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The real role of select herb and spice extracts against *Bacillus cereus* ATCC 14579 growth in cooked rice

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Abstract

This study aimed to investigate the effect of select spices and herbs added to cooked rice in the growth of *Bacillus cereus*. The results showed the actual role of herbs and spices as food flavoring ingredients rather than acting as a preservative agent. Herbs and spices extracts were added to rice before and after the cooking process, then observed the differences in *Bacillus cereus* growth. From all herb and spices extract used, clove was the most effective one against *B. cereus*. It provided a good zone of inhibition, while the others did not have similar effects. The ethanolic extracts for all tested herbs and spices at concentrations of 50-200 µg/mL showed good activity in media. The zone of inhibition was from 10 to 25 mm in diameter. Rice with Spiced/herbs was inoculated with 10^8 spores of *B. cereus* then the growth of *B. cereus* vegetative cells was detected at different temperature. As the result showed no different in the growth of *B. cereus,* We concluded that the temperature for rice cooking/reheating has main effect on the control of *B. cereus* cells while herbs and spices can be used only for flavor with no role in controlling the contamination in rice.

Keywords: herbs; spices; *Bacillus cereus*; cooked rice.

Practical Application: Control of bacterial contamination, *Bacillus cereus*, in rice using herb and Spice Extracts.

1 Introduction

Herb and spice have long been used in foods all over the world, not only for improving the taste of food, but also perhaps to increase the properties and shelf life of different foods by combating foodborne pathogens (Lai & Roy, 2004). Different part of plants containing antimicrobial agents have also been used as food preservatives (Bor et al., 2016). Early Egyptians used spices and their essential oils for food preservation, as the case in China and India as well. Moreover, spices such as clove, cinnamon, and ginger are widely used as remedies. Some herbs contain compounds that provide antimicrobial activity against a wide-range of Gram-positive pathogens. The antibacterial activity of these compounds can be enhanced by the addition of active ingredients such as phenols, alcohols, aldehydes, and hydrocarbons, thereby increasing the storage time and stability. In the 1880s, cinnamon oil discovered to have great antibacterial activity against *Bacillus anthracis* spores, which comprised the first scientific study on the preservative effects of spices (Tajkarimi et al., 2010).

Spices can be divided into three groups according to their antibacterial activities, as follows: strong agents (clove and cinnamon), medium agents (thyme, cumin, caraway, coriander, rosemary, and oregano), and weak agents (ginger, red pepper, and black pepper) (Zaika et al., 1978).

Cinnamon is extracted from cinnamon bark, fruit, leaf, and their essential oils (Eos), and many *Cinnamomum* species produce a volatile oil upon distillation that can have different compositions, and aroma characteristics.(Jayaprakasha et al., 1997; Kaul et al., 2003; Negi, 2012). The antibacterial activity of the bioactive fraction of *Cinnamomum zeylanicum* has been proven. (Alizadeh Behbahani et al., 2020). Cinnamon oil has also been found to be highly effective against some bacteria, including *Bacillus* sp (Gupta et al., 2008).In another study, black pepper (*Piper nigrum*) and turmeric (*Curcuma longa*) (a total of six extracts) showed antibacterial activity against *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus sphaericus*, and *Bacillus polymixa* (Pundir & Jain, 2010).

The natural antimicrobial agents from spices are used in food manufacturing to increase shelf life and to improve the quality of food. Antimicrobials of plant origin are extracted from the aromatic oily liquids of flowers, seeds, leaves, bark, buds, twigs, herbs, wood, fruits, and roots using different methods.. Some essential oils (EOs), such as those extracted from clove and cinnamon, have strong antimicrobial effects, whereas ginger, black pepper, cumin, and curry powder have weaker antibacterial activities (Holley & Patel, 2005; Burt, 2004).

The most effective fraction of curcumin against *Bacillus cereus*, *Bacillus coagulans*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* was the hexane extract eluted with 5% ethylacetate (Negi et al., 1999).

Food poisoning caused by *B. cereus* often happens via starchy foods such as rice (Krämer, 1992; Baumgart, 1994). This contamination in rice were depending on the content of the soil (Hamid et al., 2020). Rice is contaminated via exposure to

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B. cereus vegetative cells, which create a metabolite toxin directly on the food substrate (Kleer et al., 2001). This toxin can cause vomiting and/or diarrhea, which happens after the consumption of contaminated rice. It is necessary for *B. cereus* to grow and reach a concentration between 10^5 and 10^6 per gram for the intoxication of the toxins. However, while the vegetative cells are destroyed by the cooking temperature, spores in the air can fall into the food, leading to critical concentration levels which scan cause intoxication. *B. cereus* spores are heat resistant and a long boiling time i is required to kill such spores which may affect the quality of rice.

The aim of the study was to determine the minimum inhibitory concentration (MIC) of different herbs and spices such as clove, cinnamon, curcumin, black pepper, ginger, and saffron in the growth of *B. cereus* ATCC 14579 in cooked rice which stored at room temperature (25 °C).

2 Materials and methods

2.1 Bacterial strain and media

B. cereus ATCC 14579 was used in all preparations of the culture media and was added to nutrient broth and then sub-cultured onto a nutrient agar plate before aerobic incubation at 37 °C for 24 h. The inoculum for the antimicrobial activity was prepared by adjusting the density of the organism to approximately 10^8 colony-forming units per milliliter (CFUs/mL), and this was equal to an optical density of 0.5 at 600 nm with the help of 0.5 McFarland standards.

2.2 Enumeration of B. cereus

The total number of vegetative cells and spores was counted by using the colony count technique according to ISO 7932 (International Organization for Standardization, 2004). To enumerate *B. cereus*, a serial dilution in a sterilized saline solution of Sodium chloride (0.85%) and the nutrient agar pour plate method were used then the plates were incubated at 37 °C for 24 h. The colony-forming units per milliliter were counted.

2.3 B. cereus endospore preparation

The spores of *B. cereus* ATCC 14579 were obtained according to Beuchat et al. (1997). The cultures were grown in Brain Heart Infusion broth (Oxoid code CM375) for 24 h then spread on the surface of a solid nutrient agar sporulation medium consisting of nutrient agar (Oxoid code CM0309) and incubated at 30 °C to obtain at least 90-95% spores. Microscopic examination of spores stained by Gram stain clearly differentiates between spores and vegetative cells. Spores were collected from the surface of the nutrient agar with a sterile cotton swab, and were re-suspended in sterile distilled water (3 mL per plate). The spores were then centrifuged at 5000 rpm for 15 min at 4 °C, after that washed twice with sterile distilled water by repeated centrifugation, and finally resuspended in sterile distilled water (10^8/mL). The number of spores was determined by serially diluting the heat-shocked spore suspensions in a sterile saline solution and plating by triplicate on Tryptic Soy Agar (TSA) then for 48 h at 37 °C .

2.4 Herb and spice samples

The herbs and spices used in this study included clove, cinnamon, curcumin, black pepper, ginger, and saffron, were collected from local markets in Riyadh City, Saudi Arabia.

2.5 Preparation of the extracts

Water extraction (cold and hot) of herbs and spices

Twenty grams of each of the spices and herbs were used in the experiment. The samples were added to 100 mL of sterilized distilled water and left overnight at 4 °C (cold extraction); meanwhile, for the hot extraction, the spices were exposed to an autoclave at 110 °C for 5 min and then left overnight in the dark. The extracts were centrifuged at 4000 rpm for 10 min. The supernatants were then passed through a membrane filter of 0.45 mm; the extracts were prepared at concentrations of 25, 50,100, 150 and 200 mg/mL and used directly against the *B. cereus* standard.

Ethanolic extract of herbs and spices

Twenty grams of each herb or spice were add to 100 mL of absolute ethanol then left overnight at 4 °C. The mixture was then centrifuged at 4000 rpm to remove any residuals.

2.6 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) were determined to investigate the antimicrobial activity of herbs and spices used in this study against *B. cereus*. The broth micro-dilution technique was used. All herb and spice samples which were extracted in cold water, hot water, or ethanol were tested against *B. cereus* ATCC 14579 (10^8 CFUs/mL). Brain Heart Infusion broth diluted were prepared with different concentrations of each herb and spice (100, 50, 25, 12.5, 6.25, and 3.125 µL/mL) (Grierson & Afolayan, 1999). The previous diluent used in the wells of the microtiter plate is and an equal volume of the stock solution transferred to the plate. From the concentration of the first well, two-fold serial dilutions are made to obtain a different concentration. Six concentrations represents the achievable concentrations for the used antimicrobials (Mendoza, 1998). An equal volume of microbial culture with an optical density (OD) of 0.5 at 620 nm (10^8 CFUs/mL) is added to the wells and incubated at 37 °C for 24 h After incubation, the plates are examined for changes in turbidity as an indicator of growth (Lourens et al., 2004; Basri & Fan, 2005). The clarity was determent using spectrophotometer by measuring the absorbance at 620 nm with a negative control as a blank. The MIC of each extract is the first well that appears clear and provides a zero absorbance reading (Salie et al., 1996).

2.7 Screening for antibacterial activity against B. cereus ATCC 14579

All extracts (The ethanol and hot and cold aqueous) of different herbs and spices were used to evaluate the antimicrobial activity by agar well diffusion method. *B. cereus* ATCC 14579 was grown on nutrient agar plates and incubated at 37 °C for 24 and 72 h. The culture was transferred into normal saline (0.85%) under aseptic conditions then the density was adjusted to equal to 10^8 CFUs/mL as previously mentioned, then used as an inoculum for performing an agar well diffusion assay. One hundred microliter (100 µL) of the inoculum was spread onto the agar plates to achieve confluent growth. After inoculated plates became dry, 8 mm wells were made with a sterile borer. The lower portion of each well was sealed with molten agar medium. A 50, 100, 150, or 200 µL volume of each extract was propelled directly into the wells of the inoculated agar plates. The plates were allowed to stand for 1 h at room temperature (40 °C) for the extract diffusion into the agar then incubated at 37 and 25 °C for 24 and 72 h, respectively. Sterile water and ethanol were used as the negative and positive control. The inhibition zone (clear zone) around the well containing the extract was determined for each herb and spice and was recorded if the zone was greater than 8 mm. A zone of inhibition

2.8 Artificial contamination of herbed/spiced rice by 10^8 of B. cereus spores before the cooking process and after storage at different temperatures (4, 25, and 35 °C)

The rice was washed five times with tap water and soaked for approximately 1 h at room temperature. The herbs and spices extracts were added according to the effect of the MIC. The herbed/spiced rice was inoculated with 10^8/mL of *B. cereus* spores and mixed well. The rice was cooked at 100 °C for 20 min. After cooling down at room temperature, the cooked rice was stored at different temperatures (4, 25, and 35 °C) for 72 h. The total viable number of *B. cereus* was counted daily per 1 g of each treated rice sample by using serial dilution and the pour plate method using Brain Heart Infusion agar (Oxoid code CM375). The negative control of the rice was inoculated with 10^8 of *B. cereus* spores without any additional extracts.

2.9 Addition of herbs/spices before and after the rice cooking process and contamination with 10^4 B. cereus ATCC 14579 spores at 70 °C and stored at 25 °C

The ethanolic extracts of different spices were mixed before and after the rice cooking process according to the MIC, following which they were contaminated with 10^4 CFUs/g of *B. cereus* ATCC 14579 and stored at 25 °C. The total viable count using the pour plate method was recorded using Brain Heart Infusion agar, and the samples were incubated at 37 °C for 72 h.

3 Results

The minimum inhibitory concentrations of the studied herbs and spices are listed in Table 1. Cold water extracts (Table 1A) for most herbs/spices appeared not to have an effect on the growth of *B. cereus* ATCC 14579 except Clove and Saffron at high concentration, MIC were 25 and 100 mg/mL respectively. Table 1B shows the effect of Herbs/Spices hot water extract on the growth of *B. cereus* ATCC 14579 and the minimum inhibitory concentrations of each herbs and spices. The two effective herbs/spices in cold water also appeared to have stronger effect when hot water used to make the extract. The MIC for Clove and Saffron hot water extracts were, 6.25 and 50 mg/mL respectively. Cinnamon and Black pepper also showed to have effect at the highest concentration, 100 mg/mL. Table 1C shows the minimum inhibitory concentrations of the different herbs and spices dissolved in ethanol. Almost all of the herbs/spices appeared to have an effect on the growth of *B. cereus* ATCC 14579. Clove, black pepper, curcumin, and saffron were effective at any concentration provided the a minimum inhibitory concentration was 3.125 mg/mL for Clove, black pepper, curcumin, and saffron while for cinnamon and ginger the MIC was 6.25 mg/mL.

Figure 1 shows the zone of inhibition of each extract against *B. cereus* ATCC 14579. Only Clove water extract (cold and hot water) had clear zone. However, all herbs/spices ethanol extracts had clear effect. Table 2 shows the size of the zone of inhibition (mm) for ethanol extracts of different herbs/spices at different concentrations (50, 100, 150, and 200 mg/mL) in order to study their effect on the growth of B. cereus ATCC 14579. All of the herbs/spices extracted by ethanol affected the growth of *B. cereus*, and the largest zone was noted for cinnamon and clove at a concentration of 100 mg/mL, reaching 30 and 25 mm in diameter, respectively. Ginger, black pepper, curcumin, and saffron showed zones of inhibition of 25, 20, 20, and 15 mm in diameter, respectively, at a low concentration of 100 mg/mL.

The data pertaining to the artificial contamination of herbs/spiced rice by 10^8 of *B. cereus* spores before and after the cooking process and storage at different temperatures (4, 25, and 35 °C) showed that there was no growth of *B. cereus,* meaning that all of the spores were killed at 100 °C for 20 min.

Figure 2 shows the effect on the growth of bacteria spores of *B. cereus* ATCC 14579 of adding herbs/spices to the rice before the cooking process at 70 °C and incubating at 37 °C for three days. It was clearly observed that the *B. cereus* strain was not affected by any of the herbs/spices added, and increasing the growth contaminated the rice and led to the subsequent release of the toxin.

Figure 3 shows the effects of adding herbs/spices to the rice after the cooking process on the growth of spore of *B. cereus* ATCC 14579 contaminated at 70 °C and incubated at 37 °C for three days. It was clearly observed that the *B. cereus* strain was not affected by any of the herbs/spices added, and increasing the growth contaminated the rice and led to the subsequent release of the toxin.

4 Discussion

The use of solvents in the extraction of spices or plant materials is the most common method because of its efficiency, ease of use, and wide applicability. Some of the common solvents used for the extraction of phenolics from plants are ethanol, acetone, ethyl acetate, and their combinations, as well as different proportions of water. The choice of a suitable solvent depends on the effects of the amount and rate of polyphenols extracted (Xu & Chang, 2007). Ethanol is less dangerous compared to other extraction solvents such as methanol and acetone, particularly when used as additives in foods.

Table 1. The effect of Herbs/Spices on the growth of *B. cereus* ATCC 14579 (10^8 (CFUs)/mL) Optical density (OD) at 600 nm.

A: Cold water extracts							
Herbs/Spices	Scientific Name	MIC (mg/mL) at 600 nm					
		100	50	25	12.5	6.25	3.125
Clove	*Syzygium aromaticum*	XXX	XXX	XXX	0.122	1.095	1.237
Cinnamon	*Cinnamomum zeylanicum*	XXX	0.643	1.389	1. 400	1.572	1.650
Black pepper	*Piper nigrum*	0.664	0.908	1.507	1.613	1.651	1.732
Curcumin	*Curcurma domestica*	XXX	0.764	0.904	1.122	1.149	1.272
Ginger	*Zingiber officinale*	0.533	0.500	0.597	1.027	1.341	1.49
Saffron	*Crocus sativus*	XXX	0.592	0.816	0.828	0.850	0.895
B: Hot water extracts							
Herbs/Spices	Scientific Name	MIC (mg/mL) at 600 nm					
		100	50	25	12.5	6.25	3.125
Clove	*Syzygium aromaticum*	XXX	XXX	XXX	XXX	XXX	0.966
Cinnamon	*Cinnamomum zeylanicum*	XXX	0.721	0.754	0.915	0.942	0.810
Black pepper	*Piper nigrum*	XXX	1.100	1.230	1.303	1.523	1.213
Curcumin	*Curcurma domestica*	0.651	0.981	1.00	1.050	1.057	1.100
Ginger	*Zingiber officinale*	0.115	0.345	0.698	1.566	1.393	1.409
Saffron	*Crocus sativus*	XXX	XXX	0.834	0.954	1.049	0.105
C: Ethanolic extracts							
Herbs/Spices	Scientific Name	MIC (mg/mL) at 600 nm					
		100	50	25	12.5	6.25	3.125
Clove	*Syzygium aromaticum*	XXX	XXX	XXX	XXX	XXX	XXX
Cinnamon	*Cinnamomum zeylanicum*	XXX	XXX	XXX	XXX	XXX	0.448
Black pepper	*Piper nigrum*	XXX	XXX	XXX	XXX	XXX	XXX
Curcumin	*Curcurma domestica*	XXX	XXX	XXX	XXX	XXX	XXX
Ginger	*Zingiber officinale*	XXX	XXX	XXX	XXX	XXX	0.641
Saffron	*Crocus sativus*	XXX	XXX	XXX	XXX	XXX	XXX

XXX: no growth.

Herein, the phenolic compounds found in the herbs/spices extracted by ethanol affected the growth of *B. cereus*. The antimicrobial activity of clove on *B. cereus*, attributed to eugenol, which can be released from clove by ethanol, leads to the deterioration or disruption of the membrane of *B. cereus* and may inactivate enzymes and genetic materials, as suggested by Wendakoon & Sakaguchi (1995). Abo El-Maati et al. (2016) reported that the yield and phenolic contents of clove, dry extracted using different solvents, range from 3.9 to 11.7 g extract/100 g dried clove bud. Clove extracted by ethanol generated the highest amount (11.7 g extract/100 g), followed by water (7.5 g extract/100 g) and finally ethyl acetate (3.9 g extract/100 g). This range or variation in extraction yields with different solvents is due to the differences in the polarity of the constituents found in plant materials (Jayaprakasha et al., 2001).

The effect of cinnamon is related to the cinnamaldehyde, which is responsible for its antibacterial activity due to its lipophilicity of terpenoids and phenyl propanoids, and which can invade the membrane and reach the inner part of the cell, thereby impair the bacterial enzyme system (Helander et al., 1998). Kwon et al. (2003) reported that *B. cereus* is most sensitive to cinnamic aldehyde.

Pepper (*Piper nigrum*) consists of more than 1000 species, distributed mainly in the tropical regions of the world (Chaveerach et al., 2006). *Piper nigrum* L. (black pepper) is the king of spices, which is considered to be the most famous species of this genus, and this title is attributed to its pungent principle piperine and the popularity of its use for flavoring food throughout the world (Ahmad et al., 2012). In medicine, *P. nigrum* has been used for many purposes since ancient times. For example, *P. nigrum* is used in medicine for its antibacterial, antifungal, antiapoptotic, antidepressant, antidiarrheal, anti-inflammatory, antimutagenic, antioxidative, antipyretic, antispasmodic, antitumor, anticold, anticough, anti-intermittent fever, anticolic, and antidysentery properties, as well as to improve appetite and digestive power, to cure dyspnea and throat diseases, and to treat worms and piles (Ahmad et al., 2012; Islam et al., 2015).

Curcumin inhibits bacteria by damaging the bacterial membrane. In a study by Tyagi et al. (2015), it was concluded that the addition of curcumin to the membrane of Gram-negative bacteria, such as *Pseudomonas aeruginosa* and *Escherichia coli*, and Gram-positive bacteria, such as *S. aureus* and *Enterococcus faecalis*, leads to the leakage of contents into the membrane. However, according to a study by Yun & Lee (2016), using a high concentration of curcumin induces damage of membranes, although there is no effect at the minimum inhibitory concentration. This is in agreement with our data for the cold and hot extracts of curcumin, which did not provide a minimum inhibitory concentration in comparison to the extraction by ethanol, which showed a large zone of inhibition at a high concentration. On the contrary, some authors have noticed that *B. cereus* and *E. coli* are inactivated by the addition of curcumin due to the production of reactive oxygen species (ROS), including singlet oxygen and

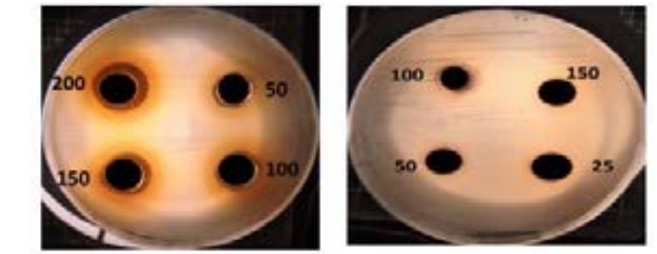

Figure 1. Effect of cold water (A), hot water (B), and ethanol (C) extracts of different herbs and spices concentrations on the growth of *B. cereus* ATCC 14579.

Table 2. Zone of inhibition (mm) of ethanolic extracts on the growth of *B. cereus* ATCC 14579.

Herbs/Spices	Scientific Name	Zone of Inhibition (mm)			
		50 µL	100 µL	150 µL	200 µL
Clove	*Syzygium aromaticum*	20	20	25	25
Cinnamon	*Cinnamomum zeylanicum*	20	25	27	30
Black pepper	*Piper nigrum*	15	18	18	20
Curcumin	*Curcurma domestica*	15	17	18	20
Ginger	*Zingiber officinale*	17	20	20	25
Saffron	*Crocus sativus*	10	12	15	15

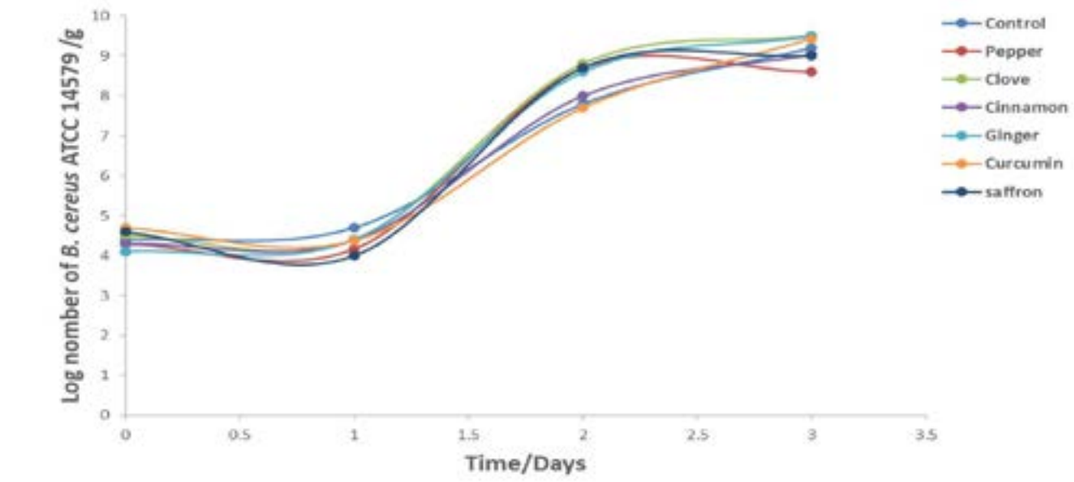

Figure 2. The contamination of herbs/spiced cooked rice by *B. cereus* ATCC 14579 spores.

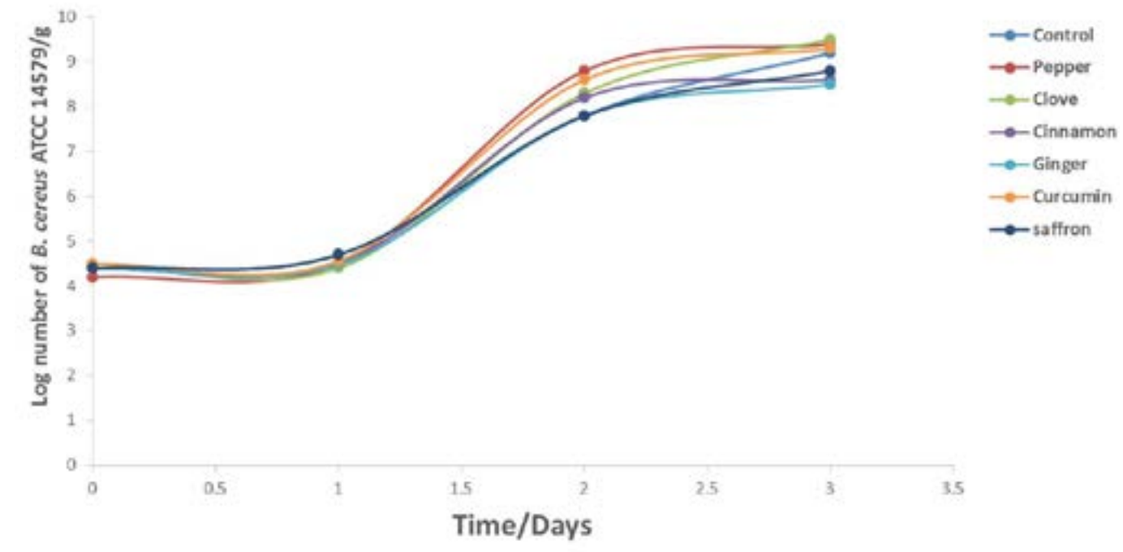

Figure 3. Effect of the spices added after cooking the rice on the contamination by *B. cereus* ATCC 14779.

hydroxyl radicals (Xu et al., 2018). At the MIC of 12 µg/mL, curcumin-treated cells display various apoptotic markers, including ROS accumulation, membrane depolarization, and Ca^{2+} influx (Yun & Lee, 2016).

In some foods, particularly traditional foods, ginger is used as a flavoring agent (Shamsi & Tajuddin, 2010; Si et al., 2018). Many studies have also evaluated the antimicrobial activity of ginger, which has been shown to have a promising inhibitory effect against some pathogenic bacteria and fung (Mascolo et al., 1989; Thongson et al., 2005; Mahady et al., 2005; Kaushik & Goyal, 2011). Our data from the ethanolic extract of ginger are in agreement with those of Harmalkar & Desai (2011), who evaluated its effect against *Bacillus cereus*, *S. aureus*, *E. coli*, *S. typhi*, and *P. aeruginosa*; however, it was shown to have an effect only against *B. cereus*, *S. aureus*, and *P. aeruginosa*. Oleoresin (solvent-free) is an essential oil and natural extract recognized as safe by the Food and Drug Administration (2017). In Arabic countries, its application in foods such as cooked rice as flavoring agent is common, but its use as a preservative agent is still scarce.

The high value of saffron is due to its ability to color and flavor foods and beverages, as well as to its aromatic strength. Saffron is a valuable spice with high added value among agricultural crops. The spice is, in fact, dried stigmata obtained from the flowering part of the *Crocus sativus* plant (Abdullaev, 2002).

Motamedi et al. (2010), by examining the antibacterial effect of the ethanolic and methanolic extracts of saffron against some pathogenic bacteria, showed that *S. aureus*, *B. anthracis*, *B. cereus*, *L. monocytogenes,* and *B. melitensis* are the most susceptible species. Meanwhile, *P. mirabilis* and *S. typhi* show resistance toward these extracts. Azami et al. (2012) showed that *S. typhimurium* is the most sensitive bacterium to saffron petals. Gandomi et al. (2012) study, the antibacterial properties of saffron petal extract, showed that the methanol extract of saffron is effective against *S. typhimurium*, *B. cereus,* and *L. monocytogenes* . Our data on the effect of the ethanolic extracts of saffron are in agreement with those of Tayel & El-Tras (2009), who mentioned that *B. cereus* is the most susceptible bacterium, while and *E. coli* and *P. aeruginosa* are the most resistant bacteria to the saffron petal extract.

The germination of spores of *B. cereus* ATCC 14579 was carried out at 70 °C, which was also intended to remove ethanol by evaporation. The germination of spores (e.g., in cooked rice) requires temperatures in the range of 5-50 °C. The temperature for the growth of vegetative forms is 15-50 °C, with optimum growth at approximately 30-37 °C (Lake et al., 2004). The data regarding the antibacterial activity of herbs/spiced cooked rice against *B. cereus* ATCC 14579 before cooking process or after addition of herbs/spices to cooked rice provide proof that not all of the spices have effect on the bacterial growth when added to rice, showing their role instead to be for flavoring, coloring, and tasting, rather than for preservation or for prolonging the shelf life of rice.

5 Conclusion

The heating temperatures used throughout the rice cooking process reached 100 °C continuously for approximately 20 min, which is considered sufficient to kill or control the growth of *B. cereus* ATCC 14579. When the herbs/spiced rice was inoculated by the *B. cereus* strain before the cooking process, or when the herbs/spices were added after the rice cooking process, there did not appear to be any effect on the control of *B. cereus,* nor was the growth of *B. cereus* in the rice stopped. Thus, the role of the addition of herbs/spices to the rice before or after the cooking process does not exceed the effects of flavoring, coloring, or tasting, meaning that it does not have any role in the preservation of rice from contamination by *B. cereus.*

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