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Application of Starch Foams Containing Plant Essential Oils to Prevent Mold Growth and Improve Shelf Life of Packaged Bread

S. Lotfinia[1], M. Javanmard Dakheli[2], A. Mohammadi Nafchi[1]*

[1]Department of Food Science, Faculty of Agriculture,Damghan branch, Islamic Azad University of Dameghan, Iran

[2]Department of Food Technologies, Iranian Research Organization for Science & Technology. Tehran, Iran

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ABSTRACT: In the recent years, considerable attention has been allocated in the area of using natural preservatives in foods, especially vegetable oils. Starch foams prepared from high amylose starch are useful for encapsulation of substances such as chemicals, liquids or solids, including flavor compounds, pharmaceuticals and essential oils. The foams have the ability to trap the active material and subsequently release the activity. Cinnamon oil is absorbed to foam starch microparticles and acts as an antimicrobial agent. This study was designed and implemented to evaluate the use of starch foam containing vegetable oil to prevent mold growth and improve packaged bread shelf life. For this purpose, first cinnamon essential oil was extracted with water by distillation method then, 250 groups of bread were prepared within polypropylene plastic bags. Various amounts of cinnamon essential oil (500, 750, 1000and1500ppm) with 1 g of starch foam powder inside sterilized filter paper were added to these packages.The obtained results of multi-way and intergroup repeated tests indicated that there was a significant difference (P <0/05) between the control groups and various groups containing cinnamon essential oil in terms of microbial load. In the groups containing essential oils, less increase was showed in microbial load and with increasing concentrations of cinnamon essential oil, mold and yeast growth rate decreased. It concluded that by using starch foam containing cinnamon essential oil in bulky bread packing at ambient temperature (25°C), the spoilage process of bulky bread can be postponed 3 to 6 days, and it can be used as an appropriate natural and antifungal preservative in packaging of bread.

KEYWORDS: Active packaging, Antifungal activity, Bread, Cinnamon bark oil, Starch foam

INTRODUCTION

Bread is one of the most popular foods. This interest is because of its high nutritional and organoleptic characteristics. Generally, bread's shelf life is confined by several destructive processes including fungi growth, moisture loss and staling.

Corresponding author: S. Lotfinia, Department of Food Science, Faculty of Agriculture, Damghan branch, Islamic Azad University of Dameghan,Iran.Email:Slotfinia@rocketmail.com

Sixty percent of bread spoilage is attributed to the mold's activity (*Penicillium* species and *Aspergillus Niger* strains). Yeasts constitute only 15% of this spoilage. In addition to their detectable growth, the fungi are responsible for the development of off flavor, producing mycotoxins and allergenic compounds. However, these compounds exist before fungal growth [13]. Furthermore, secondary contamination may occur during chilling and wrapping and spoil bread. Packaging system can be considered as an operative part in food production lines, because of its ability of improving food safety and prolonging food's shelf life. Protective actions of packaging can be divided into two classes, active and inactive. Once packaging is a part of preparation process and maintenance system, there is inactive protection against mechanical forces, microbial contamination, heat and mass transfer. When packaging of food is a combination of storage and processing and is considered as an essential element in the processing, thus it can be named active packaging. Common examples of active packaging include oxygen scavenging systems and antimicrobial packages. In recent decades, addition of antimicrobial agents, such as organic acids, inorganic compounds, enzymes and spices to packaging materials to develop active packaging has been widely studied. To produce antimicrobial packaging, antiseptic agents can be combined and integrated into packaging system by adding sachets (small plastic bags)or gas or vapor carrier pads, which coat the agent on inner surface of package, insert it into the polymer matrix, or release it directly into head space of package. When antimicrobial agents are released into the package, the microbial growth is stopped or delayed on the food surface of which maximal contamination and spoilage occurs. Protection and containment play important roles in food packaging. Food retention depends on sustainability of the packaging material against environmental factors such as heat, humidity and exposure to chemical compounds. One kinds of active packaging is adding an antimicrobial agent into package structure, and then releasing it into the product in a controlled manner, which leads to preventing the proliferation of microorganisms during storage [14]. In this study, efforts have been initiated to evaluate the effect of a herbal essential oil to inhibit mold growth on bread and prolonging leavened bread's shelf life. The study also examined the application of starch-based foam with a herbal essential oil to make an active package for bread that leads to gently releasing of the essential oil into the package and prolonging its effect on the inhibition of bread molds.

MATERIALS AND METHODS

Extraction of cinnamon essence

To obtain cinnamon essential oil, dried barks of cinnamon tree were powdered by grinding. Then 70 grams of it was accurately weighed and transferred into a 1000 ml flask. Then, 600-700 ml of distilled water was added to it. In order to form fewer bubbles resulting from boiling, several glass pearls were also added to the flask. In this work, clevenger apparatus was used. About one milliliter of cinnamon essential oil was extracted from 70 g of cinnamon powder and after dewatering, the separation was done by centrifugation [9].

Preparation of starch-based microcellular foams

A suspension of high unmodified amylose corn starch with 8% starch (w/w) was made using distilled water. Corn starch solutions (500 ml) were mixed at 75 rpm in a viscoamylograph and heated about 95 ºC at a rate of $2^{o}Cmin^{-1}$. The temperature of the starch solution was maintained at 95ºC while mixing continued until the peak viscosity was reached. The gelatinized starches were poured into cylindrical plastic molds (1.56 cm dia., 11.4 cm length), covered with aluminum foil, and refrigerated ($5^{\circ}C$) overnight to form rigid aqua gels. The water of the aqua gels was displaced with ethanol by

batch equilibration with a succession of ethanol baths. The equilibration time for each bath was 24 hr. The sequence of baths was one time in 70% (w/w) ethanol and three times in 100% ethanol for the corn starch aqua gels, which their thicknesses are less than 2.5 cm. As the water within aqua gel matrix was displaced with ethanol, the gels began to shrink. Its appearance, which had been slightly semi-translucent, turned opaque and white. Owing to the gelation process, starch has a structure like foam with tiny pores. The alcohol that existed in the pores was evaporated and released by heating at 60°C in a vacuum oven. In this way, the starch foams converted into white masses. The masses were grounded by a Mill until a white powder formed, which would be used in the next step [2].

Scanning electron micrographs of starch-based foam

Starch foam samples were cut into 1×1 cm in size and then coated with gold palladium, viewed by Scanning Electron Microscope (SEM), and photographed [2].

Baguette bread preparation method

Bread used in this study was provided from Nanavaran Company. The bread was prepared by semi-industrial method and packaged. Amounts of raw materials required for preparing bread dough on the basis of flour weigh of 30 kg include 2% oil, 1.5% salt, 1.7% sugar, 1.6% yeast, 0.29% improver, 0.1% gluten and 50-65% water. The preparation process of this type of bread included the steps of mixing, initial resting, makeup, final resting, baking and cooling. At first, flour was mixed in the mixer for two minutes. Then, the ingredients including salt, sugar, yeast, improver, gluten and oil, were put into the mixer and mixed for two minutes. Then, water was added to the flour and all the ingredients were mixed in the mixer for 20-25 minutes. The dough was transferred into the divider, in which the dough was divided into 380-400 g pieces in weight. Then divided and rounded pieces of dough were placed in the fences in two manners; one flat and the other chute shaped. After placing the dough in special trays, the trailer transferred them to the incubation room and were stored for 45-60 minutes, then transferred to the oven having the temperature 180-270ºC. The time required for baking the breads was 25-30 minutes. The breads were transferred to the cooling room, and after reaching the desired temperature, they were carried into the packaging unit [8].

Sachet preparation from starch-based foam

According to the tests already done, different concentrations of the essential oil of cinnamon (50, 100, 200, 300, 400 and 500 ppm) combined with one gram of starch foam powder used in packaging bread were evaluated in terms of microbial contamination. It was found that the antimicrobial activity of cinnamon essential oil expressed as MIC (minimum inhibitory concentration) was 500 ppm on a 250g bulky bread. Values of 500, 750, 1000 and 1500 ppm of cinnamon essential oil were added to starch foam powders of 1g in weight. The obtained mixtures were packaged in sterilized membrane papers in the form of sachets and placed inside the packages, which contained a bulky bread of 250g. The control samples consisted of packages that contained starch foam without any essential oil. Then, the control and treated samples were assessed in terms of mold and yeast contamination and sensory properties every 3 days for 15 days [1].

Experiment

On the days of 0, 3, 6, 9, 12 and 15, three samples were taken per pack and day. Bread samples were placed in sterile bags of mixer in the vicinity of flame, then put in the stomacher and homogenized for four minutes. The samples were completely mixed and becomes a uniform powder. Amount of 10 g of the powder was weighed and mixed with 90 ml of a sterile ringer solution in a sterile beaker. The dilution number was 10^{-1}. Then, it was mixed and serial dilutions were prepared. Amounts of 0.1 ml of the serial dilutions were taken by a pipette and transferred into three individual petri dishes containing Saborad Dextrose Agar. The cultured plates

were incubated at 25ºC for five days and examined from the second to fifth day of the incubation period. Then colonies contained in the petri dishes were enumerated and total number was multiplied by 10 and the reverse of the dilution number [6].

Sensory test

Sensory tests were carried out by 10 trained people according to the test 5 hedonic. The volume of bulky breads generally is varied from a moderate to high amount. The compactness of bread negatively impacts the freshness and flavor or staling. Bread crust should be crispy and thick enough. It should be golden in color and hollow and tender enough but not brittle. Internal texture of bread should be elastic and easily cut and its flavor and smell should be fresh and mild and have a good taste. The samples were placed in front of sensory evaluation panels at 25ºC with drinking water and a piece of tissue. Panels tested the samples and washed their mouths by drinking water at intervals to eliminate the traces of previous samples. By using 5-digit hedonic method, intensity and weakness of flavor, texture, color and general acceptability were studied and scored from1 to 5 [7].

STATISTICAL ANALYSIS

Due to the nature of measurement of the data at certain intervals and storage tests and also initial condition of the treatments, which were not the same, repeated measurement tests were used. In this case, time was considered as the repeated factor on the days 0, 3, 6, 9, 12, and 15. The independent variable was the cinnamon essential oil concentration at 5 levels (0, 500, 750, 1000 and 1500 ppm). The dependent variables were mold and yeast contamination. Sensory evaluation (color, odor, taste, texture and general acceptability) were tested at 5% level of confidence. In this test, the effect of time and concentration of cinnamon essence were evaluated. Due to the non-parametric data (lack of uniformity in variances and significance of the test LEVEN), the test Kruskal-Wallis replacing ANOVA was used and analyzed by means of IBM SPSS.

Figure1. High-amylose corn starch foams.

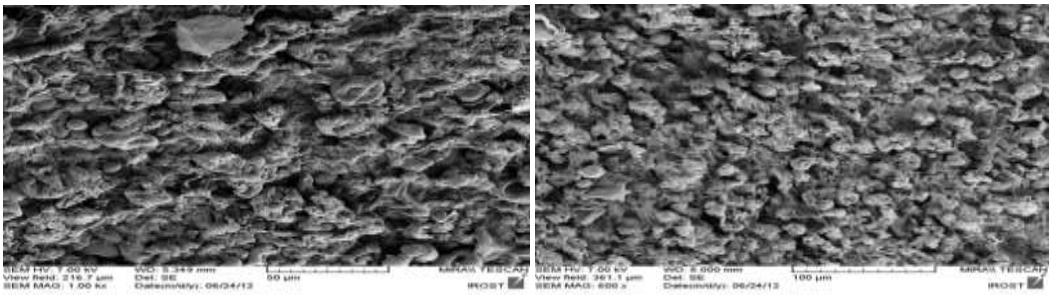

Figure2. Scanning electron micrographs of high amylose corn starch with a magnification of 100µm, 50µm.

RESULTS

Scanning Electron Micrographs of high amylose corn starch

The cinnamon essential oil at the concentrations of 500, 750, 1000 and 1500 ppm was adsorbed by the microcellular foam of high amylose corn starch and subsequently resulted in the release of its activity, which is due to the porosity of high amylose corn starch foams.

Cinnamon essential oil concentration effect on mold and yeast levels over time

The results of repeated measurements, time and cinnamon essential oil concentration effects on mold and yeast growth in bread are shown in Figure 3. There was a significant statistical difference between the control samples and cinnamon essential oil concentrations of 500, 750, 1000 and 1500 ppsm over time. The cinnamon essential oil concentrations of 500 and 750 ppm had minimal changes in yeast and mold levels over time. The concentration of 1500 ppm had the most antimicrobial effect compared to the concentrations of 500, 750 and 1000 ppm.

Organoleptic characteristics

The results of multi-way and between-groups tests of repeated measurements are shown in Table 1, which indicates that time effect on the organoleptic properties (smell, taste, color, texture and overall acceptability) of various breads is significant ($P<0.05$) and contrastive effect of cinnamon essential oil concentration during storage is also significant ($P<0.05$) (Differences in small Latin letters in each row indicate significant differences between the mean values over time at 5% level of confidence).

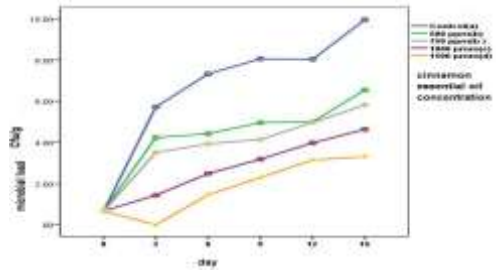

Figure 3. Mold and yeast load variations as a function of time and concentration of cinnamon essential oil obtained from the repeated measurements (indicating mean value ± standard deviation. Differences in Latin letters on the columns indicate significant differences at 5% level of confidence).

Table1. The comparison of mean value and standard deviation of cinnamon essential oil concentration (ppm) and time effect on sensory evaluation (odor, taste, color, texture and overall acceptability) of bulky bread.

		Storage time(day)					
		0	**3**	**6**	**9**	**12**	**15**
Smell acceptability	control	$5/00\pm0/00^{aA}$	$3/85\pm1/26^{bA}$	$1/00\pm0/00^{cD}$	$1/00\pm0/00^{cE}$	$1/00\pm0/00^{cE}$	$1/00\pm0/00^{cE}$
	500 ppm	$5/00\pm0/00^{aA}$	$2/52\pm0/86^{dB}$	$2/70\pm0/92^{cA}$	$2/70\pm0/79^{bA}$	$2/70\pm1/12^{bA}$	$3/10\pm0/71^{bA}$
	750 ppm	$5/00\pm0/00^{aA}$	$1/88\pm0/78^{dC}$	$2/00\pm0/91^{cB}$	$2/40\pm1/13^{bB}$	$2/10\pm1/06^{cB}$	$2/50\pm0/94^{bB}$
	1000ppm	$5/00\pm0/00^{aA}$	$1/80\pm1/06^{cD}$	$1/90\pm0/96^{cB}$	$1/60\pm0/50^{cC}$	$1/80\pm0/76^{cC}$	$2/30\pm1/02^{bC}$
	1500ppm	$5/00\pm0/00^{aA}$	$1/33\pm0/70^{dE}$	$1/70\pm0/79^{cC}$	$1/30\pm0/47^{dD}$	$1/40\pm0/50^{dD}$	$1/90\pm0/84^{bD}$
Taste acceptability	control	$5/00\pm0/00^{aA}$	$3/80\pm0/92^{bA}$	$1/20\pm0/61^{cD}$	$1/00\pm0/00^{dD}$	$1/00\pm0/00^{dD}$	$1/00\pm0/00^{dE}$
	500 ppm	$5/00\pm0/00^{aA}$	$2/65\pm0/72^{bB}$	$2/70\pm1/12^{cA}$	$2/30\pm0/79^{cA}$	$2/40\pm1/13^{cA}$	$2/50\pm0/68^{cA}$
	750 ppm	$5/00\pm0/00^{aA}$	$2/40\pm0/55^{bC}$	$2/50\pm0/82^{bA}$	$2/20\pm0/61^{cA}$	$1/70\pm0/79^{dB}$	$1/90\pm0/71^{dB}$
	1000ppm	$5/00\pm0/00^{aA}$	$1/80\pm1/19^{cD}$	$2/20\pm1/00^{bB}$	$1/90\pm0/96^{cB}$	$1/40\pm0/81^{dC}$	$1/65\pm0/72^{dC}$
	1500ppm	$5/00\pm0/00^{aA}$	$1/30\pm0/65^{dE}$	$2/00\pm0/64^{bC}$	$1/60\pm0/93^{cC}$	$1/20\pm0/41^{dC}$	$1/50\pm0/51^{cD}$
Color acceptability	control	$5/00\pm0/00^{aA}$	$3/70\pm1/39^{bA}$	$1/00\pm0/00^{cC}$	$1/00\pm0/00^{cB}$	$1/00\pm0/00^{cC}$	$1/00\pm0/00^{cC}$
	500 ppm	$5/00\pm0/00^{aA}$	$3/50\pm0/93^{dA}$	$3/30\pm0/47^{eA}$	$3/70\pm0/47^{dA}$	$4/00\pm0/64^{bA}$	$3/90\pm0/31^{cB}$
	750 ppm	$5/00\pm0/00^{aA}$	$3/43\pm1/02^{dB}$	$3/20\pm1/00^{eA}$	$3/70\pm0/47^{cA}$	$4/00\pm0/64^{bA}$	$4/00\pm0/00^{bA}$
	1000ppm	$5/00\pm0/00^{aA}$	$2/83\pm0/98^{eC}$	$3/40\pm0/67^{dA}$	$3/70\pm0/47^{cA}$	$3/70\pm0/79^{cB}$	$4/00\pm0/00^{bA}$
	1500ppm	$5/00\pm0/00^{aA}$	$2/87\pm0/56^{eC}$	$2/80\pm0/41^{eB}$	$3/60\pm0/50^{dA}$	$3/90\pm0/55^{cB}$	$4/00\pm0/00^{bA}$
Texture acceptability	control	$5/00\pm0/00^{aA}$	$3/32\pm1/10^{bA}$	$1/30\pm0/92^{cD}$	$1/00\pm0/00^{dC}$	$1/00\pm0/00^{dC}$	$1/00\pm0/00^{dD}$
	500 ppm	$5/00\pm0/00^{aA}$	$2/95\pm1/09^{cA}$	$2/80\pm0/61^{dA}$	$3/70\pm0/65^{bA}$	$3/20\pm0/61^{cB}$	$2/85\pm0/56^{dC}$
	750 ppm	$5/00\pm0/00^{aA}$	$3/08\pm0/94^{dA}$	$2/70\pm0/92^{eB}$	$3/60\pm0/67^{bA}$	$3/40\pm0/67^{bA}$	$3/30\pm0/47^{cB}$
	1000ppm	$5/00\pm0/00^{aA}$	$3/23\pm1/19^{cA}$	$2/80\pm1/19^{dB}$	$3/40\pm0/81^{cB}$	$3/50\pm0/68^{cA}$	$4/00\pm0/00^{bA}$
	1500 pm	$5/00\pm0/00^{aA}$	$3/73\pm0/89^{cA}$	$2/60\pm0/93^{dC}$	$3/60\pm0/67^{cA}$	$3/50\pm0/68^{cA}$	$4/40\pm0/50^{bA}$
Overall acceptability	control	$5/00\pm0/00^{aA}$	$4/02\pm0/94^{bA}$	$1/00\pm0/00^{cB}$	$1/00\pm0/00^{cE}$	$1/00\pm0/00^{cE}$	$1/00\pm0/00^{cE}$
	500 ppm	$5/00\pm0/00^{aA}$	$2/95\pm0/66^{bB}$	$2/60\pm0/81^{dA}$	$2/70\pm0/92^{cA}$	$2/80\pm0/89^{cA}$	$2/80\pm0/41^{cA}$
	750 ppm	$5/00\pm0/00^{aA}$	$2/70\pm0/47^{bC}$	$2/50\pm0/68^{cA}$	$2/50\pm0/82^{cB}$	$2/30\pm0/79^{cB}$	$2/60\pm0/50^{cB}$
	1000ppm	$5/00\pm0/00^{aA}$	$2/00\pm0/79^{dD}$	$2/70\pm0/92^{bA}$	$2/30\pm0/79^{C}$	$1/90\pm0/71^{dC}$	$2/35\pm0/72^{cC}$
	1500ppm	$5/00\pm0/00^{aA}$	$1/85\pm0/85^{dD}$	$2/60\pm0/67^{bA}$	$1/80\pm0/89^{dD}$	$1/60\pm0/50^{dD}$	$2/20\pm0/57^{cD}$

DISCUSSION

Evaluation of Scanning Electron Micrographs of high amylose corn starch

The image of granular structure of microcellular foam of high amylose corn starch and its porous nature which was taken by a scanning electron microscope with different magnifications is shown in Figure 2. Microcellular foam is a low density solid matrix having air-filled pores of a few micrometers or smaller in diameter (50 microns) in the porous structure. The microcellular starch foam adsorbs about 25% (w/w) of non-aqueous liquid, while remaining as individual flowing micro-particles. High amylose corn starch foams have very small cavities of 1 µm [5]. The use of edible sorbents, having microscopic cavities such as microcellular starch foam, provides a different approach to adsorb flavor compounds. The advantage of high amylose corn starch is that volatile compounds such as essential oils can be adsorbed in a relatively mild condition at room temperature [3]. Microcellular starch foams are used for encapsulating chemicals, liquids and solids such as flavoring agents, pharmaceutical, fragrances, pesticides, pheromones and also feeding bees with essential oils that have mitocidal characteristics. Thus, microcellular starch foam provides a method to protect bee colonies against Varroa mite. Microcellular foam matrix of high amylose corn starch includes a fibrous network without starch granule remnant [4].

Evaluation of cinnamon essential oil concentration effect on yeast and mold levels over time

Figure 3 shows the number of yeasts and molds of the bulky bread samples in 250g packages with starch foam powder containing cinnamon essential oil concentrations of 500, 750, 1000 and 1500 ppm and the control samples all stored at ambient temperature (25°C) on the days 0, 3, 6, 9, 12 and 15.There was a significant statistical difference between control samples and cinnamon essential oil concentrations of 500, 750, 1000 and 1500 ppm ($P<0.05$). The highest growth rate of mold and yeast was in the control sample and the lowest growth rate was in the treatment of cinnamon oil concentration of 1500 ppm. The maximum growth of mold and yeast occurred in the control sample from the third day, but no fungal growth was observed in the sample with essential oil of 1500 ppm concentration, on the third day. Among the above treatments, the cinnamon oil concentration of 1500 ppm was the best treatment for reducing the fungal load. According to Gandomi and his colleagues (2009), an essential oil reduces the contamination at least 50%, which is considered as a positive inhibitory factor. In this study, the cinnamon essential oil at the all concentrations reduced the mold and yeast growth in bread, which is one of the most important objectives of this study. However, none of the concentrations could completely inhibit the mold growth in bread. The effect of different concentrations of the essential oils on the fungal growth was statistically significant, implying that the increase of cinnamon essential oil concentration decreases the fungal growth in bread. A reduction of over 50% was observed for the fungal load at the concentrations higher than 500 ppm. In general, the rate of reduction of fungal load was from 34% for 500 ppm to 75% for 1500 ppm of essential oil. Thus, starch foam containing cinnamon essential oil is considered a positive inhibitory of fungal growth at the concentrations higher than 500 ppm. The fungal load variations in the breads packed with 1 g of starch foam containing different concentrations of cinnamon essential oil and stored for 15 days at ambient temperature suggested that antifungal effect of essential oil is reduced in the samples containing 500, 750, 1000 and 1500 ppm of cinnamon essential oil over time. This might be due to the microcellular starch foam action as a sorbent of essential oil and its porous structure which kept essential oil and released it inside the bread package. Also, during the final days, the release of

essential oil from the microcellular starch foam had been stopped so the antifungal effect of cinnamon essential oil was reduced. As a result, maximum fungal growth was observed on the fifteenth day. However, the samples showed less growth than the control samples. At the concentrations of 500, 750, 1000 and 1500 ppm, 4 log cfu/g, 5 log cfu/g, 6 log cfu/g, 8 log cfu/g reduction were observed, respectively. Researchers have investigated different cases of cinnamon essential oil antimicrobial effect, which are explained as follows:

In one study carried out by Javanmard, Golmohammadi and his colleagues (1999), the antimicrobial effect of cinnamon extract on meat packages was investigated. In this work, cinnamon extract was first obtained, and minimum inhibitory concentration was determined. Then, 50g pieces of ground meat were wrapped in sterile plastic bags. Different amounts of cinnamon extract (20, 30, 40 µlit/gr) were added to the packs. The initial microbial load of the packs was determined and inoculated with a certain amount of *Salmonella typhimurium*. Moreover, the control group included the sample of alcohol and sample without extract. All the groups were investigated on the days of 7, 14 and 21, and microbial tests were carried out. The obtained results showed that there had been a significant difference between the control and various extract groups in terms of overall microbial load ($P<0.05$) in the first week and the groups containing the extract showed a smaller increase in microbial load. There was a statistically significant difference between the control sample without the extract and with the extract in terms of *Salmonella* contamination in the first week ($P<0.05$), but the difference was not significant since the second week onwards. Thus, it seems that cinnamon extract has a desired antimicrobial effect on the overall microbial load and *Salmonella* growth in ground meat packs for one week.

Nielsen in 2000 investigated the effect of various vegetable essential oil on the fungi that are commonly found on bread. Mustard essential oil is the only compound that is strong enough to be able to inhibit the growth of all the microorganisms for seven days. Next, cinnamon and garlic were the best antimicrobial compounds, respectively. Vanilla showed no inhibitory effect. The least concentration of inhibition of mustard essential oil was 0.5 ml that inhibited 67% to 100% of the mold and yeast population. However, one milliliter of this essential oil stopped the growth of all the microorganisms for more than two weeks. Required concentrations of cinnamon and garlic essential oil for complete inhibitory of fungal growth were slightly more than that of mustard.

In a research conducted by Rezaei and his colleagues sin 2012, antibacterial effect of essential oil of cinnamon bark was investigated in in vitro condition on five strains of causing food spoilage. In this work, antibacterial activity or in other words minimal inhibitory concentration of cinnamon bark essential oil was evaluated against five foodborne pathogens including *Listeria monocytogenes, Escherichia coli, Pseudomonas fluorescens, Lactobacillus plantarum, Lactobacillus sakei*. Minimal inhibitory concentration of cinnamon essential oil to inhibit Lactobacillus sakei and other bacteria was reported as 250µg/ml and 500µg/ml, respectively. The inhibitory effects of cinnamon bark essential oil on the putrefactive and pathogenic bacteria of meat were analyzed under the certain and controlled conditions. The results of this study indicated that cinnamon essential oil and its major antibacterial compounds can potentially be used as a natural food preservative. Soliman K M, Badaeaa R I, (2002) studied the inhibitory effects of 12 pharmaceutical herbs against *Aspergillusflavus*. The essential oils of cinnamon and thyme (≤500 ppm), parsley (≤2000 ppm), mint and basil at 3000 ppm completely inhibited the growth of *Fusariummoniliforme* and *Aspergillusochraceus*.

The two researchers found that the essential oils of cinnamon, thyme and mint were more effective than

other oils in fungal growth inhibition, followed by mycotoxin production in wheat grains. In one another study by Noori and her colleagues (2009), the preservation effect of cinnamon essential oil and storage temperature was studied on *E.coli* growth rate in hamburger. In this study, the *E.coli* growth in hamburger, which was influenced by different concentrations of cinnamon essential oil (0, 0.005, 0.15 and 0.03%) at the temperatures of 8 and 25ºC for 21 days, was analyzed. The statistical results showed a significant difference between various amounts of the essential oil and bacterial growth rate ($P<0.01$). When the concentration of essential oil increased, the rate of bacterial growth decreased under identical conditions. Therefore, it can be concluded that cinnamon essential oil can be used as a natural preservative and a good antibacterial preservative in meat products. Patkar, Dwivedi and colleagues (1993) found that *Aspergillusflavus* is one of the most toxigenic foodborne fungi which can infect the flour used for bakery production. This fungus can be stopped by some herbal derivatives. Investigation of antifungal activity of essential oil of the flowering plant against *Aspergillus* species showed an important inhibitory effect of the seed essential oil (Trachyspermum) on the fungal growth at low concentrations (≤500 ppm). The cinnamon essential oil concentration of 500 ppm completely inhibits an aflatoxigenic strain of *Aspergillusflavus* in yeast extract broth and agar for seven days. The investigation results were consistent with the results obtained in this study.

CONCLUSION

Today, people demand natural preservatives derived from animal and plant sources due to their ability of increasing retention time of foods and also to be safe from the harmful effects of chemical and synthetic preservatives. Among natural compounds, there are essential oils and herbal extracts that have been interested by food safety and hygiene experts in recent years, thus their preservation and antimicrobial effects have been studied. Since the 1980s, the bread industry has put great effort to reduce the number of additives and synthetic preservatives, such as calcium propionate, and to produce natural, fresh bread. The results of this research indicated that undesired fungal growth in bread can be inhibited by adding starch foam powder containing cinnamon essential oil in active packaging, which has gained a lot of interest. However, in previous studies done by other investigators, these effects have been usually examined in culture media and rarely conducted under operational and environmental conditions.The present study showed that the fungal growth of bulky bread is reduced by increasing the cinnamon essential oil concentration. After 15 days, the fungal load decreased 8 log cfu/g compared to that of the control group, at a cinnamon essential oil concentration of 1500 ppm. According to the National Standard of Iran No. 2395, the acceptable level of fungal growth in bread is 10^2 cfu/g. In this study, starch foam powders containing 1000 and 1500 ppm of cinnamon essential oil in bulky bread packages inhibited the microorganism growth for six days. There wasn't any significant difference among the samples containing cinnamon essential oil concentrations of 500, 750, 1000 and 1500 ppm in terms of general acceptance of bread sensory evaluation on the sixth day. Therefore, cinnamon essential oil concentration of 1000 ppm can be considered as the best concentration for inhibiting mold growth and increasing the retention time of bread, which was the main objective of this study. As we know, maximum retention time of bulky bread at room temperature (25°C) is three days and there after microbial load will be higher than the acceptable level and bread spoilage can be seen. Thus, by using starch foam powder containing cinnamon essential oil in active packaging, the bulky bread spoilage is delayed from

three days to six days. The use of cinnamon essential oil on an industrial scale to preserve the quality of product provides a new approach for innovative technology on a commercial scale.

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