

Effect of ajwainseeds on pan bread quality

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Abstract

The aim of this study was to evaluate the effect of ajwain seeds (*Trachyspermum ammi* L.) and its essential oil on pan bread quality. Ajwain seeds yielded 2.8% essential oil. Thymol (35.80%) followed by ρ -cymene (34.76%) and γ -terpinene (19.20%) were identified as major constituents of the essential oil. Powder of ajwain was used at 2, 4 and 6% of wheat flour (w/w), while ajwain oil incorporated at 200 μ l/4g sunflower. Only replacement level at 6% was unacceptable by consumer panelists. No significant differences were observed in specific volume and hardness in all tested samples either contained different ajwain powder levels or ajwain essential oil compared with control. The only observed differences ($p < .05$) was in specific volume of treatment A₄ which containing 4% ajwain powder. Specific volume of pan bread was ranged between 4.11cm³/g for control bread without any addition C₀ to 3.89 cm³/g for A₄ treatment. Whereas, hardness values ranged between 7.50 and 7.73N. for samples C₀ and A₄, respectively. Crumb color was significantly affected ($p < .05$), by increasing replacement level, L* values were decreased while, b* and a* values increased. These means the darkness gradually increased, and yellowness decreased. Ajwain seeds powder at 2% and 4% exhibited good antimicrobial activity until the seventh day of storage, as also observed for positive control. While, shelf life of pan bread without any addition C₀ was not exceeded three days, and five days for A_E sample which contain ajwain essential oil.

Key words: Ajwain, Pan bread quality, Shelf life, Essential oils, Physical properties.

Introduction

According to the Codex Alimentarius, bread is the product resulting from baking dough obtained by mixing flour and water, with or without addition of edible salt, fermented bakery's yeast (Heras-Mozos *et al.*, 2019). Among bakery products, pan bread is one of common consumed product worldwide. Like to any processed food, the shelf life of bread is reduced due to many causes such as microbial growth (Mani López *et al.*, 2018). There are several methods to extend shelf life of bakery products, propionic acid and its salts are the most common synthetic preservative had been used. The growing demand of consumers for safe products without addition of chemical preservative has led to more attention to aromatic plants which generally recognize as safe (GRAS) (Lucera *et al.*, 2012). One of these valuable plants is ajwain seeds as powder and/or its essential oil.

Ajwain (*Trachyspermum ammi* L.) is an annual herbaceous flowering plant belong to family Apiaceae, which grows worldwide in arid and semi-arid regions, including Egypt (Niazian *et al.*, 2019). Ajwain seeds had essential oil known to be active and effective as anti-fungal, anti-bacterial and anti-oxidant agent (Kavoosiet *et al.*, 2013; Boskabady *et al.*, 2014; Zarshenas *et al.*, 2014; Snoussi *et al.*, 2018 and Attouet *et al.*, 2019).

Literature searches were undertaken on the effect of addition medicinal and aromatic plants and their extracts on bakery products quality. For example, the effects of grape seed extract (Peng *et al.*, 2010), effect

of ginseng powder (Chang and Ng, 2012), *Rubus coreanus* powder (Lee, 2015), effect of black tea (Zhu *et al.*, 2016) and green tea powder (Ninget *et al.*, 2019). Therefore, the aim of this study was to investigate the effect of ajwain and its essential oil on pan bread quality.

Materials and methods

Materials:

Ajwain (*Trachyspermum ammi* (L.) seeds were obtained from Harraz for Food Industry and Natural Products, Cairo, Egypt. Wheat flour 72% extract, instant yeast, salt, sugar and sodium propionate were purchased from the local market. Sunflower oil without synthetic antioxidant was obtained from Armafor Food Industry, 10th of Ramadan City, Egypt.

Methods:

1. Extraction of ajwain essential oil:

Hundred grams of dried plants were subjected to hydro-distillation using Clevenger-type apparatus, for 3:4 hr. according to the method recommended in British Pharmacopoeia (2007). The extracted essential oil was dried using anhydrous sodium sulfate and stored in dark and sealed bottles in a refrigerator at 4° C ($\pm 2^\circ$ C).

2. GC/MS analysis of ajwain essential oil:

GC-MS analyses were carried out using the methods described by Mihajilov-Krstevet *et al.*, (2009).

CapillaryGC-2010 plus Gas Chromatographs (Shimadzu Corp., Japan), coupled with Shimadzu FID 2010 plus detector (Flame Ionization Detector). The GC system was equipped with a Stabilwax column (30 m × 0.25 mm i.d., film thickness 0.25 µm); oven temperature was 40°-150° C at a rate of 4° C/min and held for 6 min; rising at 4°C/min to 210°C and held for 1 min., carrier gas was Helium with a flow rate 1mL/min, split ratio 1:10. The injector and detector were held at 210°C and 250°C, respectively. The obtained chromatogram and analysis report were analyzed to calculate the percentage of the main volatile compounds.

3. Preparation and formulation of pan bread samples:

The straight dough method for pan bread production was carried out according to the method described by (AACC, 2002) with some modification at Special Unit for Bakery Products Food Technology Research Institute (FTRI), Giza, Egypt.

Basic dough formula of 1000g flour basis was consisted of instant yeast (15g), sugar (20 g), salt (12g), sunflower oil (4g) and the required amount of water. The blends of wheat flour with three levels of ajwain powder *i.e.* 20, 40, 60gm or with 200µl essential oil as shown in Table (1). All other ingredients were mixed together. The resulted dough was let to rest for 30 min (first proofing), then divided to (350 gm) pieces, rolled and molded in molding. Each piece was placed in baking pans (28x12x7 cm) tightly greased to prevent the loaves from sticking pans and was let to ferment for 60 min in a cabinet at 30°C and 80 - 85% relative humidity, then baking process was carried out in electrically heated oven at 230°C for 25-30 min. After baking, loaves were separated from the baking pans and allowed to cool for 2 hr. at room temperature (28°C± 5°C) for physical and organoleptic evaluation. The produced loaves were weighted, and the volume was measured by the rapeseed displacement method according to (AACC, 2002).

Table 1. Ingredients of pan bread

Sample	Flour (g)	Ajwain		Yeast (g)	Sunflower oil (g)	Salt (g)	Sugar (g)
		powder (g)	Essential oil				
C ₀ Control (-)	1000	-		15	4	12	20
C ₁ Control (+)	1000+ sodium propionate (0.2%)			15	4	12	20
A ₂	980	20		15	4	12	20
A ₄	960	40		15	4	12	20
A ₆	940	60		15	4	12	20
A _E	1000	-	200µl	15	4	12	20

*C₀: Control bread without any addition; C₁: Control bread with synthetic preservative (sodium propionate); A₂, A₄ and A₆: pan bread contains ajwain seed powder 2%, 4% and 6% respectively; A_E: pan bread contains ajwain seed essential oil at 200 µl.

4. Overall acceptability

A seven-point hedonic scale was used to evaluate the overall acceptability of the bread formulations; where 1 = dislike very much, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike, 5 = like slightly, 6 = like moderately and 7 = like very much as suggested by Wójcik *et al.*, (2017), to determine the acceptable levels of replacement. Breads were considered acceptable if their mean scores for overall acceptability were above 4 (neither like nor dislike).

5. Sensory evaluation

The Organoleptic characteristics of the pan bread attributes were evaluated by fifteen panelists of Food Technology Research Institute (FTRI) for color (20), taste (20), odor (20), appearance (20) and texture (20) according to Seleemet *et al.*, (2014).

6. Physical properties of pan bread

a. Specific volume of pan bread loaves

After baking and cooling for 2 hr. The bread volume was measured according to the method of (AACC, 2002), weights were recorded by using decimal digital weighing scale. The specific volume was calculated as the ratio between the volume and the weight of the bread (cm³/g). Three replicates of each sample were analyzed.

b. Texture profile analysis (TPA)

Texture parameter (hardness) of pan bread samples was measured objectively by using a texture analyzer CT3 Texture Analyzer (Version 2.1, 10000 Gram unit, Brookfield, Engineering Laboratories, Inc. USA), according to method 74-09 (AACC, 2000) and as describe by Peng *et al.*, (2010) with some modifications. Whereas one slice of pan bread approximately 25mm thick or two slices, each approximately 12.5mm thick could be used. Set up a 36 mm diameter cylindrical probe at a test speed of 2

mm/s. The location of testing was the center of the bread slices avoiding non-representative areas of crumb. Sample was subjected to 40% deformation and trigger load 5g. Parameters (graph and data) were automatically recorded by computer software (TA-CT-PRO Software). Test was a total of three samples per treatment undergo. Results were calculated as the average of the three determinations per treatment.

c. Color measurement

The color (CIE* system) of pan bread crumb were determined using a Chroma-meter (Minolta CR-400, Minolta, Osaka, Japan) according to the method of Zhu *et al.*, (2016). Where, L* indicates the brightness-darkness. While positive and negative a* indicates the redness-greenness and b* indicates yellowness-blueness. For a comprehensive analysis comparing L*, a* and b* values together, E index was calculated using the following equation:

$E = (L^*2 + a^*2 + b^*2)^{1/2}$ as mentioned by Peng *et al.*, (2010).

7. Microbial evaluation of pan bread loaves

The microbial evaluation of pan bread loaves includes; total bacterial count (TBC), yeasts and mold counts. Cell counts were performed by plating of serial dilutions of the pan bread homogenates at 0, 4 and 7 days of storage at room temperature according to ISO, (2008). All experiments were carried out in duplicates.

8. Statistical analysis

The results were statistically analyzed using CoState statistical software (CoHort Software, Monterey, CA, USA). The statistical calculations included the analysis of variance (ANOVA) one way completely randomized. $p < 0.05$ was considered to be significant using Duncan's test. All data were

expressed as means values \pm Standard Deviation (SD), as described by Snedecor and Cochran (1982).

Results and discussion

1. The essential oil content of ajwain seeds

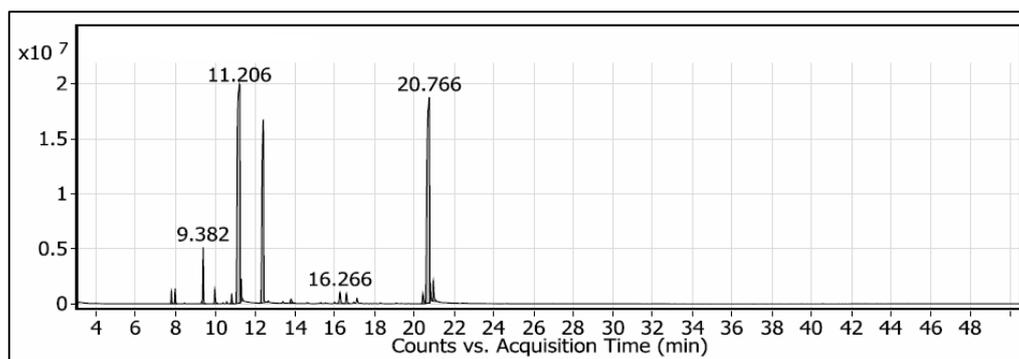
Light brownish essential oil with yield of 2.8% (on dry weight basis) was obtained from dry ajwain seeds. This findings in agreement with that obtained by Attouet *al.*, (2019), who reported that hydro-distillation of dried ajwain seeds yielded 2.58% (w/w). Also, Zarshenaset *al.*, (2014) mentioned that mean yield of ajwain essential oil obtained from ten samples of dried seeds ranged from 2.2% to 4.8% (v/w). While, Howyzehet *al.*, (2018) found that essential oil of Iranian ajwain yielded 5.7%.

2. Composition of ajwain essential oil

The essential oil composition of ajwain seeds was analyzed by GC/MS. Results are shown in Table (2) and are graphically illustrated in Fig. (1). It could be noticed that twelve compounds, representing 98.02% of total chemical composition, were identified in ajwain (*Trachyspermum ammi* (L.) essential oil. The major constituents were thymol (35.80%) followed by ρ -cymene (34.76%) and γ -terpinene (19.20%). Meanwhile, β -pinene was detected at 2.65%. These results in the same line with those obtained by Zarshenaset *al.*, (2014) and SoltaniHowyzehet *et al.*, (2018) who found that major constituents of ajwain essential oil were thymol, ρ -cymene and γ -terpinene, and these components were different in ranking amount. While, on another study by Moeinet *al.*, (2015) stated that γ -terpinene was the major compound (48.07%) followed by ρ -cymene (33.73%) and thymol (17.41%).

Table 2. Chemical components of ajwain essential oil

No.	Compound	RT	Area%
1	β -Thujene	7.78	0.53
2	α -pinene	7.97	0.64
3	β -pinene	9.38	2.65
4	Myrcene	9.97	0.78
5	α -Terpinene	10.81	0.51
6	ρ -Cymene	11.20	34.76
7	d-Limonene	11.29	0.95
8	γ -Terpinene	12.41	19.20
9	Trans-Sabinene hydrate	13.80	0.45
10	2,6-Dimethyl-3,5,7-octatriene-2-ol,E,E-	16.26	1.01
11	Terpiene-4-ol	16.59	0.74
12	Thymol	20.76	35.80
			98.02



Chromatogram of ajwain seeds essential oil.

Fig. 1

3. Consumer acceptability of pan bread samples

The consumer acceptability was conducted to evaluate the acceptable level of ajwain. The results in Table (3) showed that no significant differences were observed in treatments A₂ and A_E comparing to control samples, while there was a significant difference ($p < 0.05$) between control and A₄ and A₆ samples.

According to the scores, Table (3) and Fig (2) revealed that pan bread A₆ recorded below 4 (neither like nor dislike). Thus, A₆ sample was not acceptable

by the most of panelists probably due to its strong aroma and its bitter after taste (Limet *et al.*, 2011). On the other hand, all tested samples were accepted by panelists according to its score values more than four. Also, Lim *et al.*, (2011) reported that bread which contained 6% or 8% turmeric had low significant sensory score in overall acceptability, whereas, partial replacement of wheat flour with up to 4% turmeric powder in breads provide satisfactory overall consumer acceptability.

Table 3. Consumer acceptability of pan bread samples

Treatment	Consumer acceptability
C ₀ Control(-)	6.80±0.41 ^a
C ₁ Control(+)	6.77±0.45 ^a
A ₂	6.33±0.90 ^{ab}
A ₄	5.80±1.01 ^b
A ₆	2.00±0.92 ^c
A _E	6.47±0.74 ^a
LSD	0.56

*C₀: Control bread without any addition; C₁: Control bread with synthetic preservative (sodium propionate); A₂, A₄ and A₆: pan bread contains ajwain seed powder 2%, 4% and 6% respectively; A_E: pan bread contains ajwain seed essential oil at 200 µl.

Means±SD. Mean values in the same column with different superscript letters are significantly different ($P < 0.05$).

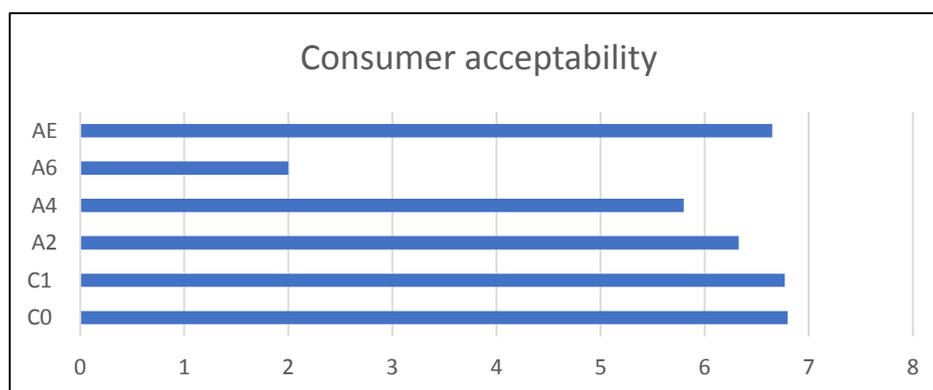


Fig. 2 The overall acceptability of ajwain pan bread samples

4. Sensory evaluation

In order to determine the differences in the previously acceptable samples more clearly, the overall quality bread was evaluated for their sensory

attributed of taste, color, odor, and texture. The mean scores of the evaluated sensory attributes are presented in Fig (3).

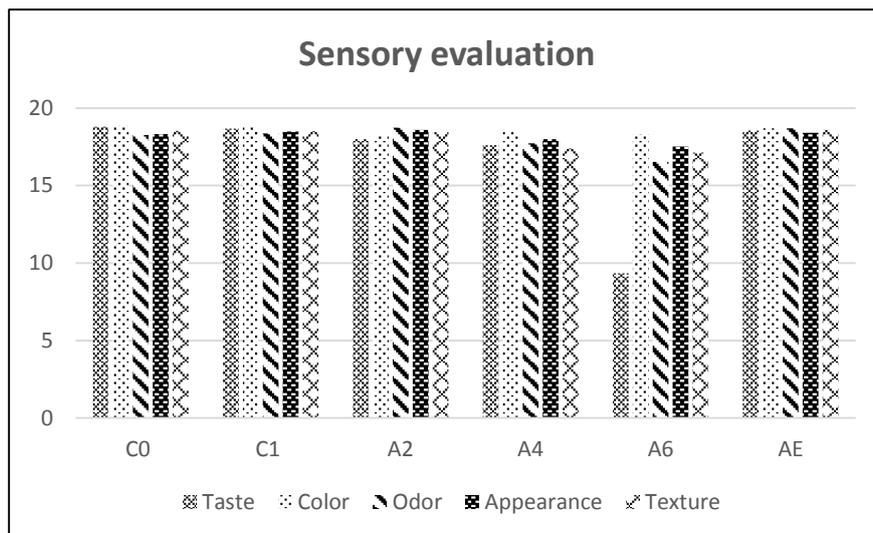


Fig. 3 Sensory evaluation of ajwain pan bread samples

The obtained results showed that no significant differences in all sensory attributes were observed in tested samples either contain 2% ajwain powder or ajwain essential oil compared with control samples. The significant differences ($p < 0.05$) was observed in taste of treatments A₄ and A₆. Whereas, in overall acceptability A₄ sample had a good score as mentioned before. These results were closest to those obtained by Bolarinwa *et al.*, (2019) who mentioned that the moringa fortified bread at level 5% was acceptable to consumer, whereas significant differences were observed in all sensory attributes of bread samples fortified with higher levels of moringa seed powder ($> 5\%$). The acceptable blends were

selected to evaluate the quality characteristics of pan bread.

5. Specific volume and texture profile (hardness)

Specific volume of pan bread was ranged between 4.11 cm³/g for control (C₀) to 3.89 cm³/g for (A₄) treatment as shown in Table 4. From the obtained results it could be noticed that the specific volume tends to decrease with increasing replacement level of ajwain powder or their essential oil. Insignificant differences were observed between control and different treatments. The decreasing of specific volume could be attributed to the effect of ajwain on gluten network (Schmieleet *et al.*, 2012 and Odunlade *et al.*, 2017).

Table 4. Specific volume and hardness of pan bread

Treatment	Specific volume (cm ³ /g)	Hardness (N)
C ₀ Control(-)	4.11±0.10 ^a	7.50±0.25 ^a
C ₁ Control(+)	4.02±0.18 ^a	7.59±0.22 ^a
A ₂	3.99±0.11 ^a	7.62±0.24 ^a
A ₄	3.89±0.02 ^a	7.73±0.10 ^a
A _E	3.95±0.07 ^a	7.69±0.13 ^a
LSD	0.20	0.36

*C₀: Control bread without any addition; C₁: Control bread with synthetic preservative (sodium propionate); A₂, A₄ and A₆: pan bread contains ajwain seed powder 2%, 4% and 6% respectively; A_E: pan bread contains ajwain seed essential oil at 200 µl.

Means±SD. Mean values in the same column with different superscript letters are significantly different ($P < 0.05$).

Hardness usually used as an indicator of bread freshness, and negatively correlated with bread quality. The results in Table (4) revealed that hardness values increase slightly by increasing the ajwain powder level and incorporated ajwain essential oil. Whereas, insignificant differences were observed in all treatments contained different ajwain powder levels or ajwain essential oil. Similar increasing trend was observed by other previous studies evaluated the effect of ramie powder (Lee and Joo, 2012) and green tea (Ning *et al.*, 2017). This increasing probably due to interactions between fibers and gluten (Feiliet *et al.*, 2013).

6. Effect of ajwain seeds on pan bread crumb color

The effect of ajwain seeds powder and its essential oil on pan bread crumb color was presented in Table 5. All color data were expressed as L* (lightness - darkness), a* (redness - greenness) and b* (yellowness - blueness) values. The obtained data revealed that crumb color of pan bread was significantly ($p < 0.05$) affected by replacement level of ajwain powder. From the results it could be noticed that as the level of ajwain increased the L* values significantly decreased, meanwhile b* and a* values were increased. These means the darkness gradually

increased, and yellowness decreased. This finding was in agreement with those obtained by Lee and Joo, (2012) and Zhu *et al.*, (2016).

Table 5. Effect of ajwain on pan bread crumb color

Sample*	Crumb			
	L*	a*	b*	E index
C ₀ Control(-)	78.35±0.70 ^a	0.84±0.07 ^c	16.55±1.11 ^c	80.08
C ₁ Control(+)	78.47±0.12 ^a	0.85±0.05 ^c	16.78±0.23 ^c	80.25
A ₂	68.90±0.40 ^b	2.33±0.11 ^b	21.28±0.66 ^b	72.15
A ₄	65.15±1.29 ^c	3.16±0.16 ^a	23.36±0.78 ^a	69.28
A _E	78.21±0.27 ^a	0.90±0.05 ^c	16.78±0.46 ^c	79.80
LSD	1.26	0.18	1.30	

*C₀: Control bread without any addition; C₁: Control bread with synthetic preservative (sodium propionate); A₂, A₄ and A₆: pan bread contains ajwain seed powder 2%, 4% and 6% respectively; A_E: pan bread contains ajwain seed essential oil at 200 µl.

Means±SD. Mean values in the same column with different superscript letters are significantly different (P< 0.05).

The observed crumb color corresponds mainly to the color of ajwain powder used. On the other hand, the changes of pan bread crumb color of (A_E) treatment showed good color values compared to control samples.

E index was introduced to describe the color changes, compared to control samples E index decreased with replacement level of ajwain powder increased. It means loss in brightness of bread samples. Furthermore, the same trend was also noticed in treatment A_E whereas, ajwain essential oil was used.

7. Effect of ajwain seeds on microbial growth of pan bread

The effect of ajwain seeds powder at substituted levels 2% and 4% and ajwain oil at concentration

200µl/4g sunflower oil on pan bread microbial growth during storage for 7 days at room temperature (28-32°C) compared to control. The obtained results are graphically presented in Fig. 4 samples without sodium propionate (C₀) or with sodium propionate at 0.2% (C₁).

Data showed that increasing in Log/CFU count by increasing storage period in all samples. After 3 days, C₀ showed a visible fungal growth which indicating the end of shelf life for this sample. This finding is in line with those reported by Selem and Mohamed (2014). Meanwhile, treatments C₁, A₂ and A₄ samples prolonged the shelf life for additional four days compared to C₀ sample.

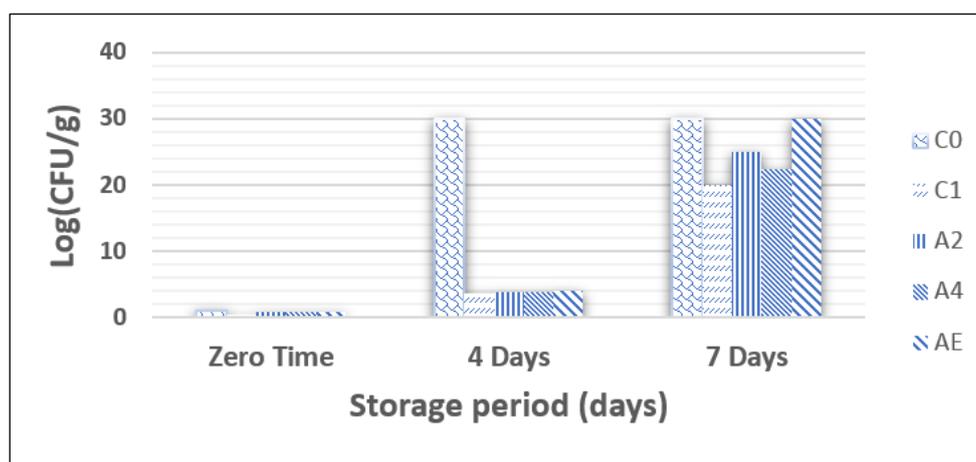


Fig. 4 Microbial growth during storage period

On the other hand, A_E exhibited microbial growth after 5 days. Shelf life of A_E sample may be extended resulted in advanced techniques as microencapsulation to protect bioactive compound of essential oil. In this respect, Saatchi *et al.*, (2014) found that ajwain essential oil was revealed as

efficient antifungal agent. Therefore, it could be concluded that ajwain showed a good antimicrobial activity and able to retard microbial growth in pan bread (p<0.05) for a considerable period of time with good manufactory practice (GMP).

Conclusion

In the present study, ajwain seeds powder and its essential oil slightly affected pan bread quality, except at 6% replacement level where, it was unacceptable by the most panelists probably due to its after taste and strong aroma. Only color characteristics was significantly affected by increasing replacement levels. These changes due to the natural color of ajwain seeds powder. As antimicrobial agent ajwain showed a good antimicrobial activity and able to retard microbial growth in pan bread ($p < 0.05$) for a considerable period of time.

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تأثير نبات الأجوين على جودة خبز القوالب

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تهدف هذه الدراسة إلى تقييم تأثير نبات الأجوين وزيتته العطري على جودة خبز القوالب. حيث وجد أن نسبة الزيت العطري تصل إلى 2.7%، وتم التعرف على مركبات الثيمول 35.80% والباراثيمين 34.76% والجاما تربينين 19.20% كمركبات رئيسية في الزيت العطري. وتم إستبدال دقيق القمح المستخدم في صناعه خبز القوالب بنسب 2 و 4 و 6% من مسحوق نبات الأجوين، بينما تم دمج الزيت العطري بنسبه 200 ميكروليتر/4 جم زيت عباد الشمس. من ناحية مدى القبول العام فكان الإستبدال بنسبة 6% غير مقبول من جانب معظم المحكمين. لم تظهر أى فروق معنوية فى الحجم النوعى ودرجة الصلابة بين كل العينات محل الدراسه، فيما عدا العينة A₄ المحتويه على 4% من مسحوق الأجوين، والتي أظهرت فرق معنوى فى الحجم النوعى مقارنة بالعينة الكونترول. وقد تراوحت قيم الحجم النوعى ما بين 4.11 سم³/جم للعينة الكونترول بدون مواد حافظه C₀ و 3.89 سم³/جم للعينة A₄ المحتويه على 4% من مسحوق الأجوين، بينما تراوحت قيم الصلابة ما بين 7.50 للعينة C₀ إلى 7.73 نيوتن للعينة A₄. كما تأثر لون لب الخبز معنوياً بدرجة الإستبدال حيث إنخفضت قيم *L* و *a* و *b*، وتشير هذه النتائج إلى أن الخبز أصبح أكثر داكنه وأقل إصفراراً ويميل إلى الإحمرار. من ناحية الخصائص الفيزيائية والحسية لم تظهر العينة A_E المحتوية على الزيت العطري لنبات الأجوين أى فروق معنوية مقارنة بالكونترول، وأظهرت أفضل النتائج بين كل العينات المعامله. أظهر مسحوق نبات الاجوين كفاءة من الناحية الميكروبيولوجيه، وأدى استخدامه إلى إطاله فترة الصلاحية حتى اليوم السابع، بينما لم تتعدى فترة صلاحية الخبز بدون أى مواد حافظه ثلاثه أيام، وخمسة أيام للعينة المحتوية على الزيت العطري A_E.

الكلمات المفتاحية: خبز القوالب، نبات الأجوين، الزيوت العطرية، خواص القوام، الصفات الفيزيائية، الصلاحية.

