (moulds). Effective preventive measures and innovative preservation methods have been intensively studied with the aim of food spoilage reduction and food shelf life improvement. The primary focus of food preservation has been on controlling microbial populations, with a specific emphasis on pathogenic microorganisms. In the last years, innovative processes and technologies have been applied in food industry as non-conventional preservative hurdles, which also respond to consumer demand for safe minimally processed food. This paper studies the effect of radiofrequency (RF) treatment on food spoilage microorganisms.

Key words: *food spoilage, radiofrequency, antimicrobial.*

INTRODUCTION

In recent years, food spoilage has been a growing problem in the food industry, mainly because of the limitation for the use of preservatives as a conservation method, in order to apply mild conservation techniques as a direct response to the consumers' requirements. Microorganisms that are present in food products continue to be a problem both for maintaining the quality of the food products and also regarding safety aspects. Therefore, food safety is a constant concern for the food industry (Deliu et al., 2013). Taking these matters into consideration, studies have been made in order to better understand the spoilage microorganisms and their phenotypic development and genetic makeup (Sanders et al., 2015). Unwanted organoleptic changes in food products may occur because of the food decomposition, which is a metabolic process that takes place inside the food matrix. These organoleptic changes consist in appearance, taste and consistency alterations of the food product, and even though generally they do not cause food borne illnesses, they affect the retail value of the food products. Some microorganisms that are involved in the food decomposition process may cause food poisoning (Shekarforoush et al., 2015).

Microbial food spoilage is characterised by random contamination with microorganisms which may lead to uncontrolled changes to the food product. The nature and character of a contaminant are not known before the food-microorganism contact happens. Consequently, in food preservation and prevention of spoilage, it is desired to account for all possible contaminants. In practice this is not feasible as many different species and strains from very different sources may cause spoilage, even in a selective environment based on a defined food formulation. This problem drives the need to better characterise possible contaminants and to describe the microbial biodiversity. Such knowledge may help to define better the boundaries for preservation of a particular food category, which is of relevance to consumers demanding minimally preserved, high quality and yet reliable food products. The importance of assessing biodiversity has been well appreciated for food pathogens (Lianou & Koutsoumanis, 2013).

The constant study and evaluation of the processing time, heating uniformity and quality influence over the final treated food product is performed due to the fact that these factors derive from the food heating processing methods. Another very important factor that is being evaluated is the energy efficiency prediction. There are several technologies (physical, chemical, and biological) used to control or reduce microorganisms in foods. Apart from the traditional processes such as thermal processing, novel technologies such as high-pressure processing, pulsed electric field processing, irradiation, ultraviolet light, ozone, others are also being developed (Deliu et al., 2013; Bermudez-Aguirre and Barbosa-Canovas, 2010).

The most common electromagnetic fields applied in the food industry are microwaves at 915 and 2450 MHz and radio frequency at a nominal 27 MHz (Brunkhorst et al., 2016). Radio-frequency (RF) heating treatment is more preferred by the food industry because it uses a longer wavelength electromagnetic energy that microwaves heating (MW). RF heating systems are primarily composed of two parts: a generator and an applicator. The generator supplies radiofrequency power, with free running oscillating systems being the most common generators used in food processing industries. The applicator consists of electrode plates that send electromagnetic currents to the products through field applicators (Rowe, 2018).

Radio-frequency (RF) heating treatment does not affect the air surrounding the product, only targeting the product itself. The overheating and over-dehydration of the treated products surface is being avoided by equalizing moisture throughout the product because the interior of the product gets hot faster than the surface, RF treatment bringing the moisture from inside outward (Orsat & Raghavan, 2014). Because of the above mentioned, the RF treatment can be applied in many applications of the food industry like drying of food products. Due to the good penetration depth, heat homogeneity and more stable control of the product temperature, in recent years, there has been an increased interest in radio-frequency thermal drying (Altemimi et al., 2019).

This paper studies the effect of radiofrequency (RF) treatment applied in food products, with special emphasis on the effect on the microorganisms involved in food spoilage.

INACTIVATION MECHANISM OF RF

Radiofrequency is a part of the electromagnetic spectrum with frequency in the range of 30-300 MHz. It is an indirect electro heating technique where initially, the electrical energy is transformed to electromagnetic radiation and slowly released as heat into the desired food sample depending on the dielectric characteristics of the food product (Rifna et al., 2019). The RF heating is achieved by using electric resistance heating and dipole heating resulted from the movement of the dissolved ions in the treated food product (Awuah et al., 2005).

Effectiveness of RF treatments on microorganism inactivation relies on few key factors such as temperature, frequency, effect of sample depth, sample moisture, capacity of equipment, microbial targets. The microbial decontamination using radiofrequency heating is based on the principle that when the heat is developed at a faster rate within the microbial cell than in another medium, the cells get thermally destroyed at a low heating rate (Rifna et al., 2019). As a consequence of exposing bacteria to RF radiations, injury may take place, which causes the leakage of some intracellular components such as ATP, nucleic acids, and proteins. This leakage leads to imbalance of the energy system, the enzymatic activity, and finally to cell death (Roohinejad et al., 2018).

The transmembrane potential is artificially increased by the exposure of a cell to an electric field that creates a charge which is stored on the cell membrane. If the transmembrane potential level is high enough and maintained for a long enough period of time, a significant increase in the cell membrane permeability to macromolecules and ions will appear.

The membranes of cells will reseal when the induced permeabilization level is moderate, and so the cell will be viable minutes after field delivery. The cells will die if the permeabilization level is made with prolonged and very high field (González-Sosa et al., 2014).

RF TREATMENT APPLICATIONS IN FOOD INDUSTRY

Lately, many studies were focused on the effect of dielectric heating processing over a large variety of food products on the microbial and pest reduction effects, such as poultry, meat, eggs and egg by-products, fish, fresh or canned fruits and vegetables, soy milk, jam, cakes, pasta, breads, spices, starch and ready to eat meals (Mitelut et al., 2011; Orsat & Raghavan, 2014).

Some examples are summarized in Table 1 and further described in more detail.

Villa-Rojas et al. (2017) studied RF heating of organic wheat flour, and evaluated *Enterococcus faecium* as a surrogate for RF inactivation of *Salmonella*. *Salmonella* reduction of 7 log was achieved at 0.45 and 0.65 a_w at room temperature, while 5 and 3 log reductions were reached for *Salmonella* and *E. faecium*, respectively, at 0.25 a_w. These data suggest that RF heating has potential as an inactivation treatment for *Salmonella*, and that *E. faecium* is a feasible surrogate to validate the efficacy of RF treatments.

Radiofrequency (RF) heating was evaluated by Chen et al. (2019) as a novel spice decontamination technology of cumin seeds. For the sample treatment a RF system of 6kW 27.12 MHz was used and two microbial strains, *Salmonella enterica* and *Enterococcus faecium* were inoculated in the cumin seeds samples in order to study the microbial change after the RF treatment. The treated samples displayed different results: *E. faecium* strain showed higher survival rate than *Salmonella* in all three batches of cumin samples, thus concluding that *E. faecium* can be used as a surrogate of *Salmonella* in the RF treatment process. In terms of quality of the treated cumin samples, no significant differences were recorded (Chen et al., 2019).

A study performed by Kou et al. (2018) analysed the use of non-thermal processing inactivation over some microorganisms with the help of RF treatments. Two types of strains, *Escherichia coli* and *Staphylococcus aureus,* that are present in apple juice and mash potatoes were treated with RF and conventional heating treatments. In order to compare their efficiency over the inactivation of the tested microorganisms, a radio-frequency treatment system that operates at 27.12 MHz frequency was designed. This system is comprised of a thermal heating block that emulates the desired heating temperature, uniformity and rate.

The survival rates of the *Escherichia coli* and *Staphylococcus aureus* strains present in the analysed food samples were similar as well for the heating block treatments as well for the radio-frequency treatments. A 1 log CFU/ml absolute difference of survival populations was observed. The results showed that the microorganism inactivation was observed only at the energy of 27.12 MHz obtained by the RF system (Kou et al., 2018).

The radio-frequency (RF) heating efficiency to inactivate *Salmonella typhimurium* and *Escherichia coli* O157: H7 on black and red pepper spice was investigated by Kim et al. (2012). A 27.12 MHz RF heating system consisted of two parallel plate electrodes was used, with the sample being placed between them. Two types of pepper samples, black peppers (whole and ground) and red peppers, were inoculated with *S. typhimurium* and *E. coli* O157:H7 strains and then treated with RF energy for different periods of time: 50 seconds for black peppers and 40 seconds for red peppers. Colour changes that occurred during the RF treatment of the samples were evaluated. The results showed that the RF heating treatment applied for 50 seconds to the black peppers resulted in 2.80 to 4.29 log CFU/g reductions of *S. typhimurium* and *E. coli* O157:H7. As for the red pepper samples that were treated with RF for 40 seconds, the pathogens were reduced by 3.38 log CFU/g to more than 5 log CFU/g (below the detection limit) without affecting the colour quality change (Kim et al., 2012).

In 2019, Wei et al. started a study that concerned the application of RF processing for pasteurization of black pepper that has been ground. At a radio-frequency treatment time of 130 seconds more than 5.98 log CFU/g reduction for *Salmonella* spp. was obtained, as for the *E. faecium* strain a reduction of 3.89 log CFU/g was obtained. Higher thermal resistance of *E. faecium* strain indicated its suitability as surrogate for *Salmonella* spp. during RF heating of ground black pepper (Wei et al., 2019).

Table 1. Effect of RF treatment applied in different food products

Product	Effect of RF treatment	Reference
Organic wheat flour	reduction of *Salmonella* and *E. faecium* in tested samples	Villa-Rojas et al., 2017
Cumin seeds	*E. faecium* strain showed higher survival rate than *Salmonella* in cumin samples	Chen et al., 2019
Black and red pepper	reduction of *S. typhimurium* and *E. coli* O157:H7 colour of samples was not affected	Kim et al., 2012
Black pepper	reduction of *Salmonella* spp. and *E. faecium* in tested samples	Wei et al., 2019
Peaches and nectarines	RF treatment effective to control *Monilinia fructicola* in tested samples	Casals et al., 2010
Cooked carrots	combined sterilization effect of ZnO nanoparticles with RF treatments prolonged the shelf life of cooked carrots up to 60 days, and reduced the loss of hardness, carotenoids and colour difference value of tested sample	Xu et al., 2017
In-shell walnuts	100% mortality rate for the instar navel orange worm larvae	Wang et al., 2007
Milk	inactivating of *Listeria* and *E. coli* in tested samples	Awuah et al., 2005
Soybean milk	reduction of *Bacillus subtilis* spores in tested samples	Uemura et al., 2010
Orange juice	reduction of *Escherichia coli* K12 in tested samples	Geveke et al., 2007
Pre-packaged bread Loaf	reduction of *Penicillium citrinum* spores shelf-life was prolonged by 28 ± 2 days	Liu et al., 2010
Peanut butter Cracker sandwiches	reduction of *S. typhimurium* and *E. coli* O157:H7 in both creamy and chunky peanut butter	Ha et al., 2013
Ham	improvement of shelf life of repacked hams by reducing the bacterial load and moisture loss	Orsat et al., 2004
Ground beef	heating to 55°C reduced *E. coli* in homogenates containing 2.5% potassium bicarbonate, 0.5% citric acid, and blends of citric acid and potassium bicarbonate at varying concentrations (0.5% citric acid and 1.5% bicarbonate)	Nagaraj et al., 2016

The growth control of brown mould in peaches and nectarines with the help of RF heating was studied by Casals et al. (2010). The samples were inoculated with *Monilinia fructicola* or with natural *Monilinia* spp. Inoculum. The results showed that a RF treatment at 27.12 MHz, with 17 mm distance between the fruit samples and upper electrode and an exposure time set at 18 min was selected as effective conditions to control brown moulds development in peaches without affecting fruit quality (Casals et al., 2010).

Effects of ZnO nanoparticles combined with RF heating on the sterilization and product quality attributes (hardness, colour, carotenoids and microstructure) of cooked carrots were investigated by Xu et al. (2017). The results of this study demonstrated that the combined sterilization effect of ZnO nanoparticles with RF treatments was greater than ZnO nanoparticles or RF heating treatment alone and prolonged the shelf life of cooked carrots up to 60 days. RF heating 20 mm/20 min (plate spacing/RF heating time) combining ZnO nanoparticles reduced the loss of hardness, carotenoids and colour difference value (ΔE) of cooked carrots (Xu et al., 2017).

A study concerning the use RF treatment as an alternative method to chemical fumigation in order to control postharvest insect contamination of in-shell walnuts was carried out by Wang et al. (2007). The RF treatment process was made possible by using a 25 kW, 27 MHz RF processing technique to achieve surface temperature of 60°C of the walnut samples. The minimum temperature has to be 52°C and the minimum treatment time needs to be 5 min. The results showed a mortality rate of 100% for the instar navel orange worm larvae, which is the most heat tolerant target pest, which is present in both air-dried and unwashed walnuts. The 100% mortality rate was achieved over a relatively wide range of walnut moisture contents (3-7.5%).

Awuah et al. (2005) used a 2 kW, 27.12 MHz RF heater, for the evaluation of the effectiveness of RF heating in inactivating surrogates of both *Listeria* and *Escherichia coli* cells in milk under continuous flow conditions. Depending on product residence time and RF power level, RF heating was found to be capable of inactivating both *Listeria* and *E. coli* in milk, with *E. coli* being the most heat sensitive. For a total residence time of 55.5 s (i.e., 29.5 and 26 s in the applicator and holding tube, respectively), up to 5- and 7-log reductions were found for heating *Listeria* and *E. coli*, respectively at 1200 W, and an applicator tube exit temperature of approximately 65°C (Awuah et al., 2005).

Uemura et al. (2010) developed an equipment for soybean milk pasteurization using RF flash heating (RF-FH). The soybean milk samples were exposed to an electrode with its surface covered in a 50 µm thick Teflon film and a 28 MHz RF-FH energy was applied. The exposure time was 0.4s and the treatment temperature was at 115°C which lead to a four-logarithm-order reduction of *Bacillus subtilis* spores in the soybean milk sample. In the same study, the soybean milk samples were processed with of RF-FH and conventional processing in order to obtain tofu. The results of the study revealed that the tofu produced using RF-FH processing had higher gel strength than the tofu made using conventional heating (Uemura et al., 2010).

Geveke et al. (2002) performed a study on non-thermal inactivation of microorganisms contained in some liquid samples. The liquid samples were treated with RF energy and in the same time heat was removed in order to control the temperature while in order to minimize localized heating the turbulent flow was maintained. Electric field strength of approximately 0.5 kV/cm was obtained from an 18 MHz RF processor, and then applied to the liquid samples. No non-thermal effects of the RF treatment energy were observed in yeast in apple cider, beer, tomato juice, liquid whole egg, deionized water, *Escherichia coli* K-12 and *Listeria innocua*. Also, there were no synergistic effects of RF energy related with heat. The low temperature effects of RF energy at 18 MHz and 0.5 kV/cm were due to heat (Geveke et al., 2002).

The effect of radio frequency electric fields (RFEF) on the inactivation of *E. coli* in apple and orange juice was investigated by Geveke et al. (2007). Pulp-free orange juice samples were processed using an 80 kW RFEF pasteurizer at flow rates of 1.0 l/min and 1.4 l/min. The orange juice samples inoculated with *Escherichia coli* K12 were treated with electric field strengths of 15 kV/cm and 20 kV/cm at three different range frequencies: 21, 30, and 40 kHz. A reduction of 3.3 log of *Escherichia coli* K12 was observed at the orange juice samples compared to the control sample, at an outlet temperature of 65°C. It was shown that by increasing the temperature and time of the RF treatment and decreasing the frequency, enhanced the inactivation levels. The variation of the electric field strength over the conditions used in the experiments had no effect on the inactivation of the *Escherichia coli* K12. After the RFEF thermal processing of the samples, no enzymatic browning or diminution of the ascorbic acid concentrations were observed. The voltage and the current used in the experiments resulted in an electric energy of 180 J/ml (Geveke et al., 2007).

A study concerning the applications of radio frequency (RF) treatment combined with conventional hot air treatment used to provide uniform heating for control of mould in pre-packaged bread loaf samples was done by Liu et al. (2010). In order to conduct the experiments a 6 kW, 27.12 MHz RF system was used. The treatment parameters used in the experiments were selected in a way that they did not influence the bread quality and based on minimum time–temperature conditions that were required for 4-log reduction of *Penicillium citrinum* spores. The core and periphery of bread loafs samples were subjected to heat with approximately the same heating rate during the combined hot air and RF treatments, thus the maximum difference of heating temperatures was less than 5°C in one bread slice. The overall differences in sample qualities between RF treated bread samples and control samples were not significant, the water activity and moisture contents of RF treated samples increased in the beginning of the treatment and then decreased, compared to the untreated samples. The firmness values showed an increasement during the storage of the both types of samples, treated and untreated. In order to control the *P. citrinum* spores, because of greater heating uniformity, a lower mean product temperature and a shorter holding were used with combined RF and hot air treatment in order to compare with the conventional heating alone. A reduction of 4-log of *P. citrinum* spores was obtained when heating bread samples at about 58°C. The shelf-life was prolonged by 28 ± 2 days for the treated white bread samples at room temperature (23°C) (Liu et al., 2010).

Ha et al. (2013) investigated the efficacy of RF heating to inactivate *S. typhimurium* and *E. coli*

O157:H7 in peanut butter cracker sandwiches. The treatment consists of a 27.12 MHz RF heating system with a maximum time of exposure of 90s. After treatment, a log reduction (CFU/g) of 4.29 was registered for *S. typhimurium* and 4.39 for *E. coli* O157:H7 in the case of creamy peanut butter. Regarding chunky peanut butter, the treatment led to a log reduction of 4.55 for *S. typhimurium* and 5.32 for *E. coli* O157:H7 (Ha et al., 2013).

Orsat et al. (2004) studied the radio-frequency heating at 27.12 MHz for the pasteurization of ham samples repacked in plastic films. The samples were brought to internal temperatures of 75°C and 85°C in 5 min and maintained at those temperatures for an additional 5 min. The ham samples were vacuum-packed in three different plastic films and stored at 4°C for 1 to 28 days. The study indicates that RF heating, combined with suitable packaging system, can lead to an improvement of the shelf life of repacked hams by reducing the bacterial load, moisture loss and maintaining an overall greater product sensory quality and acceptance (Orsat et al., 2004).

Nagaraj et al. (2016), investigate the inactivation of *E. coli* in ground beef homogenate using RF treatment both individually and in combination with antimicrobial agents. The RF heating of inoculated beef homogenate without antimicrobial agents to end-point temperatures of 50°C and 55°C resulted in 0.84 log reduction, respectively 0.94 log reductions. Adding antimicrobials (such as potassium, sodium, and ammonium bicarbonates (0.5 and 1.5%); potassium lactate (2.5%); citric acid (0.5%); and a blend of citric acid (0.5%) and potassium bicarbonate (1.5%) to the beef homogenates before RF treatment did not inactivate *E. coli*. Heating to 55°C reduced *E. coli* more than 5 log (CFU/mL) in homogenates containing 2.5% potassium bicarbonate, 0.5% citric acid, and blends of citric acid and potassium bicarbonate at varying concentrations (0.5% citric acid and 1.5% bicarbonate) (Nagaraj et al., 2016).

A simulation of RF heating was performed using a finite element shell for microbial decontamination of egg shell immersed in deionized water by Lau et al. (2017). In all the investigated configurations, concentrated heating occurred in the yolk. After 20 min of RF heating, some configurations reached about 60°C in the yolk. The cooling effect of the water together with lower electric field intensity in the egg caused the focused heating in the yolk. Extrapolation of the model demonstrated that a RF heating process (10.5 kV at top electrode) followed by a hot water immersion process can achieve a minimum of 3 log reductions of *Salmonella* in the yolk within 37 min (Lau et al., 2017).

CONCLUSIONS

Nowadays, these novel processing techniques are being intensively tested to be used in food industry in order to improve the safety and quality of the food products that we eat, as they are capable of inactivating microorganisms, promoting chemical reactions, changing cell permeability, and even inactivating enzymes. Due to major advantages offered by RF treatment, including the possibility to fast penetration up to 20 cm or more into food for more uniform and efficient heating and limited negative side effects (reduced food quality or objectionable sensory perception), RF technology has considerable potential to replace traditional (water and steam) and microwave heating for food processing.

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