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Effect of fennel (*Foeniculum vulgare* Mill.) and coriander (*Coriandrum sativum* L.) on microbial quality and sensory acceptability of frozen paratha

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Abstract

Fennel (*Foeniculum vulgare* Mill.) and coriander (*Coriandrum sativum* L.) are known to possess good antimicrobial properties. In the present work, spice-infused frozen parathas were formulated to investigate the effect of fennel and coriander on microbial (aerobic mesophilic bacteria, yeast and mould, and *Bacillus cereus*) reduction and sensory acceptability of frozen paratha throughout the storage at -18°C. The present work was also aimed at determining the relationship between spice concentrations and storage durations on microbiological quality of the samples. Fennel and coriander seed powder were used at concentrations of 2, 4 and 6% of wheat flour (w/w). The microbiological analysis was performed by total plate count, yeast and mould count, and *Bacillus cereus* count after 9, 12 and 15 weeks of storage. Sensory evaluation was conducted using hedonic scales at the end of storage durations. Results showed that spice infusion in frozen paratha significantly delayed the growth of aerobic mesophilic bacteria, yeasts and moulds, and *Bacillus cereus* during storage. The lowest log count was demonstrated by coriander at 6% in total plate count (3.85, 3.90 and 3.91 log10 CFU/g), and yeast and mould count (2.54, 2.59 and 2.60 log10 CFU/g) after 9, 12 and 15 weeks, respectively. *Bacillus cereus* was not detected throughout the storage durations. Fennel exhibited minimum activity against *Bacillus cereus* with no significant difference on log count reduction when compared with control. Coriander showed the highest decrease in both total plate count and *Bacillus cereus* count during the storage duration. Sensory evaluation result indicated that control sample exhibited the highest preference over all attributes when compared with fennel and coriander. Coriander-infused paratha was slightly darker in colour due to high concentration of 6%. Fennel yielded the lowest score in terms of taste among all samples. Fennel and coriander showed no significant difference for sensory acceptability. Overall, all frozen parathas were in good quality after 15 weeks of frozen storage. It can thus be concluded that fennel and coriander can be used as potential natural preservatives to inhibit the growth of microorganisms in paratha during frozen storage. Nevertheless, the optimum spice concentration should be determined to minimise the effects on the sensory attributes.

Introduction

Paratha is a type of unleavened Indian flatbread, and traditionally served as breakfast item and eaten hot alongside dishes such as curry and chutney. Over the past decades, growing demands towards convenience foods have heightened the interest on commercialising frozen ready-to-cook paratha to accommodate the current busy lifestyle (Bhoir *et al.*, 2015). Frozen paratha offers minimal preparation; a brief heating prior to serving to save time and ease consumption. However, this product is easily exposed to deterioration and microbial growth, thus adversely affect the product's shelf life.

The stability and shelf life of these foods can generally be measured by microbiological, chemical, physical and temperature-related deteriorative changes. For example, yeast can cause spoilage due to growth of white spots or chalk bread through post-baking contamination, conveyor belts, slicer

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and bread cooler (Saranraj and Geetha, 2012). Improper temperature control of frozen bakery products will also allow the survival of fungal species and psychrotrophic microorganisms causing their deterioration (Kilcast and Subramaniam, 2000). Growth of spoilage microorganisms with high resistance towards low temperature will result in slime or mould formation on food surfaces, off-odour and flavours, and discoloration (Aneja *et al*., 2014).

Shelf life limitation had become one of the main obstacles faced by many Small and Medium Enterprises (SMEs) food companies and manufacturers including FF Sdn. Bhd., one of frozen food-based company specialised in the production of Halal instant frozen pastries. Microbial spoilage of bakery products had adversely affected their intention to expand the market share. They failed to facilitate long distance shipments to countries such as Europe and United Arab Emirates (UAE) since their product did not fulfil the requirements of few importing countries. Mould or white spots were found to form on the food surfaces within eight weeks of shipment.

Numerous studies on paratha were more focused on nutritional value, rheological characteristic and microstructure, quality of wheat flour, influences of enzymes, seed powder, whey protein concentrated, surfactant, and additives (Indrani and Venkateswara Rao, 2000; 2003; 2007; Indrani *et al*., 2000; 2007; 2010; 2011; Prabhasankar *et al*., 2004; Bhargava *et al*., 2012). Limited scientific studies however are available on frozen paratha storage. Therefore, the objectives of the present work were to formulate frozen paratha infused with fennel and coriander, and identify the antimicrobial effectiveness of fennel and coriander against aerobic mesophilic bacteria, yeasts and moulds, and *Bacillus cereus* in frozen paratha stored for 9, 12 and 15 weeks. It also aimed to determine the effect of the spices on sensory acceptability of frozen paratha and identify the relationship between type of spices, spice concentration and storage time on microbial count reduction in frozen paratha.

Materials and methods

Materials

Commercial wheat flour, margarine, milk powder, baking powder, sugar, salt and vegetable shortening were obtained from FF Sdn. Bhd. (Gombak, Kuala Lumpur). Packaged fennel (*Foeniculum vulgare* Mill.) and coriander (*Coriandrum sativum* L.) seeds were purchased from local grocery shops in Seri Kembangan, Selangor.

Preparation of paratha dough

Ten types of samples were prepared by adding fennel and coriander powder at 2, 4 and 6% on 100 g flour basis (Balestra *et al*., 2011). Dough formulated without spices powder (0%) served as control. The percentage of spices powder was selected based on the result of preliminary test conducted previously at 3, 5 and 7%. Result showed that samples at 7% indicated lower score of acceptability as compared to other concentrations. Therefore, 6% concentration was selected as the maximum percentage in the present work.

Basic formula for paratha consisted of wheat flour (49.14%), water (22.60%), margarine (4.91%), milk powder (2.95%), baking powder (0.06%), vegetable shortening (15.72%), sugar (3.93%), and salt (0.69%). The formulas were chosen according to an existing and well-defined recipe from FF Company. The ingredients were initially weighed and mixed with selected spice powder in a mixer medium machine (HOBART, Ohio, USA) for 20 min to produce homogenous and soft dough. The resulting dough was sheeted with 16% vegetable shortening using dough sheeter machine, and divided into 70 g dough balls. Each ball was formed into circle swirl. The dough was pressed into a flattened circular dough sheet at 0.3 cm thickness, and air packed in polyethylene terephthalate/linear low-density polyethylene (PET/LLDPE) packaging material before sealed. Each pack contained five sheets and labelled accordingly before stored in a chest freezer at -18°C for 9, 12 and 15 weeks.

Preparation of spice powder

Both spices were heated to 50°C for 1 h using cabinet dryer (M-CD0106, MALCHEM, Malaysia), and dried until residual moisture content was less than 10% (XM-120, Precisa Instruments Ltd, Switzerland). This level appears to be the best conditions for preparing dried spices or powder because low moisture content limits microbial growth (Saohin *et al*., 2007). Dried spices were ground into powder using commercial grinder since this form can easily release spice flavour and more readily dispensable in foods (Balestra *et al*., 2011). Spice powder was then sifted for size less than 150 µm according to Das *et al*. (2012) with slight modifications.

Microbiological analysis

Briefly, 25 g paratha sample was suspended in 225 mL buffered peptone water, and aseptically homogenised using a stomacher (Bagmixer 400-P, Interscience, France) for 1 min at speed of 8 strokes per second (Bhoir *et al*., 2015). The suspensions

were further diluted in 0.1% of peptone water ranging from 10^{-2} to 10^{-6} dilution. Next, 1 mL of each sample homogenate was pipetted into a Universal bottle containing 9 mL buffered peptone water. Sample dilution (0.1 mL) was then pipetted and spread onto plate count agar (PCA, Difco-Becton, Dickinson, Sparks, MD, USA), potato dextrose agar (PDA, Difco-Becton, Dickinson, Sparks, MD, USA) and CHROMagar *B. cereus* (CHROMagar™, Paris, France) using spread plate method. PCA and CHROMagar *B. cereus* plates were incubated at 30°C for 24 h, and PDA plates were incubated at 25°C for 48 h in inverted position. After incubation, visible colonies were counted using a colony counter (Galaxy230 Colony Counter, Rocker Scientific Co., Ltd., Taiwan). Each analysis was performed in triplicate, and result was reported as log10 CFU/g for all samples (Downes and Ito, 2001).

Sensory evaluation

Samples at -18°C for 15 weeks were subjected to sensory evaluation which was carried out at Sensory Laboratory, Faculty of Food Science and Technology, Universiti Putra Malaysia to select attribute that best described the paratha. Samples were pan-fried prior to sensory analysis, coded with 3-digit random numbers and balanced ordered testing to keep samples anonymous and avoid bias (Lawless and Heymann, 2010). Sensory attributes; taste, aroma, appearance, texture and overall acceptability, were evaluated by 50 untrained panellists, using 9-point hedonic scale with 1 representing the least score (extremely dislike) and 9 representing the highest score (extremely like). The 50 panellists were chosen from both staff and students of the Faculty.

Statistical analysis

Each analysis was carried out in triplicate. Microbiological analysis data was analysed using General Linear Model (GLM) and sensory evaluation was analysed using one-way ANOVA. Differences between all variables were assessed by Fisher test ($p > 0.05$). The data analysis was carried out using Minitab 16 software, and expressed as mean ± standard deviation.

Results and discussion

Frozen paratha samples were evaluated by three microbiological analysis including total plate count (TPC), yeast and mould count (YMC) and *B. cereus* counts. *B. cereus* is considered as microbiological concern in paratha samples as this pathogenic spore-forming bacterium is mainly associated with rope spoilage of bakery products, causing discoloration, undesirable odour and extremely moist, stringy crumbs and foodborne illness (Saranraj and Geetha, 2012). All microbiological results of TPC, YMC and *B. cereus* counts, expressed as log10 (CFU/g), are presented in Tables 1, 2 and 3. It was found that the infusion of fennel and coriander significantly decreased ($p < 0.05$) aerobic mesophilic bacteria, yeast and mould, and *B. cereus* growth in the samples during frozen storage. The log count reported a declining pattern with increasing spice concentration. All samples were found within the acceptable range of microbial quality (<105 CFU/g) and yeast and mould (<103 CFU/g) for ready-to-eat foods (FEHD, 2014).

Table 1. Effect of spice infusion on total plate count (log10 CFU/g) of frozen paratha during storage.

Type of Spices	Conc. (%)	Storage time (week)		
		9	12	15
Fennel	0	4.48 ± 0.15^{cdA}	4.61 ± 0.20^{bA}	4.72 ± 0.03^{aA}
	2	4.15 ± 0.17^{cdB}	4.21 ± 0.08^{bB}	4.24 ± 0.30^{aB}
	4	4.09 ± 0.03^{cdC}	4.15 ± 0.01^{bC}	4.17 ± 0.01^{aC}
	6	4.08 ± 0.02^{cdC}	4.11 ± 0.01^{bC}	4.16 ± 0.02^{aC}
Coriander	0	4.48 ± 0.15^{eA}	4.61 ± 0.20^{dA}	4.72 ± 0.03^{cA}
	2	4.03 ± 0.08^{eC}	4.08 ± 0.04^{dC}	4.17 ± 0.04^{cC}
	4	3.98 ± 0.01^{eD}	4.03 ± 0.30^{dD}	4.07 ± 0.06^{cD}
	6	3.85 ± 0.04^{eE}	3.90 ± 0.02^{dE}	3.91 ± 0.04^{cE}

Mean values with different lowercase within the same column indicate significant difference ($p < 0.05$) between each storage time. Mean values with different uppercase within the same row indicate significant ($p < 0.05$) difference between each concentration.

The highest TPC was recorded by control sample which ranged from 4.48 to 4.72 log10 CFU/g as compared to all spice-infused samples (Table 1). TPC of 2% coriander was 4.03 to 4.17 log10 CFU/g and further decreased to 3.98 to 4.07 log10 CFU/g and 3.85 to 3.91 log10 CFU/g as the concentration increased up to 6%. Similar results were observed in fennel samples. The presence of natural antimicrobial compounds as trans-anethole and linalool in fennel and coriander provides excellent antioxidant and antimicrobial properties against spoilage flora and Gram-positive and Gram-negative microorganisms such as *Staphylococcus aureus*, *Listeria monocytogenes*, *B. cereus* and *Salmonella enteritidis* (Ceylan and Fung, 2004; Lo Cantore *et al*., 2004; Da Cruz Cabral *et al*., 2013; Mendez-

Vilas, 2013, Mandal and Mandal, 2015). Udensi *et al*. (2012) also claimed that higher spice concentrations were found to significantly reduce microbial load and extend the shelf life of soymilk samples for 5 days when stored at ambient temperature. The mechanisms of this antimicrobial activity may relate to the ability of the compounds to disrupt bacterial structures and alter the cell permeability, further penetrate the membranes and cell interior, resulting in the inhibition of cell functional properties and causing bacterial cell death (Tongnuanchan and Benjakul, 2014; Nazzaro *et al*., 2013). The numbers of TPC were also found to significantly increase ($p < 0.05$) from week 9 to week 15. Cross-contamination from poor handling practices and storage of frozen samples may have contributed to the increase of TPC. Yadav *et al*. (2008) showed a similar finding where both treated and control samples of ready-to-bake frozen chapatti showed a slight increase of TPC after stored at -18°C for six months.

Table 2. Effect of spice infusion on yeast and mould count (log10 CFU/g) of frozen paratha during storage.

Type of Spices	Conc. (%)	Storage time (week)		
		9	12	15
Fennel	0	2.89 ± 0.07^{cA}	3.02 ± 0.09^{abcA}	3.09 ± 0.19^{aA}
	2	2.67 ± 0.03^{cB}	2.76 ± 0.12^{abcB}	2.82 ± 0.11^{aB}
	4	2.57 ± 0.11^{cCD}	2.65 ± 0.09^{abcCD}	2.65 ± 0.29^{aCD}
	6	2.52 ± 0.00^{cD}	2.54 ± 0.26^{abcD}	2.65 ± 0.09^{aD}
Coriander	0	2.89 ± 0.07^{bcA}	3.02 ± 0.09^{abA}	3.09 ± 0.19^{aA}
	2	2.74 ± 0.07^{bcB}	2.76 ± 0.08^{abB}	2.85 ± 0.21^{aB}
	4	2.65 ± 0.07^{bcBC}	2.69 ± 0.10^{abBC}	2.74 ± 0.00^{aBC}
	6	2.54 ± 0.09^{bcD}	2.59 ± 0.16^{abD}	2.60 ± 0.00^{aD}

Mean values with different lowercase within the same column indicate significant difference ($p < 0.05$) between each storage time. Mean values with different uppercase within the same row indicate significant ($p < 0.05$) difference between each concentration.

As shown in Table 2, YMC of spice-infused samples increased much slower than the control sample. Fennel and coriander were found to significantly reduced ($p < 0.05$) the count even at their lowest concentration of 2%. Fennel at 6% showed the lowest YMC among all samples which was 2.52, 2.54 and 2.65 log10 CFU/g. These are in line with previous study by Seleem and Mohamed (2014) who highlighted that the addition of aromatic plants such as coriander and fennel at 2% were able to inhibit total fungal count and prolong the shelf life of pan bread during storage at room temperature. The shelf life was extended to 5 days while control sample recorded 4 days. The antimicrobial properties in fennel and its major constituents, anethole have proven to be efficient in controlling fungal growth (Singh *et al*., 2006; Soylu *et al*., 2007). According to AbduRahim *et al*. (2017), fennel essential oil was active against all tested fungal strains and showed complete zone inhibition against *Aspergillus* and *Fusarium*, the main contaminant in bread at 6 µL dose.

Table 3. Effect of spice infusion on B. cereus count (log10 CFU/g) of frozen paratha during storage.

Type of Spices	Conc. (%)	Storage time (week)		
		9	12	15
Fennel	0	4.06 ± 0.08^{aA}	3.71 ± 0.12^{bA}	0.00 ± 0.00^{bA}
	2	3.43 ± 0.02^{aAB}	0.00 ± 0.00^{bAB}	0.00 ± 0.00^{bAB}
	4	3.41 ± 0.08^{aAB}	0.00 ± 0.00^{bAB}	0.00 ± 0.00^{bAB}
	6	3.40 ± 0.00^{aAB}	0.00 ± 0.00^{bAB}	0.00 ± 0.00^{bAB}
Coriander	0	4.06 ± 0.08^{abA}	3.71 ± 0.12^{bA}	0.00 ± 0.00^{bA}
	2	3.48 ± 0.00^{abAB}	0.00 ± 0.00^{bAB}	0.00 ± 0.00^{bAB}
	4	0.00 ± 0.00^{abB}	0.00 ± 0.00^{bB}	0.00 ± 0.00^{bB}
	6	0.00 ± 0.00^{abB}	0.00 ± 0.00^{bB}	0.00 ± 0.00^{bB}

Mean values with different lowercase within the same column indicate significant difference ($p < 0.05$) between each storage time. Mean values with different uppercase within the same row indicate significant ($p < 0.05$) difference between each concentration.

Coriander at 4% and 6% completely inhibited *B. cereus* (100%) during storage (Table 3). In contrast, the addition of fennel insignificantly affected the number of *B. cereus* as compared to coriander. Manonmani and Khadir (2011) classified fennel as weak source of antimicrobial agents against *B. cereus* as less inhibition on *B. cereus* was observed using disc diffusion method. Lo Cantore *et al*. (2004) compared the antibacterial activity of coriander and fennel essential oils using agar diffusion method against bacterial species that were responsible for cultivated mushroom diseases. Coriander showed higher antibacterial activity towards majority of the bacterial strains especially *E. coli* and *Bacillus* spp. as compared to fennel. This contradicts Silva *et al*. (2011) who investigated antimicrobial activities of coriander against 12 bacterial strains using microdilution broth susceptibility assay, and found that coriander essential oil showed good antimicrobial

activity against all tested bacteria except *B. cereus* and *Enterococcus faecalis*.

Fennel and coriander samples also showed no growth of *B. cereus* after nine weeks while control exhibited the highest log counts; 4.06 and 3.71 log CFU/g, respectively when stored at -18°C. These results match those observed by Sani and Tan (2006), where all of the bread samples demonstrated negative result on *Bacillus* spp. detection after three months of storage at -18°C. Gram-positive bacteria such as *Bacillus* spp. are resistant to freezing as compared to gram-negative bacteria. Nevertheless, the psychotropic strain could only survive at minimum temperature of 7-10°C (Rajkowski and Bennett, 2003; Erickson and Hung, 2012). This highlighted the major role of storage temperature, and spices could hinder the growth of *B. cereus*. Synergistic of both factors have strongly influenced the shelf life and quality of samples. Findings from Table 3 may be somewhat limited by the survival of *B. cereus* spores in samples. These heat-resistant spores are usually induced under unfavourable survival conditions and can survive for long period of time before germinating and produce emetic toxins (Silva *et al*., 2013). However, Ryu *et al*. (2005) identified that growth and sporulation of *B. cereus* did not occur at 8°C. Growth was detected with no sporulation at 12°C, and *B. cereus* were found to produce spores at 22°C. These observations enable to conclude that temperature required for sporulation is higher than the minimum temperature for *B. cereus* growth, thus sporulation can be prevented using temperature control during processing.

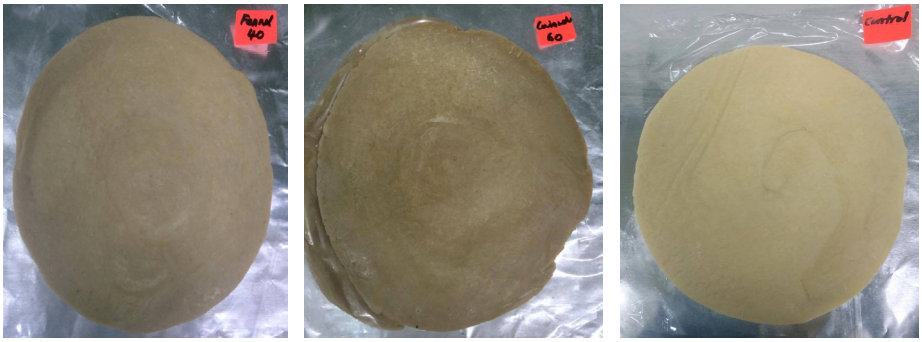
A review by Gottardi *et al*. (2016) on the *in vitro* activity of extracts from various spices showed that various compounds isolated from the spices demonstrated antimicrobial activity against common microorganisms that affect the quality and safety of foods. Anethole, a main compound in fennel responsible for the activity against *B. cereus* was also found in star anise (Shan *et al*., 2007). The presence of other compounds; curcumin (Moghadamtousi *et al*., 2014), cuminal (Ceylan and Fung, 2004; Sethi *et al*., 2013) and piperine (Shiva Rani *et al*., 2013) in garlic, turmeric, cumin and black pepper, respectively have been reported to inhibit *B. cereus*. Similarly, for *in vivo* activity, although there has been not much information available on the enhancement of microbial shelf life in bread by adding spice extracts, few studies reported that range of concentrations of extracts (2 - 4%) were needed to enhance the shelf life of bread. The results presented in the present work coincide with a study by Yadav *et al*. (2016) that showed the addition of 4% of clove, ginger and cinnamon extracts in bread inhibited the growth of bacteria and fungi during six and four days refrigerated storage, respectively. Similarly, Dhillon *et al*. (2013) reported that breads added with 2% oregano had no mould growth during six days ambient temperature storage. To some extent, the results of the present work concur with another finding that the addition of clove, oregano, cinnamon, and black mustard at 0.33 – 1.00% concentrations was effective in reducing microbial growth on raw chicken meat during storage at 4°C for 15 days (Radha Krishnan *et al*., 2014). Another study (Zhang *et al*., 2016) reported similar findings for dried cloves and rosemary.

Table 4. Sensory evaluation scores for different types of paratha samples.

Attributes	Samples		
	Control	Fennel (4%) *	Coriander (6%) *
Colour	7.78 ± 0.95^{a}	6.56 ± 1.31^{b}	5.40 ± 1.818^{c}
Aroma	7.36 ± 1.35^{a}	6.02 ± 1.56^{b}	6.00 ± 1.63^{b}
Texture	7.26 ± 1.14^{a}	6.24 ± 1.49^{b}	5.98 ± 1.72^{b}
Taste	7.74 ± 1.14^{a}	5.72 ± 2.20^{b}	5.82 ± 1.76^{b}
Overall acceptability	7.78 ± 0.98^{a}	6.00 ± 1.69^{b}	5.94 ± 1.61^{b}

Mean values with different lowercase within the same row indicate significant difference ($p > 0.05$) between each sample. Concentrations of fennel (4%) and coriander (6%) were selected for sensory acceptability evaluation based on their microbial quality results.

The results for colour, aroma, texture, taste and overall acceptability of paratha samples are shown in Table 4. Fennel at 4% and coriander at 6% were selected for sensory acceptability based on the microbiological analysis results. Control sample scored the highest preference over all attributes (7.78), followed by fennel (6.00) and coriander (5.94). It is apparent that the acceptability order of frozen parathas was control > fennel > coriander. Infusion of spices was seen to be a factor that significantly ($p < 0.05$) affected each sensory attributes of the frozen paratha. This could be related to high concentration of fennel and coriander used where the level of organic compounds in spices could mask positive attributes of foods (Parthasarathy *et al*., 2008). Similar idea was opined in previous studies by Balestra *et al*. (2011) and Przygodzka *et al*. (2015) who suggested that excessive infusion of spices could result to the least sensory acceptability and significantly enhanced the bitterness and pungency taste, strong spicy aroma and texture of samples. It can be seen in the present work that fennel and coriander exhibited insignificant differences of score among each other. Both spices recorded lower acceptability over aroma (6.02 and 6.00), texture (6.24 and 5.98), taste (5.72 and 5.82) and overall acceptability (6.00 and 5.94). However,



a b c

Figure 1. Sensory acceptability (colour attribute) of paratha samples added with 4% fennel (a) and 6% coriander (b) compared to the negative control (c).

a significant colour variation was observed where coriander scored the lowest acceptability among the samples. The addition of high concentration of coriander (6%) resulted in a darker coriander sample than control and fennel (Figure 1). According to Das *et al*. (2012), increasing coriander concentration will reduce the brightness value in colour analysis and enhance discoloration of bread as the spice had darker shade than wheat flour. It was also observed that fennel scored the lowest taste acceptability among the samples. This might be strongly related to the high percentage of estragole and fenchone in fennel which play a role in imparting bitterness (Przygodzka *et al*., 2015), which might have reduced the panellists' preference in the present work. This is also in line with Das *et al*. (2013) who indicated that high percentage of fennel (15%) in bread was found to be lower in consumer acceptability, and caused negative effect on the bread sample's taste as it resulted in impaired taste due to bitterness. Overall, the addition of spices into frozen paratha formulation was seen to significantly affect the scores of sensory evaluations.

Conclusion

In conclusion, the present work demonstrated that all samples remained within the acceptable range of microbial quality stipulated by Food and Environmental Hygiene Department (FEHD, 2014). Samples also showed no appearance of mould growth and physical changes such as ropey, discoloration, extremely moist texture or produced rotten fruit odour showed in another study after 15 weeks of storage (Saranraj and Geetha, 2012). It can be concluded that infusions of fennel and coriander were suitable to be used in frozen paratha to suppress the growth of microorganisms during frozen storage. Nevertheless, lower concentration should be considered to minimise the effects on sensory attributes. By balancing both microbiological stability and sensory acceptability, this new formulated frozen paratha could win huge score on consumer preferences and company's profits. Further research in this field would be of great help in improving quality and prolonging the shelf life of frozen foods. Similar studies should be carried out by considering different varieties of factors such as new spices, herbs or plant extracts, packaging materials, storage temperature and time. These would propose new pathways of antimicrobial preservatives and offer different alternatives for shelf life extension.

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