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Production of Low Calorie Bakery Product with Pleasant Flavour, Antioxidant and Antimicrobial Activities

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Aromatic plants are considered sources of antioxidants, antimicrobial and flavouring agents. Four aromatic plants (*Thymus vulgaris L., Foeniculum vulgare, Pimpinella anisum L.* and *Trigonellafoenum–graecum L.*) were analysed in the study. Yoghurt was used to produce a low calorie pie. Chemical and rheological parameters, baking performance, staling rate and sensory properties of the pie were investigated. Volatile aroma compounds were analysed with GC and GC/ MS, and antioxidant activity was evaluated by DPPH and β–carotene assays. The incorporation of yoghurt and some aromatic plants in the pie improved protein, fat, fibre, ash, and minerals contents and allowed achieving about 19% reduction in calories. Sensory evaluation of pie containing the mixture of aromatic plants showed its superior sensory quality. In addition, it could be concluded that aromatic plants were able to inhibit the growth of yeast, mould and bacteria and to prolong the storage periods of pie compared with the control.

INTRODUCTION

Bakery products are consumed all over the world. Fat is an important ingredient in bakery products and its major functions are to tenderise the product and soften the texture, add moistness and richness, increase keeping quality, add flavour, and to assist in leavening when used as creaming agents or give flakiness to products such as puff pastry, pie and dough [Gisslen, 1994]. In recent decades, the supply and consumption of bakery products with reduced energy content has been increasing in response to the demand for products with lower calorie content [Sandrou & Arvanitoyannis, 2000].

Fat is important because it affects food taste and texture and also affects human health depending on intake. Some trends have emerged in diets around the world, including excess calories, lack of essential nutrients, imbalanced nutrition, deterioration in meal quality, declines in physical activity, and increased dependence on processed, fast, and instant food rather than natural.

Unhealthy dietary trends have led to an increase in obesity, metabolic syndrome, and non–communicable disease morbidity; thus, causing health problems [Shin *et al.,* 2013]. Cheng *et al*. [2009] reported that the rise in cardiovascular disease and obesity and in other diet–related illnesses has led to consumers taking a greater interest in the ingredients of food products and valuing those with a reduced caloric value more positively. An effect of the lower consumption of herbal aromatic plants in recent decades is insufficient intake of antioxidants [Nanditha & Prabhasankar, 2009].

Attempts have, therefore, been made to use yoghurt instead of fat in bakery products. Yoghurt is used to confer colour and flavour to baked products. It should be fully heat–treated at 220°C to avoid problems associated with the loss of product volume [Cauvain & Young, 2001]. Reducing fat in every–day's diet has become a public health issue and a concern for most consumers. While in some product sectors reduced fat alternatives are both widespread and acceptable to the consumer, other sectors such as bakery are generally behind in producing successful reduced fat alternatives. Pie may be described as a savoury fermented product produced mainly from wheat flour, water, yeast, shortening and salt by a series of processes involving mixing, kneading, proofing, shaping and baking [Dewettinck *et al*., 2008].

The flavour of fresh baked products is central to consumer acceptability. Among the various intrinsic properties of bakery products, volatile compounds play a key role in the perception of fresh bakery flavour. However, the perceived fresh bakery flavour often relies on the type of bakery, ingredients, method of production and shelf life.

Aromatic plants and their essential oils have been used extensively as flavour ingredients in a wide variety of foods, beverages, and confectionery products. Several studies ex-

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amined the antioxidant activities of aromatic plants [Hudaib *et al.*, 2002; Mahjoub *et al.*, 2010]. For this reason, these substances can be used as safe and effective alternatives to synthetic preservatives [Wilson *et al*., 1997]. Aromatic plants have been recognised to possess biological activities, including antibacterial, antifungal and antioxidant properties. Several spices (cinnamon, clove and marjoram), herbs (thyme, sage, oregano, rosemary and basil) and fruits (berries) contain volatile antimicrobial compounds [Carraminana *et al*., 2008]. The aromatic plants are widely used for their antioxidant activities and as alternative to synthetic preservatives [Busatt *et al*., 2008; Carraminana *et al*., 2008; Burt, 2004]. Pie manufactories face a major problem of lipid oxidation which reduces the shelf life. For this reason, these substances can be used as safe and effective alternatives to synthetic preservatives [Wilson *et al*., 1997]. Antioxidants and preservatives may be used to overcome this problem. So the objective of this study was to produce calorie–reduced bakery products fortified with aromatic plants as thyme, fennel, anise and fenugreek because they are rich with phenolics, antioxidants and with the use of yoghurt to improve the rheological, nutritional, and flavour properties as well as to extend the shelf life of the produced pie.

MATERIAL AND METHODS

Material

Wheat flour (72% extraction) was purchased from the North Cairo Flour Mills Company, Egypt. Ground thyme, anise, fennel and fenugreek seeds were obtained from local herbal shop (Dokki, Egypt). Sugar, shortening, active dry yeast, salt, and skimmed milk were purchased from the local market, Cairo, Egypt. Also, sodium sulphate anhydrous, dichloromethane, methanol, hexane, n–hydrocarbons (C_7– C_{22}), 2, 2–diphenyl–1–picrylhydrazyl radical (DPPH), β–carotene, linoleic acid, chloroform and Tween80 were purchased from Sigma–Aldrich company (St. Louis, MN,USA).

Preparation of dried blends

Pies (control and fortified) were prepared by preparing different blends (Table 1).

Rheological properties

Rheological properties of doughs were evaluated using farinograph and extensograph according to AACC [2000].

Preparation and evaluation of pie

Different blends (Table 1) were mixed at the rate of 100 g blended flour with 1.5 g active dry yeast, sodium chloride (1.5 g), and sugar (10 g). The dough was left to ferment for 1 h at 30°C and 85% relative humidity. The dough was divided to pieces each weighing 150 g. The pieces were arranged on trays and left to ferment for further 30 min at the same temperature and relative humidity. The pieces of fermented dough were left again for 15 mins at the same temperature and relative humidity, and baked at 230°C for 10 min. Pies were allowed to cool on racks for about 1 h before evaluation. They were evaluated organoleptically by 15 trained panellists according to Kulp *et al*. [1985]. The samples were characterised according to taste (20), aroma (20), mouth feel (10), crumb texture (15), crumb colour (10), crust colour (10), break & shred (10) and symmetry shape (5).

Identification of volatile constituents

The ground thyme, anise, fennel and fenugreek seeds were subjected to hydrodistillation for 3 h using a Clevenger–type apparatus. The obtained essential oil was dried over anhydrous sodium sulphate, filtered and stored at –4 °C until analysed.

Isolation of fortified pie volatile components

Simultaneous distillation extraction (SDE) method was used for isolation of the volatiles followed by solvent/ solvent extraction apparatus (SE) of pie samples [Lee & Shibamoto, 2001]. The ground pie (100 g) of each sample was mixed with 500 mL of distilled water and subjected to SDE then SE using dichloromethane. The solvent containing volatiles was dried over anhydrous sodium sulphate overnight and concentrated using a rotary evaporator at 40°C to the final volume (100 μL) under nitrogen before GC and GC/ MS analyses.

Preparation of fortified pie extracts

Lipid was extracted from pie samples before extraction process to overcome their turbidity in the antioxidant assays.

TABLE 1. Composition of formulas used in manufacture of pie.

Formulas	Ingredients (g)									
	Wheat flour	Fat	Yeast	Yoghurt	Salt	Sucrose	Thymus	Fenugreek	Anise	Fennel
1. Basic formula (control)	100	26	2	–	1.5	10	–	–	–	–
2. Pie with Thymus (Thy)	95	10	2	16	1.5	10	5	–	–	–
3. Pie with fenugreek (Fen)	95	10	2	16	1.5	10	–	5	–	–
4. Pie with anise (Ani)	95	10	2	16	1.5	10	–	–	5	–
5. Pie with fennel (Fenn)	95	10	2	16	1.5	10	–	–	–	5
6. Pie with fen+Thy	95	10	2	16	1.5	10	2.5	2.5	–	–
7. Pie with fen+fenn	95	10	2	16	1.5	10	–	2.5	2.5	–
8. Pie with Fen + ani	95	10	2	16	1.5	10	–	2.5	–	2.5
9. Pie with Fen +Thy+Fenn+ani	95	10	2	16	1.5	10	1	2	1	1

Well–ground sample of each treatment (1, 2, 5 and 10 g/L) was extracted by using methanol (HPLC grade).

Gas chromatographic analysis (GC)

GC analysis was performed by using Hewlett–Packard model 5890 equipped with a flame ionization detector (FID). A fused silica capillary column DB–5 (60 m x 0.32 mm id) was used. The oven temperature was maintained initially at 50°C for 5 min, and then programmed from 50 to 250°C at a rate of 3°C/min. Helium was used as the carrier gas, at a flow rate of 1.1 mL/min. The injector and detector temperatures were 220 and 250°C, respectively. The retention indices (Kovats index) of the separated volatile components were calculated using hydrocarbons (C7– C21, Aldrich Co.) acc. to Adams [1995].

Gas chromatographic – mass spectrometric analysis (GC/ MS)

The analysis was carried out by using a coupled Varian gas chromatography/ mass spectrometery. The ionization voltage was 70 eV, mass range m/z 39–400 a.m.u. The GC was carried out as mentioned above. The isolated peaks were identified by computer with Wiley 275 and National Institute of Standards Technology (NIST 3.0) libraries provided with the computer controlling GC/ MS. The retention indices were calculated using a homologous series of *n*–alkanes C_7–C_{21}. The quantitative determination was carried out based on peak area % [Adams, 1995].

Determination of antioxidant activity

DPPH radical scavenging assay

Antioxidant activity was also determined by DPPH assay using a spectrophotometer at 517 nm [Tepe *et al.*, 2005]. Each extract of different concentrations (100, 200 and 400 μg/mL, respectively) and TBHQ (100, 200 and 400 μg/mL) was taken in different test tubes. Four millilitres of 0.1 mmol/L methanol solution of DPPH were added to these tubes, that were next shaken vigorously. The tubes were allowed to stand at room temperature for 30 min. The control was prepared as the same without any extract and MeOH. The changes in the absorbance of the prepared samples were measured at 517 nm. Radical scavenging activity was estimated as the inhibition percentage and was calculated using the following formula:

$$\% \text{ Inhibition} = [(A_B - A_A)/A_B] \times 100$$

where: A_B – absorption of blank sample (t=0 min), and A_A – absorption of sample solution (t=30 min).

β–Carotene– linoleic acid scavenging assay

The antioxidant activities of the essential oil and fortified pie samples were also determined in terms of measurement of the percentage inhibition of peroxidation in the linoleic acid system according to Iqbal *et al.* [2007] with some modifications.

In this respect, a stock solution of β–carotene–linoleic acid mixture was prepared as follows: 0.5 mg of β–carotene was dissolved in 1 mL of chloroform and 25 μL linoleic acid and 200 mg Tween 40 were added. After evaporation of chloroform, 100 mL of oxygen–saturated distilled water was added with vigorous shaking. Then, 2500 μL aliquots were dispensed into the test tubes, 200 μL of the extracts at different concentrations were added and the emulsion system was incubated for 48 h at room temperature. The same procedure was done on both BHT (as positive control) and blank. In turn, absorbance of the mixture was measured at 490 nm. Antioxidative capacities of the extracts were compared with those of BHT and blank.

Analytical methods

Moisture, protein, fat, crude fiber and ash of raw materials and different pies were determined according to AOAC [2000]. Carbohydrates were calculated by differences. Caloric value was calculated according to dietary guidelines for Americans, 2010, U.S. Department of Agriculture.

$$\text{Energy} = 4\ (\text{protein\%} + \text{carbohydrate \%}) + 9\ (\text{fat \%})$$

Pie volume and weight

The volume of pie was measured by rapeseed displacement according to [AACC, 2000]. Rapeseed displacement [AACC, 2000] is a common method for measuring volumes of irregular solids and has been used for bread [Keskin *et al.,* 2004]. Rapeseeds have a diameter of approximately 1.7 mm. In the rapeseed method, first the tapped bulk density of rape–seeds is determined by filling a glass container of known volume uniformly with rapeseeds through tapping and smoothing the surface with a ruler. Then, the sample and rapeseeds are placed together into the container. The container is again tapped and the surface is smoothed with a ruler. Tapping and smoothing is continued until constant weight is reached between consecutive measurements. The volume of the sample is calculated as follows for volume of bulk solids, $V_{sample:}$

$$m_{seeds} = m_{total} - m_{sample} - m_{container};\ V_{seeds} = m_{seeds} / \rho_{seeds}$$

and

$$V_{sample} = V_{container} - V_{seeds}$$

where: m is mass and ρ is density.

Both weight and volume were determined according to the method described by Kulp *et al*. [1985]. Specific volume = volume / weight.

Freshness of pies

Pies freshness was tested after wrapping using polyethylene bags and storing at room temperature (0, 3 and 7 days) using Alkaline Water Retention Capacity test (AWRC) according to the method of Yamazaki [1953], as modified by Kitterman & Rubenthaler [1971].

Microbiological analysis

The tested pie samples (10 g) were aseptically weighed and a 1:10 dilution was prepared using Maximum Recovery Diluent (sterile physiological saline can be used as an alterna-

tive to neutralizing solution). A decimal dilution series was then performed using nutrient agar (NA) and 1 mL pour plates prepared. For the bacteria examined, a total aerobic count was performed using Plate Count Agar. The plates were allowed to set, inverted and incubated at 30°C±1 for 48 h, and all resultant colonies were counted. For the yeast and fungi examined, Czapeks–Dox yeast extract agar (CDYEA) was used. The pour plates were allowed to set, then inverted and incubated at 25±1°C for 5 days and all resultant yeast and filamentous fungi were counted.

Effect of selected spices on growth of the inculcated moulds in pie product

Preparation and inoculation of the pie analogues
Isolates

The isolates used were *Penicillium verrucosum* and *Aspergillus niger* isolated from cereal grain and bakery products because they are the most contaminated fungi in cereals and bakery products. The two moulds are maintained in the culture collection of the Laboratory of Department of Microbiology, National Center for Irradiation Research and Technology Cairo, Egypt.

Preparations of suspensions of test microorganisms

Under sterile conditions, fungal spores of the *Penicillium verrucosum* and *Aspergillus niger* from 7–10 day old cultures from the growth media were harvested and each species placed into sterile plastic universal bottles containing 20 mL of distilled water. Bottles were shaken vigorously for 3–5 min and centrifuged in a bench top microfuge for 15 min at 850 × g. After discarding the supernatant, another 20 mL of sterile water was added and the washing process was repeated three times. After the third wash, spores were resuspended with the required buffer (0.85%, NaCl) sterile solution. Spore concentrations were assessed using a haemocytometer slide and then adjusted to 10^3 spores m/L.

Inoculation treatment

Penicillium verrucosum and *Aspergillus niger* were used in artificial inoculation. Pie was sterilized by irradiation at 10 kGy. The irradiation was performed in the National Center for Radiation Research and Technology, NCRRT (Nasr City, Cairo, Egypt) using Russian gamma cell, Model Issledovatel utilizing Cobalt 60 as an irradiation source (2.5 kGy.h–1). Stock cultures of all test fungi were grown in nutrient broth (CzapeksDox yeast extract broth, CDYEB) for 18 h. Pie (25 g) was aseptically packed in sterile polythene bags to which thyme, anise, fennel and fenugreek and test culture were added. The control samples did not contain test spices. All the packets were then stored at room temperature. The total viable counts of the control and treated samples were determined immediately and at intervals of 3 days.

Statistical analysis

The obtained results were evaluated statistically using analysis of variance as reported by McClave& Benson [1991].

RESULTS AND DISCUSSION

Chemical composition and energy value of raw materials and pie

Data presented in Table 2 show gross chemical compositions and energy value of both ingredient and the final products prepared with different blends. It is clear that fenugreek had the highest level of protein while anise was high in fat and crude fibre, while thyme was highest content of ash. For instance, the protein content of control pie that was 10.56% increased to 14.65 for formula 3. Our results were in agreement with Hassannen [1998], Mohammad [2004], Abdel–Aziem [2007] and Hussein *et al*. [2011a]. The results also showed that energy values kcal/100 g decreased in all pie samples in comparison to control due to the substitution of shortening by yoghurt. Formula 8 had a low energy value reaching 401.08 kcal/100 g that may be attributed to its high content of dietary fibre.

TABLE 2. Proximate composition and energy value of raw and pie (on dry weight basis).

Samples	Moisture	Protein	Fat	Crude fiber	Ash	Total carbohydrate	Energy value k cal
Wheat flour	12.56±0.09	11.65±0.06	1.22±0.01	0.46±0.01	0.51±0.02	86.16±0.82	367.27±1.62
Thymus	8.33±0.07	7.9±0.09	8.67±0.08	8.3±0.11	9.6±0.07	65.53±0.52	371.75±2.16
Fenugreek	6.87±0.03	27.20±0.16	7.00±0.03	7.00±0.05	4.28±0.11	54.52±0.42	389.88±2.11
Fennel	7.54±0.05	18.82±0.21	12.26±0.13	10.52±0.09	7.85±0.22	50.55±0.48	387.82±1.84
Anise	6.54±0.02	14.83±0.11	17.89±0.10	22.16±0.15	6.6±0.06	38.52±0.56	374.41±2.23
Formula 1	34.15±0.13	10.56±0.09	26.60±0.12	0.65±0.003	1.12±0.01	61.07±0.13	494.24±1.16
Formula 2	35.56±0.17	13.40±0.15	10.80±0.09	0.75±0.03	1.35±0.02	73.7±0.16	405.40±2.65
Formula 3	34.96±0.11	14.65±0.11	10.65±0.16	0.72±0.01	1.32±0.02	72.66±0.18	401.14±1.22
Formula 4	35.21±0.26	14.02±0.09	10.90±0.11	1.00±0.09	1.35±0.07	72.73±0.22	403.04±2.18
Formula 5	34.77±0.22	13.58±0.03	11.12±0.09	0.66±0.03	1.28±0.05	73.36±0.13	407.10±1.65
Formula 6	35.87±0.33	14.35±0.04	10.52±0.06	0.75±0.01	1.25±0.03	73.13±0.42	401.55±1.73
Formula 7	36.08±0.26	14.53±0.07	10.65±0.03	0.69±0.05	1.26±0.09	72.87±0.25	401.86±2.11
Formula 8	36.28±0.19	14.60±0.09	10.56±0.02	0.75±0.02	1.23±0.03	72.86±0.17	401.08±1.81
Formula 9	36.54±0.13	13.55±0.07	10.82±0.04	0.82±0.03	1.19±0.01	73.62±0.26	405.41±1.17

Volatile constituents of thyme, anise, fennel and fenugreek essential oils

The main components identified by GC and GC/ MS analyses are shown in Table 3. The major components in thyme oil were thymol (22.0%), *cis*–sabinene hydrate (7.85%) and trans–*p*–mentha–3–en–8yl acetate (7.35%), *trans*–anethole and anethole were the predominant volatile components in anise and fennel essential oils (65.94% and 77.43%, respectively). While fenugreek oil contained linoleic acid (44.79%) and hexadecyl acetate (13.19%). Farag *et al.* [1989] reported

TABLE 3. Volatile constituents of thyme, anise, fennel and fenugreek.

Compound [a]	Area %					Method of identification
	KI[b]	Thyme	Anise	Fennel	Fenugreek	MS, KI
α–Pinene	943	2.16	0.42	0.46	nd	MS, KI
Camphene	952	1.58	nd	nd	nd	MS, KI
Myrcene	994	0.28	0.05	nd	nd	MS, KI
Δ–3–Carene	1011	nd	4.28	nd	nd	MS, KI
(E)–2–Heptenal	1041	0.28	nd	nd	nd	MS, KI
cis–Sabinene hydrate	1070	7.85	nd	nd	nd	MS, KI
α–Terpinene	1018	0.96	nd	nd	nd	MS, KI
Phellandrene	1031	0.36	5.12	nd	nd	MS, KI
DL–Limonene	1047	22.75	nd	4.16	4.77	ST, MS, KI
γ–Terpinene	1059	0.18	nd	nd	nd	MS, KI
trans– Sabinene hydrate	1068	0.78	nd	nd	nd	MS, KI
α–Terpinolene	1088	1.2	nd	nd	nd	MS, KI
trans–*P*–Mentha–3–en–8–yl acetate	1140	7.35	nd	nd	nd	MS, KI
α–Terpineol	1185	5.43	nd	nd	nd	ST, MS, KI
Linalool acetate	1257	1.56	nd	nd	nd	MS, KI
cis–Anethole	1269	nd	2.40	nd	nd	MS, KI
trans–Anethole	1283	nd	65.94	0.85	2.12	ST, MS, KI
Anethole	1289	nd	nd	77.43	nd	MS, KI
(E,E)–2,4–Decadienal	1311	9.95	nd	nd	nd	MS, KI
cis–Carvyl acetate	1362	0.29	nd	nd	nd	MS, KI
2–Undecenal	1365	1.45	nd	nd	nd	MS, KI
α–Copaene	1376	nd	nd	1.78	nd	MS, KI
Geranyl acetate	1383	0.77	nd	nd	nd	MS, KI
Cubenene–α	1389	nd	7.06	nd	nd	MS, KI
β–Elemene	1391	nd	nd	1.75	nd	MS, KI
β–Carryophyllene	1418	nd	7.51	0.19	nd	MS, KI
p–Menth–1–en–9–ol acetate	1424	0.15	nd	nd	nd	MS, KI
α–*trans*–Bergamotene	1434	nd	3.51	nd	nd	MS, KI
Aromadendren	1439	nd	nd	1.31	nd	MS, KI
γ–Cadinene	1512	nd	1.1	nd	nd	MS, KI
(–)–Caryophyllene oxide	1573	0.31	nd	nd	nd	MS, KI
Hexadecanoic acid	1984	1.18	nd	nd	nd	MS, KI
Hexadecyl acetate	2009	nd	nd	nd	13.19	MS, KI
Linoleic acid methyl eater	2097	nd	nd	nd	44.79	ST, MS, KI
(Z)–9–Octadecenoic acid	2161	9.30	nd	nd	4.77	MS, KI
Octadecanoic acid ethyl ester	2194	0.12	nd	nd	8.21	MS, KI
Docosane	2200	0.54	nd	nd	3.16	MS, KI
Tetracosan	2400	nd	nd	nd	8.56	MS, KI

a; Values expressed as relative area percentage to total identified components; b; compounds listed according to their elution on DB5 column; nd: not detected; a: Kovat index; c: compounds identified by GC–Ms (MS) and / or Kovat index on DB5 (KI) and / or by comparison of MS and KI of standard compounds run under similar GC/MS conditions.

that thyme (*Thymus vulgaris*) essential oil has 43% thymol and 36% *p*–cymene. Cosentino *et al.* [1999] found among four samples of thyme essential oil, thymol as the major component but with concentrations ranging from 20% to 50%, while the composition of star anise essential oil isolated by the same method was similar as that reported in CuPerinau *et al.* [1999].

A literature search revealed *trans*–anethole (62.0%), fenchone (20.3%), estragole (4.90%) and (D)–limonene (3.15%) to be the main components of essential oils from fennel seed native to the Podgorica region, central south Montenegro [Damjanovic *et al.*, 2005]. Mimica–Dukic *et al.* [2003] also reported *trans*–anethole (74.18%), fenchone (11.32%), estragole (5.29%), (D)–limonene (2.53%) and α–pinene (2.77%) as the major compounds identified in the essential oil from *Foeniculum vulgare* Mill. In turn, Ozcan *et al*. [2006] reported that estragole (61.08% and 40.49%), fenchone (23.46% and 16.90%) and limonene (8.68% and 17.66%), respectively, were the major constituents in the essential oil of bitter fennel (*F. vulgare* spp.) grown in Turkey. Such variations in the chemical composition of essential oil across countries might be attributed to the varied agro–climatic (climatical, seasonal, geographical) conditions of the regions, stage of maturity and adaptive metabolism of plants. In the essential oil of fenugreek, 8 compounds were identified, representing 89.48 % of the total oil. In turn, linoleic acid methyl ester (44.79%), hexadecyl acetate (13.19), tetracosan (8.56%) and octadecanoic acid ethyl ester are the main compounds present in the essential oil fraction followed by D–limonene (4.77%) as reported in previous work [Mebazaa *et al.,* 2009; The Pherobase Database, http:// www.Pherobase.com].

Rheological prosperities of pie dough

Data presented in Table 4 showed the estimated rheological properties of the dough for pie processing. From the table it could be noticed that there were no apparent differences in water absorption, arrival time, dough development time, weakening and mixing tolerance index between all pie dough formulas and control sample. The results of analyses made with extensograph showed that there were no apparent differences in extensibility, resistance to extension and dough energy index between all pie dough formulas and control sample. In general, the obtained results are in agreement with findings of Hassannen *et al*. [1998] and Abdel–Aziem [2007].

Baking quality and freshness of pie

The physical characteristics of the produced pie are presented in Table 5. There were no apparent differences in pie volume and specific volume between all pie dough formulas and control sample. The changes in alkaline water retention capacity (AWRC) for different pie samples stored at room temperature for 0, 3 and 7 days are shown in Table 5. It could be observed that the control sample had the highest value of AWRC, being 305, 297.6, and 290.4% at 0, 3 and 7 days of storage, respectively. However, all pie formulas caused a noticeable decrease in AWRC value at 0, 3 and 7 days of storage compared with the control. The maximum decrease in AWRC value after 7 days storage was observed in formula 7, which may be due to dilution of gluten in all formulas.

Sensory characteristics of pie

Results of the sensory evaluation of pie are shown in Table 6. The obtained results showed that there were no significant differences in mouth feel, texture, break and shred, crust colour, and symmetry shape scores between control pie and all different formulas. Taste and palatability of the produced pie were improved for the formulas (4.5, 7.8 and 9) compared with control. This might be attributed to the higher content of volatile aromatic or essential oils in these blends. Similar findings were also reported by Opawale *et al*. [2011] and Giwa *et al.* [2012]. Generally, it could be observed that the addition of aromatic plants to bakery products affected the highest scores of sensory evaluation [Basuny *et al*., 2012].

Volatile aroma compounds of fortified pie

Major volatiles of different fortified pie are listed in Table 7. They include: twelve Strecker carbonyls, six alcohols,

TABLE 4. Farinograph and extensograph parameters of wheat flour dough as affected by addition of ground fenugreek, thymus, fennel and anise.

Rheological properties	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6	Formula 7	Formula 8	Formula 9
A. Farinograph parameters									
Water absorption (%)	63.5	64.0	64.0	64.1	64.2	64.0	64.1	64.0	64.2
Arrival time (min)	1.5	1.5	1.0	1.0	1.0	1.5	1.5	1.0	1.0
Development time(min)	2.5	3.0	3.0	3.0	3.0	2.5	2.5	3.0	3.0
Stability time(min)	3.0	2.5	2.5	2.5	2.0	2.5	3.0	2.5	3.0
Weakening (BU)	100	90	95	90	95	100	100	90	100
Mixing tolerance index (MTI)	50	40	55	60	60	55	50	40	40
B. Extensograph parameters									
Extensibility (E)(mm)	145	143	140	142	145	142	141	143	145
Resistance to Extension (R)(BU)	270	275	280	278	275	277	279	277	277
Ratio (R/E)	1.9	1.92	2.0	1.96	1.89	1.95	1.98	1.92	1.89
Energy $(Cm)^2$	50	45	48	48	52	47	48	45	52

TABLE 5. Baking quality and freshness properties of produced pie.

Samples	Baking quality			Water retention capacity (Freshness)		
	Weight (g)	Volume (cc)	Specific volume	Zero time	3 days	7 days
Formula 1	120±0.65	370±0.68	3.08±0.03	305.0±0.36	297.6±0.22	290.4±1.16
Formula 2	119.2±0.62	380±0.45	3.18±0.01	295.0±0.35	288.8±0.63	279.3±1.25
Formula 3	120.8±0.13	385±0.38	3.18±0.05	287.0±0.56	278.6±0.85	270.5±0.96
Formula 4	121±0.22	387±0.32	3.19±0.03	280.2±0.59	271.8±1.19	263.6±0.72
Formula 5	122±0.35	390±0.63	3.2±0.01	274.8±075	264.8±1.05	252.8±0.75
Formula 6	125±0.32	375±0.38	3.0±0.07	260.4±0.68	247.6±1.13	238.9±0.32
Formula 7	128±0.18	380±0.95	2.97±0.03	257.6±0.76	248.6±0.62	232.1±0.96
Formula 8	128±0.75	385±0.62	3.01±0.01	302.2±0.13	297.6±0.96	290.4±1.13
Formula 9	130±0.39	385±0.86	2.96±0.03	302.2±0.16	297±1.35	289±1.65

TABLE 6. Sensory characteristics of produced pie.

Samples	Taste (20)	Aroma (20)	Mouth feel (10)	Crumb texture (15)	Crumb colour (10)	Break & Shred (10)	Crust colour (10)	Symmetry shape (5)
Formula 1	16.2^{b}±2.74	18.1^{a}±1.66	8.4±1.17	13.3±0.82	8.6^{a}±0.84	8.4±1.34	8.7±0.82	4.4±0.52
Formula 2	15.8^{b}±2.49	17.4^{a}±1.03	7.9±0.99	13.1±0.74	8.2^{a}±0.63	8.6±0.69	8.0±0.94	3.8±0.63
Formula 3	16.0^{b}±1.49	16.5^{b}±0.88	7.6±0.52	11.9±1.79	7.7^{a}±1.06	8.7±1.34	8.0±1.05	4.2±0.79
Formula 4	17.6^{a}±1.07	17.7^{a}±0.65	8.5±0.53	13.6±0.84	8.5^{a}±0.53	8.6±0.69	8.2±0.79	4.1±0.74
Formula 5	17.2a±0.92	16.5^{b}±1.16	7.9±0.56	13.1±1.10	8.0^{a}±0.82	8.6±0.84	8.5±0.71	4.3±0.82
Formula 6	15.5^{c}±2.95	16.3^{b}±1.03	8.4±1.26	12.1±1.37	6.9^{b}±0.99	8.2±0.92	7.5±0.84	4.1±0.74
Formula 7	17.8^{a}±1.48	15.8^{c}±0.85	7.9±0.99	12.2±1.62	7.2^{b}±0.92	8.2±1.32	8.6±0.84	4.1±0.88
Formula 8	18.2^{a}±1.47	18.0^{b}±0.76	8.0±1.05	12.4±1.96	8.0^{a}±1.33	8.3±1.05	8.6±1.26	4.0±0.82
Formula 9	17.8^{a}±1.48	17.6^{a}±0.96	8.2±1.23	12.9±1.37	7.9^{a}±1.19	8.4±0.84	8.1±0.99	4.3±0.67
LSD at 0.5	1.753	1.47	NS	NS	0.873	NS	NS	NS

Mean values in columns with different letters differ significantly. NS = not significant.

five hydrocarbons, three oxygen–containing compounds, four nitrogen–containing compounds, six acids and four miscellanies compounds. Most of them were identified in bakery products [Mohsen *et al.*, 2009]. The volatiles in bakery products were generated either from Maillard reaction (MR) or lipid degradation [Mottram, 1994].

Table 7 showed the difference in the volatile constituents between different blends. The increased wheat flour and fat levels may indirectly influence the rate of Maillard reaction products where the presence of protein may indirectly influence the rate of MR either by hydrolysis or by deamination of the bound amino acids [Pozo–Bayon *et al*., 2006]. (*E*)–4–Heptenal (31.9%, in F3) was the most abundant aldehyde among others in all pie samples followed by 3–methylbutanal (9.08%, in F4).

The four Strecker aldehydes identified in our study, 2/3–methylbutanal, acetaldehyde and phenyl acetaldehyde (Table 7), may be derived from protein degradation in wheat flour [Mohsen *et al*., 2009]. Other aldehydes such asnonanal, (*E*)–2–nonenal, (*E*)–2–decenal, (*E*)–4–heptenal and 2–undecanal were generated from lipid degradation. The addition of yoghurt caused the incensement of these aldehydes, which might be due to the condensation reaction between amino

TABLE 7. Antioxidant activity of thyme, fennel, anise, fenugreek and fortified pie extracts.

Samples	DPPH (ASA%)	Beta–carotene (EC_{50}, μg/mL)
Thyme	36.33	3.98
Fennel	23.83	7.81
Anise	18.16	9.67
Fenugreek	14.33	11.62
Formula 1	4.13	56.15
Formula 2	16.86	14.58
Formula 3	9.76	19.75
Formula 4	14.6	14.15
Formula 5	12.0	16.87
Formula 6	12.8	16.74
Formula 7	11.7	19.18
Formula 8	8.83	21.12
Formula 9	12.26	12.4
TBHQ	95.46	0.001

ASA, Anti–scavenging activity; EC_{50}, concentration in μg/mL required to inhibit free radicals formation by 50%.

group in flour and added sugar to produce oxygen/ nitrogen––heterocyclic volatiles [Bredie *et al.,* 1998].

2**–**Octanone was identified in all pie samples and its high percentage (2.0%) was assayed in formula 1 (F1), as probably generated due to lipid degradation [Mottram, 1994]. Furan methanol was the predominant oxygen–containing heterocyclic compound especially in F6 with 12.26%, but a dramatic decrease was observed in all pie samples. Furfural, furanone and furanmethanol were derived from sugar degradation or MRPs and characteristic with caramel–like, sweety

TABLE 8. Volatile compounds of control and fortified pie with some aromatic plants.

Compounds	Area %										ID [b]
	RI [a]	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6	Formula 7	Formula 8	Formula 9	
2–Methylbutanal	632	0.3	0.05	0.28	–	0.35	0.9	0.4	0.23	–	KI,MS
3–Methylbutanal	648	1.4	2	1.5	9.08	2.43	2.1	1.16	2.41	3.21	KI,MS
Acetaldehyde	703	1.06	0.24	0.22	1.63	–	0.4	1.4	0.63	0.65	KI,MS
(Z)–2–pentenol	758	1.0	0.8	0.8	–	3.26	0.2	1.8	2.22	–	KI,MS
Octane	779	0.4	0.36	0.2	3.61	1.66	0.6	0.8	3.54	0.92	KI,MS
α–Pinene	918	–	1.22	–	–	–	–	–	–	–	KI,MS
Benzaldehyde	957	0.3	0.13	0.09	0.22	–	0.4	0.6	0.8	0.58	KI,MS
Phenylacetaldehyde	1035	0.3	0.34	nd	3.29	0.44	1.03	0.99	0.48	0.36	KI,MS
Nonanal	1097	0.2	0.22	1.85	4.4	2.97	2.74	2.06	0.9	0.42	KI,MS&ST
(E)–2–nonenal	1157	1.2	0.3	1.4	0.85	0.14	0.3	1.0	0.6	1.0	KI,MS
Dodecane	1195	0.2	0.65	0.6	0.51	0.56	0.17	0.2	0.55	0.56	KI,MS
(E)–4–Heptenal	1220	1.45	12.03	31.90	24.1	20.28	20.11	14.44	30.08	30.4	KI,MS&ST
(E)–2–Decenal	1268	0.2	1.13	–	–	1.1	1.13	0.8	–	1.4	KI,MS
2–Octanone	1270	2.0	1.3	1.13	0.74	0.42	0.4	1.26	1.55	0.71	KI,MS
Thymol	1280	–	1.27	–	–	–	0.5	–	–	0.1	KI,MS&ST
Trans–anethole	1283	–	–	–	–	2.1	–	–	0.7	0.1	KI,MS&ST
Anethole	1289	–	–	–	1.5	–	0.3	0.27	–	0.11	KI,MS&ST
(E,E)–2,4–Decadienal	1317	0.4	54.9	–	20.3	25.83	33.38	23.05	28.01	34.17	KI,MS&ST
2–Undecanal	1360	1.2	2.1	5.19	1.59	1.2	1.18	1.3	1.47	1.2	KI,MS
Trimethylpyrazine	1393	2.29	0.8	0.71	0.21	0.5	0.15	0.7	–	1.07	KI,MS
(Z)–2–Hexenol	1402	1.2	0.37	0.46	0.25	0.35	0.2	0.31	0.24	3.8	KI,MS
2–ethyl–2,5–dimethylpyrazine	1438	0.6	0.4	0.87	0.56	0.36	0.3	0.61	0.23	0.19	KI,MS&ST
Furfural	1470	0.2	0.48	0.55	0.29	0.36	0.63	0.69	0.85	0.13	KI,MS
Pyrrole	1516	0.81	0.27	5.2	0.43	0.2	1.05	0.48	0.67	0.91	KI,MS
1–Octanol	1561	0.4	0.11	0.43	0.52	0.5	0.11	0.3	0.25	0.14	KI,MS
Furanmethanol	1688	6.0	2.38	10.36	3.06	0.12	9.68	12.26	8.01	8.48	KI,MS
N–acetyl–4–(H)–pyridine	1730	0.12	0.11	2.19	0.2	–	1.59	0.9	0.73	1.66	KI,MS
2–(5H)–Furanone	1769	0.28	0.13	0.18	0.29	0.36	0.27	0.5	0.15	0.42	KI,MS
Hexanoic acid	1862	3.49	0.62	0.85	0.95	2.59	0.7	4.82	2.14	0.13	KI,MS
Benzyl alcohol	1886	0.2	0.63	0.3	0.12	0.24	0.4	0.2	0.4	0.1	KI,MS
Phenyl ethanol	1931	0.81	0.29	0.67	0.8	0.4	0.6	0.3	0.6	0.3	KI,MS
Hexadecanoic acid	1984	25.4	3.3	2.07	11.6	13.09	1.96	2.32	0.73	2.50	KI,MS
Octanoic acid	2052	8.16	3.6	2.11	1.0	–	2.22	–	0.62	–	KI,MS
Linoleic acid	2097	–	–	2.1	–	–	–	1.2	1.0	0.4	KI,MS
Tetradecanol	2116	3.19	0.52	1.74	0.58	0.23	3.38	0.83	2.33	0.38	KI,MS
9–Octadecanoic acid	2164	1.92	0.73	1.7	0.49	11.14	0.19	30.14	0.19	0.2	KI,MS
Octadecanoic acid methyl ester	2194	21.9	0.11	0.62	0.6	0.88	0.19	3.30	1.0	0.58	KI,MS

a; Retention indices calculated from GC results on DB5 column; b; identification method; RI, identification by comparison with published GC retention index; MS, identification by comparison with published MS spectra in NIST library & (http:// WWW.Pherobase.com; http:// WWW. flavornet.org); STD, identification supported by co–injection of standard compounds and Formulas from (1–9) see Table 1.

and fruity odour [Flament, 1991]. These results confirm those of the sensory evaluation (Table 6).

Two pyrazines as trimethyl pyrazine and 2–ethyl–2,5–dimethylpyrazine were identified in pie samples (Table 7). Pyrazines have nutty, roasted flavour in thermally–treated cereal products [Bredie *et al*., 2002]. It is clear that the added fat in control sample gave rise to a remarkable increase in pyrazine percentage (Table 7). The incensement of pyrazine content led to the development of toasted/ roasted odour in bakery products *via* thermal treatment [Bredie *et al*., 1998]. Pyrrole and *N*–acetyl–4–(*H*)–pyridine were also generated in all samples in our study as MRPs [Whitfield, 1992].

Acids were generated in pie samples as hexanoic acid, hexadecanoic acid, octanoic acid and 9–octadecanoic acid methyl ester. The generation of fatty acids and ester in pie samples (Table 7) may be attributed to lipids. The fortification of pie samples with aromatic plants influenced the overall volatiles profiles of pie and revealed considerable volatiles related to those herbal plants and their ratio.

Antioxidant activity assays

Two spectrophotometric antioxidant assays were applied in our study to evaluate the AOA namely, DPPH and β–carotene. Table 8 shows the radical scavenging capacities of the aromatic plants and fortified pie extracts. The extracts analysed inhibited varying degrees of scavenging capacities. Thyme extract showed the strongest ($P<0.05$) antiradical scavenging activity (ASA) with 36.33%, which is lower than that observed for the positive control, TBHQ (95.46%). The addition of aromatic plants as flavouring additive to pie samples increased their ASA in comparison with control sample (F1) which had the lowest ASA (4.13%). Lee & Shibamoto [2002] reported that thyme oil and its extract were able to reduce the stable DPPH radical and demonstrated that the compound most responsible for this activity was the oxygenated monoterpene (thymol) which has steric hindrance of the phenolic group and possesses high antioxidant activity [Viuda–Martos *et al*., 2011].

Also, the addition of fennel, anise and fenugreek showed significant increase in their ASA in comparison with control pie (14.6, 12.0 and 9.76, respectively, Table 8). The presence of aromatic plants in pie formula made a synergetic effect in their EC_{50} in β–carotene–linoleate assay, both thyme and fortified pie extracts were recorded to have the highest EC_{50} (concentration in μg/mL required to inhibit free radicals formation by 50%) reaching 3.98 and 14.58 mg/mL, respectively. While, TBHQ was recorded at 0.001 mg/mL. As mentioned above, the control had a lower EC_{50} (56.15 mg/mL, Table 8).

Effect of aromatic plants on the microbial load of the pie

Aromatic plants and herbs have been safely used since ancient times as food flavouring agents and also as herbal medicines. Recently there has been considerable emphasis on studies involving effects of aromatic plants and extracts of herbs on inhibiting the growth of microbes [Ozcan *et al.*, 2006]. Spoilage of bakery products is caused mainly by moulds and yeasts and occasionally by bacteria such as the rope causing heat–resistant endospore–forming *Bacillus subtilis* [Ozcan *et al.*, 2006].

Mould and yeast and total bacterial counts (TBC) were used to evaluate the microbiological quality of pie products. Figures 1 and 2 indicate that the total bacterial count (TBC) and total yeasts/moulds counts of control pie at zero time were 2.22 and 1.51 log cfu/g, respectively. Latou [2010] reported the yeasts and moulds counts of sliced wheat bread were 2.0 log cfu/g. Also, the survival of TBC, mould and yeast encountered in pie samples was investigated in the presence of aromatic plants and their mixture (thyme, fennel, fenugreek and anise). Generally, the aromatic plants caused a significant *($P<0.05$)* decrease in all microbial counts under investigation. The inhibition effect of the aromatic plants in pie products may be attributed to the antimicrobial activity of phenolic, flavonide and flavouring constituents that are present in thyme, anise, fennel and fenugreek.

Nielsen & Rios [2000] reported that cinnamon, garlic, and clove were potential inhibitors of microorganisms as active packaging in rye bread. In the present study at zero time, there was almost no difference between treatments, the control samples were rejected in the ninth day where the TBC reached to log 7 cfu/g, which is the acceptable limit as defined by ICMFS [1986]. In our results, the shelf–life of fortified pie products was extended to more than 12 days. Plessas *et al.* [2005] reported mould spoilage in baker's yeast–leavened bread after 3 days of storage at 25°C.

In bakery products, fungi are the most common spoilers. In unpreserved bread a shelf–life of 3–4 days may be expected especially if the hygiene in the factory is not sufficiently high [Lund *et al.,* 1996]. Microbiological spoilage of pie samples was delayed by addition of aromatic plants and reached more than 12 days compared to 9 days for the control group (Figures 1 and 2).

Our study revealed that the synergistic effects between aromatic plants when added together to pie samples were more effective than each treatment alone against yeasts and mould and TBC. Figures 1 and 2 showed that the combinations between aromatic plants were the most effective inhibitors followed by two mix and aromatic plants alone had a lesser effect. Synergistic effects were reported by Abdel–El-Kalek [2008] and Hussein *et al.* [2011b]. They reported that the combinations of spices extracts and herb tea infusions (berry leaves, dom and kharoub extracts) showed a greater inhibitory effect towards tested bacteria and fungi than that of individual extract.

Effect of thyme, anise, fennel and fenugreek on growth of the inculcated moulds in pie product

Moulds spoilage is a serious and costly problem for the bakery industry [Suhr & Nielsen, 2004]. Contaminants of bakery products are mainly *Penicillium* species (90–100%), and *Aspergillus* species also occur [Legan &Voysey, 1991].

Our study was conducted using important pathogens and spoilage fungi frequently found in bakery products. The survival of test cultures inoculated with *Aspergillus niger* and *Penicillium verrucosum*) into sterile pie containing thyme, anise, fennel and fenugreek was studied. There was a pronounced decrease in the fungi cell numbers in all fortified pie products. Figures 3 and 4 showed a decrease in log count of *Aspergillus niger* and *Penicillium verrucosum* by al-

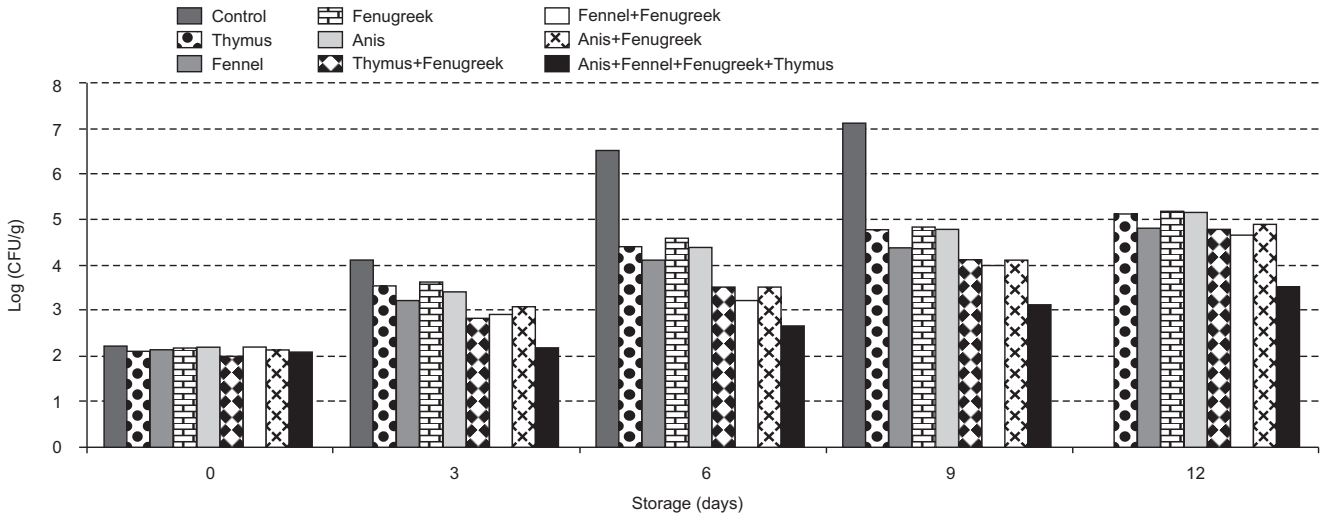

FIGURE 1. Effect of some spices and their mixtures on the total bacterial counts in pie product.

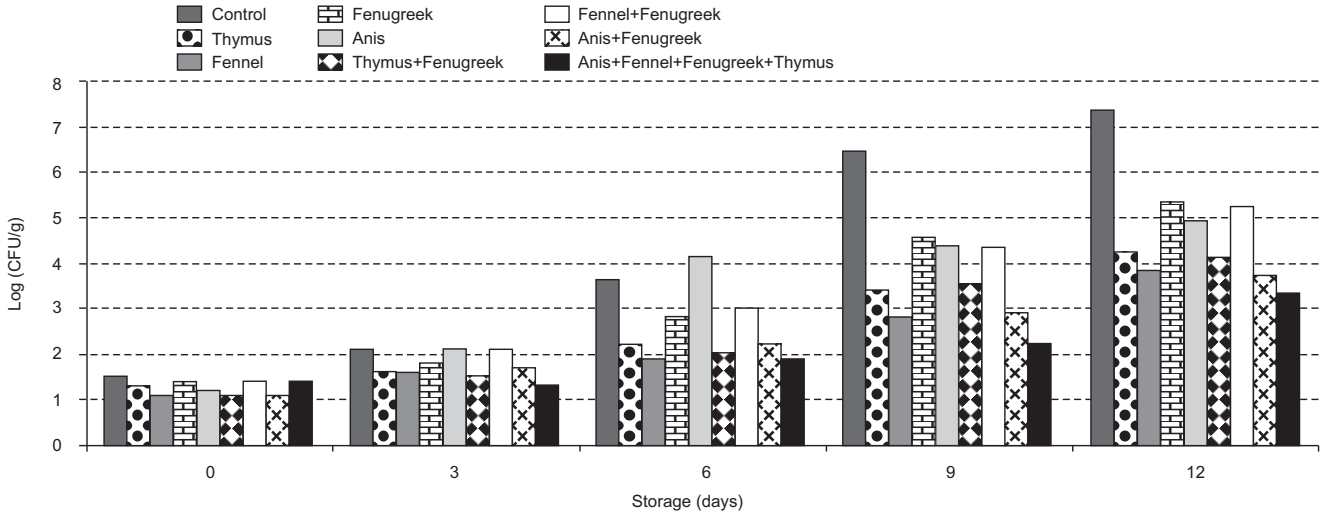

FIGURE 2. Effect of some spices and their mixtures on the total moulds and yeasts counts in pie product.

most 1.5–3.5 and 1–5 log cycles, respectively compared with the control during 12 days of storage at 25°C in pie fortified with aromatic plants.

CONCLUSION

A successful and novel formulation of pie with aromatic plants and yogurt was developed. Aromatic plants are good sources of antioxidants and antimicrobial activities. Pie formulated with partial replacement of fat with 20% yogurt had a healthy, pleasant flavour and nutritional value compared with the control sample. No significant differences were found in rheological, technological and baking quality of pie in addition to improvement in its nutritional values and sensory quality. Overall, aromatic plants could be incorporated into pie to ensure more functional prosperities and more effective antioxidant characters.

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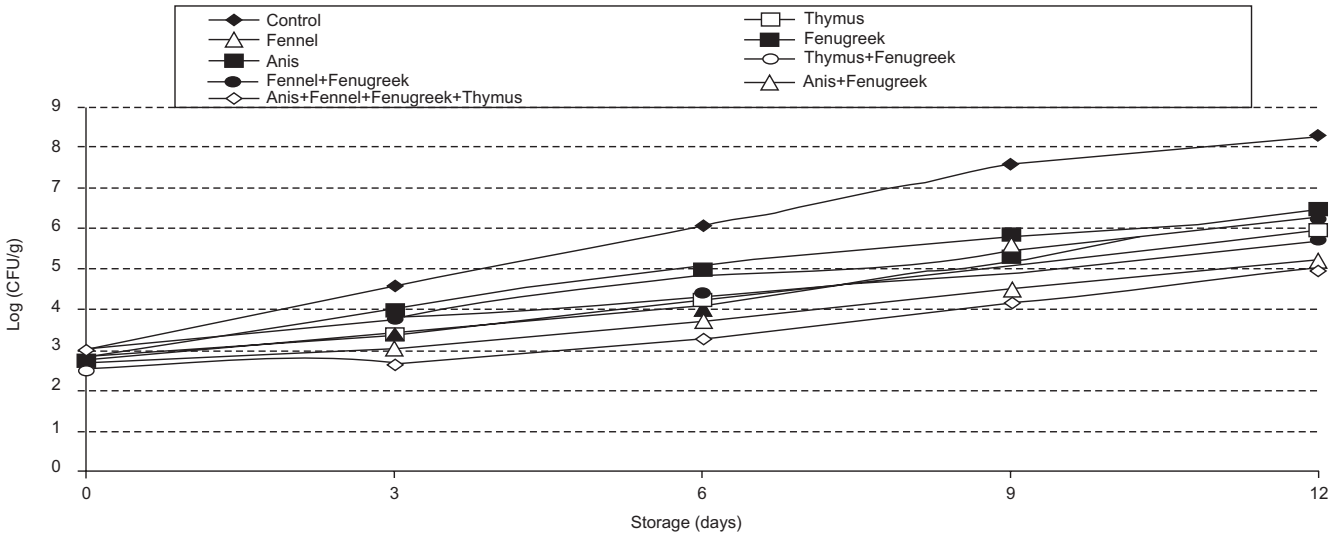

FIGURE 3. Growth of *Aspergillus niger* in pie at 25°C in the presence of some spices and those mixtures.

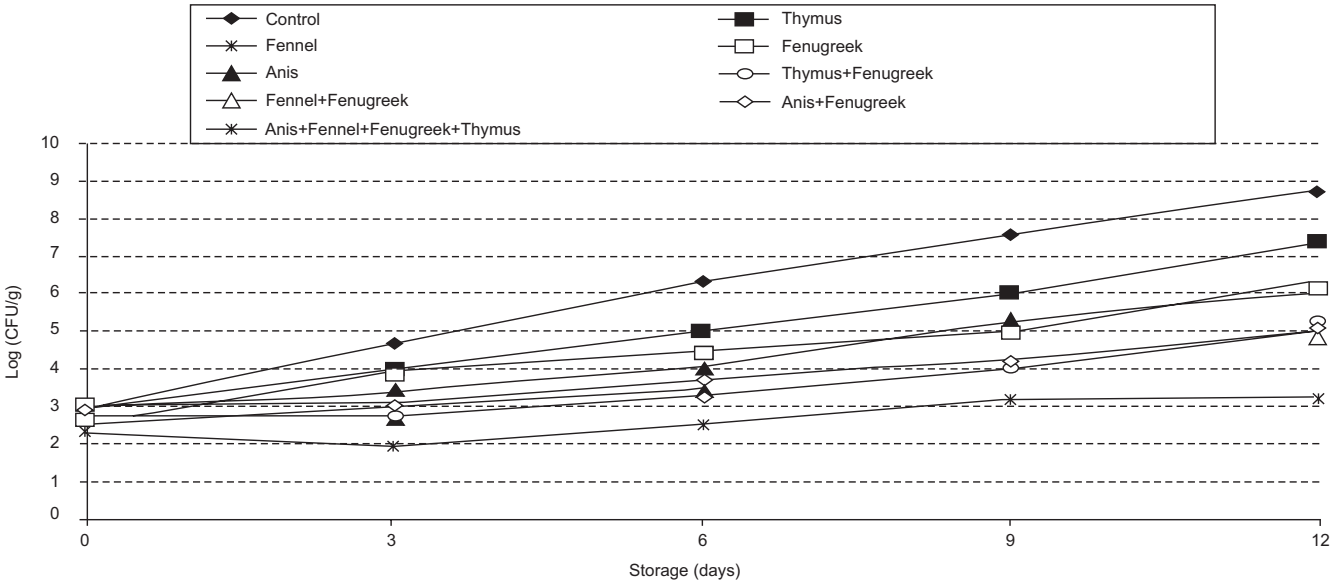

FIGURE 4. Growth of *Penicillium verrucosum* in pie at 25°C in the presence of some spices and those mixtures.

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