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Application of Natural Extracts in Beef Meatballs to Prevent Chemical and Bacteriological Spoilage Agents, and Extend its Storage Life

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Abstract The present work was carried out to study the antioxidant activity of natural products (onion skin, potato peel, marjoram, fennel, cinnamon, black seed and olive leaf) in relation to their content of total phenolic compounds. Also, their aqueous extracts will be applied in meatballs to prevent chemical and bacteriological spoilage agents and extend its storage life within the scope of this investigation. Such data indicated that the natural products extract showed considerable differences in antioxidant activity (AA= 61.83 to 92.75%) and total phenolics content (297.94 to 1207.32 mg of GAE.$100g^{-1}$). When all extracts were included in the statistical analysis, there was a positive [Total phenolics (mg/100g, d.b.)= 23.083 (Antioxidant activity, %) –1082.4, r^2= 0.6306] and significant ($p\leq 0.01$) relationship between total phenolics and antioxidant activity. Activity in a lamb fat system was established for all the extracts and further determination of the development of rancidity as malonaldehyde consistently showed that more that 50% of the rancidity can be controlled by the onion skin, potato peel, cinnamon, black seeds preparations after a period of 12 days of storage at 4°C. The same behavior was recorded for the protein quality parameter, total volatile-base nitrogen (TVBN). Also, such extracts activity against lactic acid bacteria was demonstrated in an agar diffusion test in the product and its counts were significantly ($p<0.05$) reduced. Sensory analysis results, particularly acceptability scores, indicated the significant advantages in using all extracts in rancidity-susceptible meat products. Furthermore, the use of mixtures of these extracts gave more effective results compared to the use of extracts in a single way, which proves the interactional effects of the different groups of bioactive compounds contained in those extracts in the case of mixing. Finally, the results of the study are an important step for using these natural extracts in the food industry as a substitute for synthetic antioxidants, especially after suspicions have been raised about the harmful and devastating effects of these chemically synthesized compounds on public health.

Keywords: *plant parts, malonaldehyde, total-base volatile nitrogen, lactic acid bacteria, acceptability scores*

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1. Introduction

Meat is considered an important and excellent source of protein of high biological value. Its food value depends on its large proportion of high grade proteins with all the non-convertible amino acid essential for health. The other nutrients in meat, minerals and vitamins are like- wise important. Although meat is relatively high cost compared with other sources of protein it is considered the main diet for many world people for its high nutritive value. Meat is a perishable product and during storage, the actions of microorganisms and endogenous enzymes result in chemical compositional changes. One of the most important of these chemical compositional changes is the degradation of proteins and other nitrogen (N)-containing compounds as a result of the spoilage mechanisms mentioned above cause accumulation of organic amines that are commonly known as total volatile basic nitrogen (TVB-N) [1]. These compounds are toxic and cause considerable colour and flavour changes [2]. that affect the acceptability of meat products. The TVB-N content increases with storage time of meat and often its accumulation pattern somewhat parallels other biomarkers of spoilage, such as microbial count and changes in sensory acceptability. Otherwise, lipids of meat become rancid as a consequence of oxidation and oxidative rancidity takes place by microorganisms [3,4]. Recently, interest in the effect of malonaldehyde, one of the major products of the oxidation of polyunsaturated fatty acids, on human health has been reported by many authors that is mutagenic and carcinogenic [5,6,7]. Also, Several studies reviewed that O_2^- can initiate lipid peroxidation, leading to the formation of prooxidant substances capable of reacting with oxymyoglobin (OMb) and resulting in metmyogobin (MMb) formation [8,9]. The susceptibility of myoglobin to autoxidation is the main factor in explaining colour stability in meat and meat products [10].

Although synthetic additives have been widely used in the meat industry to inhibit the process of lipid oxidation, protein degradation and microbial growth, the trend is to decrease their use because of the growing concern among consumers about such chemical additives [11,12]. Consequently, search for natural additives, especially of plant origin, has notably increased in recent years [13]. Extensively studied sources of such natural compounds are plant parts i.e. fruits, vegetables, seeds, cereals and aromatic plants. Attempts are also made to identify and evaluate these bioactive compounds (natural antioxidants) in agricultural by-products that have nutritional importance and/or the potential for applications in food preservation. Amongst of these agricultural by-products, onion skin and potato peel are producing in large quantities in food-processing plants. The major by-products resulting from industrial peeling of onion (*Allium cepa* L.) bulbs are brown skin, the outer two fleshy leaves and the top and bottom bulbs. The outer dry layers of onion bulbs (Onion skin, OS), which are not edible and removed before processing, have been shown to contain a wide spectrum of polyphenolic components [14]. Also, it is a source of flavour components and fiber compounds and particularly rich in flavonoids including quercetin glycosides [15,16]. Since quercetin from onions and their skins is rapidly absorbed and slowly eliminated, it could contribute significantly to antioxidant defense system [17]. Also, several studies have been established for using the onion skin in different food processing applications include pectin production, pigments extraction, natural antioxidants and anticarcinogenic, vingare production, biogas productions, natural fertilizers, protection of ultraviolet adverse effects etc. [18-23]. Potato is the largest vegetable crop worldwide, amounting to approximately 320 million metric tons annually [18]. Producing of potato (*Solanum tuberosum*, L.) products mainly chips, French fries, and powder has presented a steady increase during the last decades, exceeding considerably the amount of the vegetable consumed as fresh. Solid waste generated during processing consists mostly of potato peels and amounts to 10% depending on the procedure applied. Some investigations suggested the use of water extracts from potato processing waste for the recovery of antioxidants [24]. Others reported that potato skin due to containing compounds mainly phenolics with highly antioxidant properties, can play potential roles in several food technology application [25-27]. Marjoram, (*Origanum marjorana* L., Family *Labiatae* is a perennial herb, cultivated around the world [28].Quantitative composition of the essential oil, volatile compounds, bioactive compounds and the antioxidant activity of marjoram were reported by Komaitis *et al.*, [29]; Baser *et al*., [30]; Novak *et al*., [31] and El-Safty, [32]. Fennel (*Foeniculum vulgare*) is a plant species in the genus *Foeniculum* and a member of the family *Apiaceae* (formerly the *Umbelliferae*). It is a widely distributed plant in most tropical and subtropical countries and has long been used in folk medicines to treat obstruction of the liver, spleen and gall bladder and for digestive complaints such as colic, indigestion, nausea and flatulence. In recent years the interest in this plant has increased considerably with substantial progress on its chemical and pharmacological properties [33]. Several compounds including *trans*-anethole, estragole, fenchone and polyphenolics were isolated from this plant and some of these interact with potential mechanisms of the body including antioxidant, antimicrobial and inhibition of lipid peroxidation Badgujar et al., [34]. Black seed *(Nigella sativa*) seed is variously called fennel flower, nutmeg flower, Roman coriander, black seed or black caraway. It is used as part of the spice mixture in food processing, most recognizably in bread. Several studies also indicated that black seed contains different classes of bioactive compounds so used as antioxidants, immunostimulant and antibacterial drugs [35]. Cinnamon (*Cinnamomun verum*) is a famous beverage a long history and is one of the world's most . It was imported to Egypt from China as early as 2000 BC. Cinnamon is principally employed in cookery as a and flavouring material, being largely used in the preparation of some desserts, chocolate, spicy candies and liqueurs, and meat products [36]. It contains condensed tanins, oil, coumarins, cinnamaldehyde and flavonoids which have been shown potent antioxidant and anticarcinogenic activities [37,38]. Extracts of cinnamon have also been shown to have antioxidant effects in part through activating antioxidant enzymes, prevent free radical formation, remove radicals before damage can occur, repair oxidative damage, eliminate damaged molecules, inhibit lipid peroxidation [39-42] Olive leaves have known as a symbol of Mediterranean olive tree (*Olea europaea*). Large a mounts of leaves are principally generated during pruning of the trees and harvesting and working of the olives [43]. The olive leaf rich in polyphenols and flavonoids composition which are similar to that of olive oil [43-45]. The olive leave extracts was shown to have an antioxidant capacity 400% higher than vitamin C and almost double that of green tea or grape seed extract [46].

For all the above reasons, the present work was carried out to study the antioxidant activity of natural products (onion skin, potato peel, marjoram, fennel, cinnamon, black seed and olive leaf) in relation to their content of total phenolic compounds. Also, such natural products will be applied in meat products to prevent chemical and bacteriological spoilage agents and extend their storage life within the scope of this investigation.

2. Materials and Methods

2.1. Materials

2.1.1. Plant Parts

Onion skin (*Allium cepa* L.) were obtained as a donation from New Bani Suef Company for Prsevation, Dehydration and Industratzation of Vegetables, Bani Suef El-Goudida City, Nile east, Bani Suef, Egypt. Potato Peel (*Solanum tuberosum*, L.) fruits and basil (*Ocimum basilicum*) were purchased from Benha City markets, Al Qalyubia Governorate, Egypt, washed and peeled by using sharp stainless steel knife for peel obtaining. Marjoram (*Origanum marjorana* L.), Fennel (*Foeniculum vulgare*), Black seed (*Nigella sativa*) and Cinnamon (*Cinnamomun verum*) were purchased from The Company of Agrcultural Seeds, Perfumery and medical Plants (Harraz), Ahmed Maher St ‹El-Darb El-Ahmar, Cairo, Egypt. Olive leaves (*Olea europaea*) were obtained by special arrangements with some Olive Farms, Sadat City, Minoufiya Governorate, Egypt.

2.1.2. Chemicals

Chemicals and solvents (Except as otherwise stated), were of analytical grade were purchased from El-Ghomhorya Company for Trading Drug, Chemicals and Medical Instruments, Cairo, Egypt.

2.1.3. Meat Samples

Rose meat samples were obtained from the Egyptian local markets, Benha City, Al Qalyubia Governorate, Egypt.

2.2. Methods

2.2.1. Plant Parts Extract Preparation

All the selected plant parts (Figure 1), were washed and dried in oven (BIOBASE, BOV-T125F, China) at 60°C until arriving by the moisture in the final product around 10%. The dried samples were ground into a fine powder and the material that passed through an 80 mesh sieve was used in extract preparation according to Elhassaneen and Esa, [47].

2.2.2. Meatball Manufacture

2.2.2.1. Product Formulation

Meatball samples were manufactured according to Fernandez-Lopez *et al.*, [48]. A set of five treatment samples differing only by the plant parts extracts added were prepared as follow: minced meat (control samples), minced meat + 0.25% (w/w) black seeds extract (BSE), minced meat + 0.25% (w/w) basil (BE), minced meat + 0.25% (w/w) cinnamon (CE), minced meat + 0.25% (w/w) onion skin extract (OSE), minced meat + 0.25% (w/w) potato peel extract (PPE), minced meat + 0.25% (w/w) olive leaves extract (OLE), minced meat + 0.25% (w/w) fennel extract (FE), minced meat + 0.25% (w/w) marjoram extract (ME), minced meat + 0.25% (w/w) mixture BSE+ ME+ OSE+ BE by equal parts (Mix 1), and minced meat + 0.25% (w/w) mixture CE+ PPE+ FE+ OLE by equal parts (Mix 2). Plant parts extracts were used at the concentrations suggested by Elhassaneen and Esa, [47] and Essa, [49].

Onion skin (*Allium cepa* L.)   Potato Peel (*Solanum tuberosum*, L.)   Marjoram (*Origanum marjorana* L.)

Fennel (*Foeniculum vulgare*)   Black seed (*Nigella sativa*)   Cinnamon (*Cinnamomun verum*)

Basil (*Ocimum basilicum*)   Olive leaves (*Olea europaea*)

Figure 1. Plant parts used in the preparation of natural extracts

2.2.2.2. Product Processing

The products were prepared in a pilot plant resembling to commercial processing conditions such as described in Elhassaneen and Esa, [47] and Essa, [49].

2.2.3. Phytochemicals and Biological Assays

Antioxidant activity (AA) and β-carotene bleaching (BCB) assay of the extract samples and standards (α-tocopherol and BHT) was determined and calculated according to the procedures described by Marco, [50], Al-Saikhan et al., [51], Marinova et al., [52] and Mallet et al., [53]. Total phenolics in selected extracts were determined using Folin-Ciocalteu reagent [54].

2.2.4. Biochemical/Quality Indices Assays

The total volatile base-nitrogen (TVB-N.) was extracted using the method of Winton and Winton [55] such as follow: 50 g of ground meatball sample were mixed with one liter of distilled water and left to stand for 24 h at 4°C. The sample was then vigorously shaked and filtered through cheese cloth. Then, the resulted filtrate was used for determination of the TVB-N. as follow: 400 ml at the previous filtrate were put into a one liter of distillation Erlenmeyer flask, plus 30 ml of ethanol and 2 gm of Mg 0. The distillate was collected in 25 ml at 0.1N $H_2 SO_4$. After the distillation was completed, the distillate was boiled for 10 - 15 min to remove the CO_2. The distillate was cooled to room temperature, 0.2 ml of resolic acid indicator (0.2%) was added and the excess of H_2SO_4 was immediately titrated with NaOH, 0.1 N. Results were presented as mg total volatile-basic nitrogen /100 g sample according to the following equation: TVN (mg / 100 g sample) =[(V_1xN_1- V_2xN_2) x 0.014 x 1000/400 x W]x100. Where: V_1 = volume of H_2SO_4 by ml, V_2 = volume of NaOH by ml, N_1 = normal concentration of H_2SO_4, N_2 = normal concentration of NaOH and W = weight of sample. On the other side, lipid oxidation was assessed as malonaldehyde content in triplicate by the 2-thiobarbituric acid (TBA) method of Tarladgis *et al.*, [56].

2.2.5. Microbiological Analysis (Lactic Acid Bacteria, LAB)

LAB counts were determined on meet samples, control and treatments, following the standard methodologies of Gerhardt et al., [57]. A Culture media were from Oxoid (Oxoid Unipath Ltd., Basingtoke, Hampshire, UK).

2.2.6. Sensory Evaluation

Sensory evaluation for the cooked meatballs samples was carried out such as reviewed in Fernandez-Lopez et al., [48] by a trained ten member panel.

2.3. Statistical Analysis

All assays were preformad in triplicates and presented as mean ± standard deviations (SD). Statistical analysis for the data was achieved by using the Student *t*-test through MINITAB program (Minitab Inc., State College, PA).

3. Results and Discussion

3.1. Antioxidant Activities and Total Phenolics in the Selected Natural Extracts

The antioxidant activities and total phenolics of eight natural aqueous extract and their mixture are shown in Table (1). Such data indicated that the natural extracts showed considerable differences in antioxidant activity (AA = 61.83 – 92.75%) when it was calculated by the four different methods used in this study (Table 1). Black seeds, cinnamon, onion skin, potato peel and marjoram showed strong activities (AA= 92.75, 85.01, 84.88, 83.05 and 86.29%, respectively) because of their high phenolic content (783.78, 1207.32, 1029.41, 986.93 and 879.51 mg GAE/100 g, respectively). Basil, olive leaves and fennel extracts showed medium ratios of antioxidant activity (AA= 68.54, 61.83 and 64.82 %, respectively). On the other side, mixing of some such natural products extract by equal quantities [Mix-1 (black seeds, marjoram, onion skin and basil), Mix-2 (cinnamon, potato peel**,** fennel and olive leaves)] exhibited the highest antioxidant activities which recorded AA= 93.21 and 91.67, respectively. Many studies indicated that the levels of phenolic compounds vary dramatically, especially as influenced by factors such as variety, germination, ripening, storage, processing and extraction technique [19,23,32,58].

On the other, the antioxidant activity of selected natural aqueous extracts and their mixture as well as well-known antioxidants used as standards was assayed by β-carotene bleaching (BCB) method. BCB method is based on the coupled oxidation of β-carotene and linoleic acid which estimates the relative ability of antioxidants to scavenge the radical of linoleic acid peroxide (LOO•) that oxidizes/lost the double bonds of β-carotene such as mentioned by Marco [50]. The decrease in absorbance of β-carotene in the presence of different aqueous extracts of the selected natural products as well as antioxidants used as standards, BHT and α-tocopherol with the oxidation of β-carotene and linoleic acid is shown in Figure (2). Such data indicated that almost of the natural products extract (black seeds, cinnamon, onion skin, potato peel and marjoram) and the mixtures recorded the lowest decreasing in absorbance. The values of the these extracts absorbance through 120 min are coming well i.e. closing the line of 50 mg α-tocopherol, and 50-100 mg /L of butyhydroxytoluene (BHT). Such data proved the high stability of those natural products extract when comparing with that well knowing standards, α-tocopherol and BHT. Also, the rest of the extracts (basil, fennel and olive leaves) exhibited relatively medium absorbance/stability when comparing with that standard antioxidants, α-tocopherol and BHT.

Such data are accordance with that observed by several studies. For example, black seeds analysis indicated that huge bioactive compounds were found including polyphenoles, essential oils and steroids which exhibited strong antioxidant capacities [35]. Onion skin was rich in several bioactive compounds including phenolics which exhibited antioxidant and free radical scavenging activities [14,59]. Potato peel contains polyphenols and phenolic acids possess antioxidant activity in both *in vitro* and in vivo studies [23,60-63]. Cinnamon has seven phenolic acids (tannic, gallic, caffeic, cinnamic, chlorogenic, ferulic and vanillic acids) and polyphenols which have been shown to have antioxidant effects. A total of 45 compounds were identified in essential oils of marjoram and the most prominent component was 4-terpineol, carvacrol, linalool, monoterpene alcohols beside phenolic compounds which observed high antioxidant activity [29,32,64]. Several compounds including *trans*-anethole, estragole, fenchone and polyphenolics were isolated from fennel which interact with potential mechanisms of the body including antioxidants [33]. Olive leaves contains several polyphenols (Oleuropein and other secoiridoids), flavones, luteolin-7-glucoside, apigenin-7-glucoside and phenolic acids (vanillic acid and caffeic acid)with several biological activities including antioxidant activity [44,45,65]. The antioxidant activities and total phenolics of 31 plant products, including vegetables, fruits and essential products in commonly distributed in Egypt were determined. The total phenolic content of selected products varied from 89 to 3701 mg GAE/100 g. Antioxidant activity (AA,%) of methanolic extract was determined ranged 31.56 to 92.63 [19].

Table 1. Antioxidant activity and total phenolics of selected natural aqueous extract and their mixture

Extracts		Antioxidant value [a] AOX (A/h)		Antioxidant activity [b] AA (%)		Oxidation rate ratio [c] (ORR)		Antioxidant activity coefficient [d] (AAC)		Total phenolics (mg of GAE.100g^{-1})	
Black seeds	BSE	0.018 ±	0.02	92.75±	1.85	0.069 ±	0.01	1157.04 ±	30.59	783.78 ±	66.70
Basil	ME	0.155 ±	0.026	68.54 ±	6.15	0.111 ±	0.03	736.16 ±	16.14	423.55 ±	36.54
Cinnamon	CE	0.062 ±	0.029	85.01 ±	3.14	0.120 ±	0.01	1022.48 ±	26.16	1207.32 ±	39.04
Onion skin	OSE	0.062 ±	0.02	84.88 ±	4.33	0.169 ±	0.01	1020.22 ±	36.45	1029.41 ±	41.22
Potato peel	PPE	0.073 ±	0.018	83.05 ±	4.47	0.205 ±	0.04	988.41 ±	40.10	986.93 ±	15.01
Olive leaves	BE	0.193 ±	0.026	61.83 ±	2.27	0.289 ±	0.03	619.51 ±	36.41	297.94 ±	17.26
Fennel	FE	0.176 ±	0.03	64.82 ±	2.07	0.326 ±	0.02	671.49±	58.57	308.63 ±	12.93
Marjoram	OLE	0.054 ±	0.018	86.29 ±	0.00	0.390 ±	0.08	1044.73 ±	36.44	879.51 ±	23.80
Mix-1	Mix (BSE+ ME+ OSE+ BE)	0.015 ±	0.01	93.21 ±	7.49	0.145	0.01	1165.04 ±	51.82	813.97 ±	20.92
Mix-2	Mix (CE+ PPE+ FE+ OLE)	0.024 ±	0.006	91.67 ±	5.59	0.196 ±	0.01	1138.26 ±	40.88	735.08 ±	21.92
Control		0.565 ±	0.031	0.00 ±	0.00	0.998 ±	0.07	0.00 ±	0.00		
BHT, 50 mg/L		0.084 ±	0.014	85.11 ±	1.16	0.148 ±	0.01	953.45 ±	28.68		
BHT, 200 mg/L		0.012 ±	0.002	97.89 ±	1.21	0.019 ±	0.01	1175.63 ±	39.66		
α-tocopherol, 50 mg/L		0.006 ±	0.002	98.91 ±	0.95	0.013 ±	0.01	1193.36 ±	26.31		

Each value represents mean of three replicates ±SD.

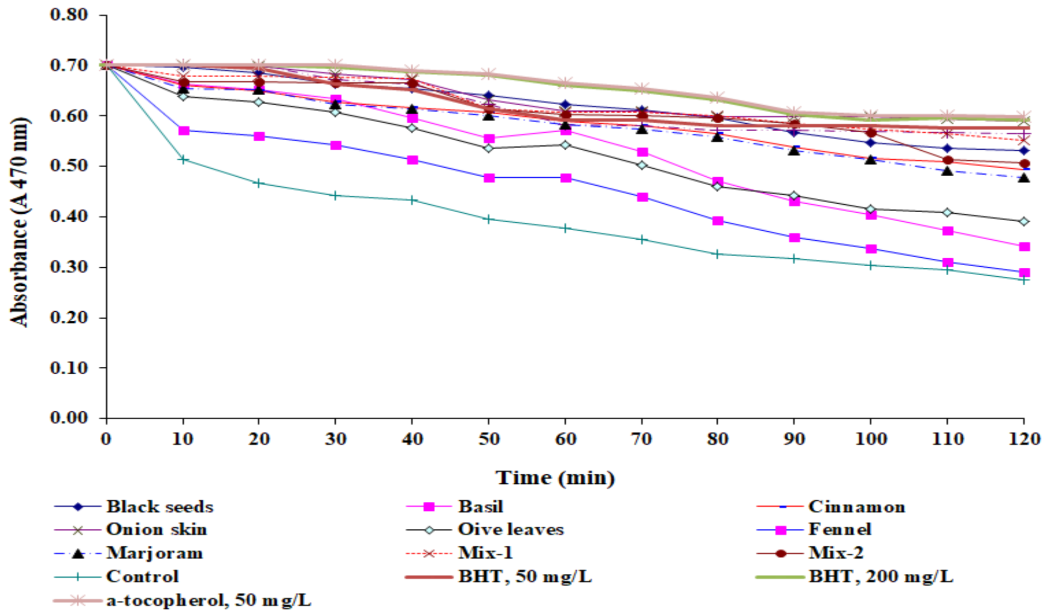


Figure 2. Activity of natural aqueous extracts and their mixture determined by the BCB method (BHT and α-tocopherol were used as references)

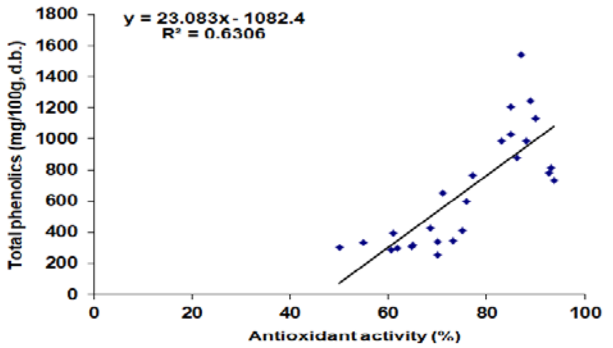


Figure 3. Relationship between antioxidant activities (AA) and total phenolic contents of selected natural extracts and their mixture (n=24)

3.2. Relationship Between Total Phenolic Content and Antioxidant Activity

The relationship between total phenolic content and antioxidant activity of the selected natural products aqueous extract is shown in Figure (3). The results indicated that when all extracts were included in the statistical analysis, there was a positive [Total phenolics (mg/100g, d.b.)= 23.083 (Antioxidant activity, %) – 1082.4, r^2= 0.6306 and significant (p≤ 0.01) relationship between total phenolics and antioxidant activity. This indicates that phenolics can play a significant role in the antioxidant activity of selected natural products. Also, the present data indicates that compounds beside the total phenolics can play important auxiliary roles in the antioxidant activities of that natural products such nutrients, vitamins, essential/volatile oils, minerals etc. Such observation was confirmed by several studies who found that beside the phenolics, other compounds such as alkaloids, essential oils, flavonoids and carotenoids helps with antioxidant activity [23,32,35,63,66]. Data of the present study also confirms this interactive effects resulted from those active groups/compounds that were contained in the mixture samples (Mix-1 and Mix-2), which had a major role in recording the highest levels of antioxidant activity in those products. In similar study, Velioglu et al., [67] reported that the correlation coefficient between total phenolics and antioxidative activities of twenty eigh plant products, including medicinal plant parts was statistically significant. Also, Lee et al., [68] reported that phenolic compounds correlated well with antioxidant activity in five cultivars of fresh pepper (*Capsicum annuum*).

In general, the data of this study with the others proved the importance of using selected natural products extract as natural antioxidants in food technology applications. For examples, several decades ago, natural products are traditionally used to improve food-flavour. In addition to this function, natural products extract are exhibit a wide range of biological effects, including antioxidants, antimutagenic, anti-carcinogenic, antithrombotic, anti-oedematous, antiviral, anti-inflammatory, antiallergic, antioedematous, antiaging, antidiabetic, antimicrobial, vasodilatory actions analgesic, activities and immunomodulator actions [23,67-78]. Also, many of natural products exhibit antibiotic properties and therefore, are considered by some to play a role in disease resistance [79]. The exhibition of these properties in different natural products related to their higher contents of natural antioxidants called phytochemicals including principally phenolic compounds. Regarding to food technology applications, Serag El-Din, [80]and Badawy, [81]found that adding of phenolic acids to vegetable oils leads to significant decrease in the rate of hydrolysis, rancidity and formation of the toxic and carcinogenic substances during the deep frying process. Also, Mohamed, [82] used some by-products rich in phenolics including red onion skin powders and potato peel powder as food additives in an important meat product i.e. meatballs. The adding of such by-products leads to significant decrease in the formation of potential toxic and carcinogenic polycyclic aromatic hydrocarbons during charcoal broiling process. Antioxidant evaluation, hydroperoxide formation in sunflower oil-in-water emulsions, of some by-products rich in phenolics showed considerable activity for potato peel powder while strong activities for the onion skin powders [84]. Finally, there was a clear improvement in the properties of the dough (farinograph and extensograph parameters) as a result of adding the by-products of the food processing factories (onion peel - potato peel) and/or black seeds to the flour, which resulted in the production of loaves and bakery products of high nutritional and sensory quality [84-87].

3.3. Rancidity Evolution in Meatballs with Different Natural Products Extracts Added, During Storage Time

Rancidity (malonaldehyde content, MDA) evolution in meatballs with different natural products extracts added, during storage time is shown in Table (2) and Figure (4). The analysis of variance for the MDA data indicates that the MDA values in meatball samples were significantly affected (P≤0.05) by both the extracts treatment and the storage period. Initial MDA values for all extracts treated meatball samples were significantly (P≤0.05) lower than those for the control ones. For the control meatball samples, the MDA was recorded 0.32 mg/kg sample which increased to 4.44 mg/kg sample (990.46%) at the end of storage period (12 days at 4 ^{0}C). All the selected natural products extract treatments leads to decrease in the formation of MDA in meatball samples after storage periods. The high decreasing rates were recorded for samples treated with onion skin, potato peel, cinnamon, black seeds by 293.07, 293.07, 312.03, 316.52% (as a percent of starting point), respectively. The rest of extracts i.e. basil, fennel and olive leaves treated samples were recorded minimally decreasing rates by 692.49, 717.85 and 736.87% (as a percent of starting point), respectively. As for mixing the extracts to form Mix-1 and Mix-2 and treating the meatballs with them, it was more effective in reducing the rates of malonaldehyde formation during the storage period until its end. The rates of MDA formation in the meatball samples were 245.52 and 267.71% (as a percentage of starting point), which were treated with Mix-1 and Mix-2 at the end of storage, respectively.

Several decades ago, there is an increasing international concern about the presence and the adverse effects of malonaldehyde. It is formed in fresh and ready to eat foods including meats as a consequence of oxidation of their contents of polyunsaturated fatty acids during storage, processing and cooking [88]. The effect of malonaldehyde on human health has been reported by many authors that is mutagenic and carcinogenic [5,6,89]. Results of the present study suggest that the natural selected products i.e. natural antioxidants retarded lipid oxidation during and immediately after cooking. These results are in accordance with that observed by Ahn *et al.*, [90] and Fernandez-Lopez *et al.*, [48] for other natural antioxidants applied to cooked beef. Also, Essa, [49] reported the partially same results for herbs/spices, natural products (coriander, cumin, dill, thyme, rosemary and carnation) applied to cooked buffalo meatballs. With this context, Chen et al., [12] reported that iron was released from heme pigments during cooking and proposed that the resultant increase in non-heme iron was responsible for lipid oxidation. Also, Sato and Hegarty, [91] found that non-heme iron was the active catalyst in cooked meats. At the end of the storage time (day 12) all the selected extracts treatments resulted in significantly ($P \leq 0.05$) lower MDA values when compared to the control, which indicates that all the tested natural extracts added to meatballs showed antioxidant properties. Data of the present study with the others indicated that all the natural products extracts applied in cooked meatball shows a strong antioxidant activity property, which is due to the fact that it contains many bioactive compounds, including phenolics, alkaloids, volatile compounds, essential oils and vitamins [14,32,35,45]. Also, such bioactive compounds exhibited free radicals scavenging activity and inhibition the lipid oxidation [23,92,93].

Table 2. Rancidity evolution (MDA, mg/kg sample) in meatballs with different natural products extracts added, during storage time (12 days at 4°C)

Treatment		Storage period (days)									
		0		3		6		9		12	
Meatballs	MB	0.32 ±	0.07	1.71 ±	0.10	2.61 ±	0.13	3.09 ±	0.04	3.44 ±	0.20^{a}
Black seeds	MB + BSE	0.32 ±	0.07	0.54 ±	0.06	0.86 ±	0.31	0.92 ±	0.24	1.31 ±	0.38^{c}
Marjoram	MB + ME	0.32 ±	0.07	0.54 ±	0.03	0.64 ±	0.07	0.96 ±	0.13	1.30 ±	0.22^{c}
Cinnamon	MB + CE	0.32 ±	0.07	0.55 ±	0.11	0.77 ±	0.10	1.03 ±	0.12	1.42 ±	0.22^{c}
Onion skin	MB + OSE	0.32 ±	0.07	0.71 ±	0.15	0.75 ±	0.10	1.19 ±	0.20	1.24 ±	0.22^{c}
Potato peel	MB + PPE	0.32 ±	0.07	0.69 ±	0.13	0.72 ±	0.02	1.32 ±	0.06	1.28 ±	0.32^{c}
Basil	MB + BE	0.32 ±	0.07	1.27 ±	0.22	2.04 ±	0.14	2.50 ±	0.22	2.50 ±	0.31^{b}
Fennel	MB +FE	0.32 ±	0.07	1.38 ±	0.32	2.01 ±	0.30	2.27 ±	0.09	2.58 ±	0.21^{b}
Olive leaves	MB + OLE	0.32 ±	0.07	1.46 ±	0.22	2.17 ±	0.05	2.09 ±	0.20	2.64 ±	0.22^{b}
Mix-1	Mix (BSE+ ME+ OSE+ BE)	0.32 ±	0.07	0.42 ±	0.40	0.67 ±	0.23	0.73 ±	0.20	1.09 ±	0.21^{d}
Mix-2	Mix (CE+ PPE+ FE+ OLE)	0.32 ±	0.07	0.44 ±	0.32	0.70 ±	0.21	0.75 ±	0.29	1.16 ±	0.24^{cd}

[*] Each value represents mean of three replicates ±SD. Means with various superscript letters in the same column are different significantly at $p \leq 0.05$.

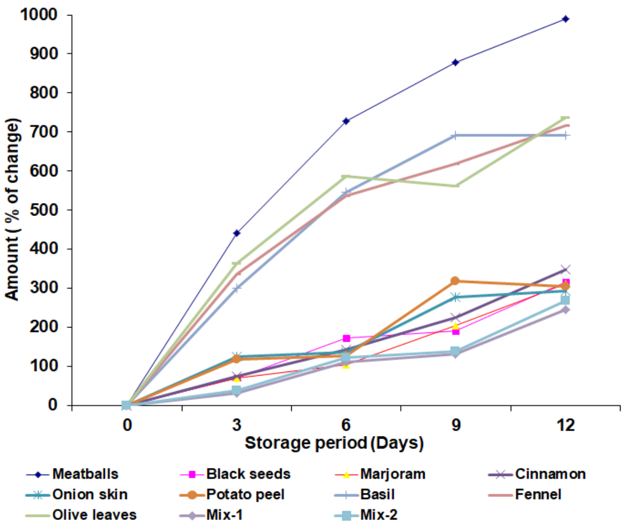
Table 3. The effect of adding natural products aqueous extract and their mixtures on total volatile nitrogen content (TVN, mg/kg sample) of meat samples storage at 4°C for 12 days [*]

Treatment		Storage period (days)									
		0		3		6		9		12	
Meatballs	MB	5.90 ±	0.21	7.97 ±	0.33	8.89 ±	0.42	11.50 ±	0.12	15.81 ±	0.66^{a}
Black seeds	MB + BSE	5.90 ±	0.21	7.04 ±	0.18	8.21 ±	1.00	8.72 ±	0.75	11.28 ±	1.51^{b}
Marjoram	MB + ME	5.90 ±	0.21	6.53 ±	0.09	8.67 ±	0.21	9.15 ±	0.42	11.34 ±	0.69^{b}
Cinnamon	MB + CE	5.90 ±	0.21	6.57 ±	0.34	7.40 ±	0.33	8.85 ±	0.39	12.65 ±	0.6b ^{b}
Onion skin	MB + OSE	5.90 ±	0.21	6.84 ±	0.48	8.35 ±	0.33	9.63 ±	0.63	12.71 ±	0.69^{b}
Potato peel	MB + PPE	5.90 ±	0.21	6.90 ±	0.42	8.25 ±	0.06	9.48 ±	0.18	12.39 ±	1.03^{b}
Basil	MB + BE	5.90 ±	0.21	7.07 ±	0.69	8.38 ±	0.45	10.32 ±	0.72	12.94 ±	0.50^{b}
Fennel	MB +FE	5.90 ±	0.21	6.66 ±	1.03	7.79 ±	0.97	9.23 ±	0.30	12.05 ±	0.69^{b}
Olive leaves	MB + OLE	5.90 ±	0.21	6.82 ±	0.70	8.19 ±	0.15	9.31 ±	0.66	11.92 ±	0.69^{ab}
Mix-1	Mix (BSE+ ME+ OSE+ BE)	5.90 ±	0.21	6.94 ±	1.30	7.18 ±	0.72	9.91 ±	0.64	10.54 ±	0.66^{c}
Mix-2	Mix (CE+ PPE+ FE+ OLE)	5.90 ±	0.21	6.43 ±	1.03	8.25 ±	0.66	10.03 ±	0.93	10.79 ±	0.75^{c}

[*] Each value represents mean of three replicates ±SD. Means with various superscript letters in the same column are different significantly at $p \leq 0.05$.

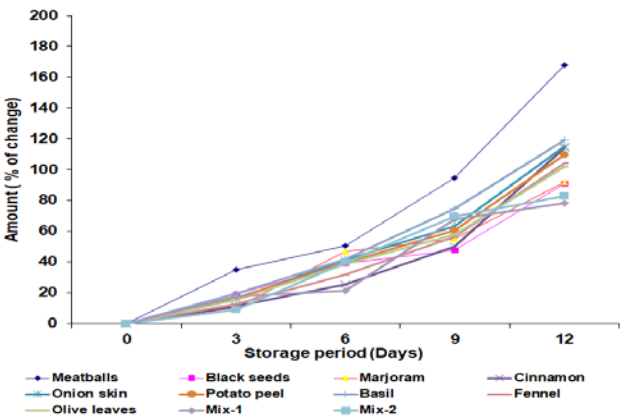
3.4. Total Volatile Basic Nitrogen (TVB-N) Content in Meatballs with Added Natural Products Extracts, During Storage Time

TVB-N) content in meatballs with added natural products extracts, during storage time is shown in Table (3) and Figure (5). The analysis of variance for the TVB-N data indicates that these values in meatball samples were significantly affected ($P \leq 0.05$) by both the extracts treatment and the storage period. Initial TVB-N values for all extracts treated meatball samples were significantly ($P \leq 0.05$) lower than those for the control ones. For the control meatball samples, the TVB-N was recorded 5.90 mg/kg sample which increased to 15.81 mg/kg sample (167.77%) at the end of storage period (12 days at 4°C). All the selected natural products extract treatments leads to decrease in the formation of TVB-N i.e. increase the degradation of protein, in meatball samples after storage periods. The high decreasing rates were recorded for samples treated with black seeds, marjoram, olive leaves, potato peel, cinnamon, onion skin and basil by 91.04, 92.06, 101.84, 109.86, 114.25, 115.32, 119.15% (as a percent of starting point), respectively. As for mixing the extracts to form Mix-1 and Mix-2 and treating the meatballs with them, it was more effective in reducing the rates of TNB-N formation during the storage period until its end. The rates of TVB-N formation in the meatball samples were 78.51 and 82.74% (as a percentage of starting point), which were treated with Mix-1 and Mix-2 at the end of storage period, respectively. Data of the present study are in accordance with that reported by several authors. For example, El-Dashlouty, [94] showed that the TVB-N content increased as the time of storage increased, during cold storage of sausage. Also, El-Kholie, [95] reported that TVN increased during frozen storage at -20°C in fermented and non-fermented sausage samples after 3 months storage was lower than that of the cold-stored ones, due to extremely less formation of alkaline protein breakdown products during freezing.



Each value represents mean of three replicates ±SD. Mix-1, meatballs with black seeds, marjoram, onion skin and basil aqueous extracts, Mix-2, meatballs with cinnamon, potato peel, fennel and olive leaves

Figure 4. Rancidity evolution (MDA, % of change) in meatballs with different natural products extracts added, during storage time (12 days at 4°C)



Each value represents mean of three replicates. Mix-1, meatballs with black seeds, marjoram, onion skin and basil aqueous extracts, Mix-2, meatballs with cinnamon, potato peel, fennel and olive leaves.

Figure 5. Total volatile basic nitrogen (TVB-N, % of change) content in meatballs with added natural products extracts, during storage time (12 days at 4°C)

Degradation of proteins and other nitrogen (N)-containing compounds as a result of the spoilage mechanisms mentioned above cause accumulation of organic amines that are commonly known as total volatile basic nitrogen (TVB-N) [96,97,98]. TVB-N often applied as an objective biomarker for the loss of muscle foods freshness and safety, and to determine the suitability for consumption [99].These compounds are toxic and cause considerable colour and flavour changes that affect the acceptability of meat products[1]. Also, Jurado et al., [100] reviewed that the TVB-N content increases with storage time of meat and often its accumulation pattern somewhat parallels other biomarkers of spoilage, such as microbial count and changes in sensory acceptability. Through these previous studies, it is clear that the rate of formation of TVB-N is affected by several factors, the most important of which are microbial contamination and conditions and length of storage period. The results of the current study, along with other studies, also proved that there are many technological treatments that can delay the degradation of proteins and reduce the rate of TVB-N formation such as fermentation process, applied the tenderization agents and used of the natural antioxidant extracts[95,96,101]. Findings from this study have revealed that natural aqueous antioxidant extracts of black seeds, marjoram, cinnamon, onion skin, potato peel, basil, fennel, olive leaves and their mixtures has antioxidative effect on protein degradation in refrigerated meatball samples. The strong antioxidant activity property of these natural products is due to the fact that their contains many bioactive compounds, including phenolics, alkaloids, volatile compounds, essential oils and vitamins [14,32,35,45]. Beside that such bioactive compounds exhibited free radicals scavenging activity and inhibition the lipid peroxidation [23,92,93].

3.5. Lactic Acid Bacteria (LAB) Counts in Meatballs with Added Natural Products Extracts, During Storage Time

LAB counts in meatballs with added natural products extracts during storage time are shown in Tables (4) and Figure (6). The analysis of variance for the lactic acid bacteria (LAB) data indicates that the LAB values in meatball samples were significantly affected ($P \leq 0.05$) by both the extracts treatment and the storage period. Initial LAB values for all extracts treated meatball samples were significantly ($P \leq 0.05$) lower than those for the control ones. For the control meatball samples, the LAB was recorded 1.06 $\log_{10}$ cfu/g sample which increased to 4.78 $\log_{10}$ cfu/g (352.12%) at the end of storage period (12 days at 4°C). All the selected natural products extract treatments leads to decrease the LAB in meatball samples after storage periods. The high decreasing rates were recorded for samples treated with black seeds, onion skin, potato peel, cinnamon, marjoram and fennel by 191.01, 248.83, 277.36, 285.54, 287.43, 297.17 % (as a percent of starting point), respectively. The rest of extracts i.e. basil and olive leaves treated samples were recorded minimally decreasing rates by 315.09 and 320.75% (as a percent of starting point), respectively. As for mixing the extracts to form Mix-1 and Mix-2 and treating the meatballs with them, it was more effective in reducing the rates of LAB reproduction during the storage period until its end. The rates of LAB reproduction in meatball samples were recorded 107.55 and 117.92% (as a percentage of starting point), which were treated with Mix-1 and Mix-2 at the end of storage, respectively. These results are in accordance with that observed by Essa, [49] for other herbs/spices, natural products (coriander, cumin, dill, thyme, rosemary and carnation) applied to cooked buffalo meatballs. With the same context, some spore formers and heat resistant strains which have been linked with contaminate of meats and meat products are likely to contribute to LAB counts [102,103]. Despite the presence of LAB, there was no evidence of strong lactic fermentation in any product, as confirmed by very low (<15 mM) lactic acid as determined by colorimetric methods, which also indicated the absence of significant amounts of sugars. Therefore, some bacteria may be present but their growth on the product is controlled under storage conditions. The variation in antibacterial activity effect among the tested natural products applied in meatball samples could be attributed to several factors including bioactive compounds in extract, dilution of the extract necessary for its use, fat content in meat and water activity of the product (meatballs) [103-106]. Also, Hegazy, [107] found that appling of the gum Arabic (GA) with the phyto-extracts to the meatball samples exhibited more decreasing in LAB compared with the similar samples without GA which due to the effect of GA in lowering the water activity within the product for its high content of dietary fiber with high water absorption. For the extract bioactive compounds composition, onion skin was rich in several bioactive compounds including phenolics which possess many other biological activities including antimicrobial activity [108]. Cinnamon extract contains several classes of bioactive compounds such phenolics, phenolic acids, alkaloids and cinnamaldehyde which had potent antimicrobial activities against microorganisms [109].

Table 4. Lactic acid bacteria (LAB) counts (log$_{1°C}$fu/g) in meatballs with added natural products extracts, during storage time (12 days at 4 $^{°C}$)

Treatment		Storage period (days)				
		0	3	6	9	12
Meatballs	MB	1.06 ± 0.12	2.50 ± 0.11	2.99 ± 0.16	3.39 ± 0.24	4.78 ± 0.20^{a}
Black seeds	MB + BSE	1.06 ± 0.12	1.57 ± 0.05	1.70 ± 0.13	2.19 ± 0.21	3.08 ± 0.20^{c}
Marjoram	MB + ME	1.06 ± 0.12	2.04 ± 0.14	2.65 ± 0.16	2.97 ± 0.16	4.10 ± 0.22^{b}
Cinnamon	MB + CE	1.06 ± 0.12	2.32 ± 0.18	2.89 ± 0.12	3.24 ± 0.20	4.08 ± 0.23^{b}
Onion skin	MB + OSE	1.06 ± 0.12	1.93 ± 0.20	2.43 ± 0.24	2.82 ± 0.22	3.70 ± 0.09^{bc}
Potato peel	MB + PPE	1.06 ± 0.12	2.26 ± 0.23	2.82 ± 0.58	3.14 ± 0.06	4.00 ± 0.16^{b}
Basil	MB + BE	1.06 ± 0.12	2.38 ± 0.24	2.91 ± 0.19	3.31 ± 0.08	4.40 ± 0.30^{a}
Fennel	MB +FE	1.06 ± 0.12	2.54 ± 0.33	2.93 ± 0.07	3.47 ± 0.11	4.21 ± 0.30^{ab}
Olive leaves	MB + OLE	1.06 ± 0.12	2.56 ± 0.29	2.95 ± 0.14	3.17 ± 0.37	4.46 ± 0.12^{a}
Mix-1	Mix (BSE+ ME+ OSE+ BE)	1.06 ± 0.12	1.12 ± 0.35	1.29 ± 0.25	1.80 ± 0.23	2.20 ± 0.41^{d}
Mix-2	Mix (CE+ PPE+ FE+ OLE)	1.06 ± 0.12	1.18 ± 0.05	1.79 ± 0.12	2.10 ± 0.21	2.31 ± 0.32^{d}

[*] Each value represents mean of three replicates ±SD. Means with various superscript letters in the same column are different significantly at $p \leq 0.05$.

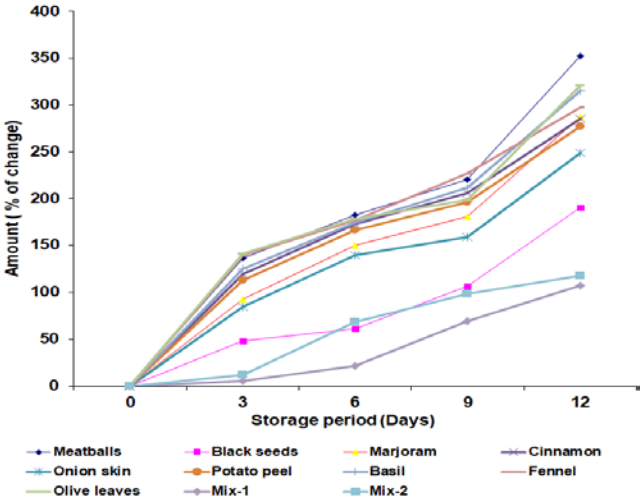
Table 5. Evolution of sensory scores for perception of overall acceptability (scores) of meatballs with added natural extracts during storage time

Natural products		Storage period (days)				
		0	3	6	9	12
Meatballs	MB	7.13 ± 0.21	6.69 ± 0.61	6.27 ± 0.28	4.21 ± 0.08	3.67 ± 0.43^{e}
Black seeds	MB + BSE	7.13 ± 0.21	6.87 ± 0.12	6.47 ± 0.65	6.00 ± 0.49	5.20 ± 0.79^{a}
Marjoram	MB + ME	7.13 ± 0.21	6.85 ± 0.06	6.42 ± 0.14	5.14 ± 0.28	4.66 ± 0.45^{b}
Cinnamon	MB + CE	7.13 ± 0.21	6.94 ± 0.22	6.40 ± 0.22	6.12 ± 0.26	5.75 ± 0.45^{a}
Onion skin	MB + OSE	7.13 ± 0.21	6.77 ± 0.32	6.40 ± 0.22	4.62 ± 0.42	3.92 ± 0.45^{d}
Potato peel	MB + PPE	7.13 ± 0.21	6.77 ± 0.28	6.38 ± 0.04	4.37 ± 0.12	3.88 ± 0.17^{d}
Basil	MB + BE	7.13 ± 0.21	6.81 ± 0.30	6.41 ± 0.30	4.73 ± 0.47	3.99 ± 0.65^{cd}
Fennel	MB +FE	7.13 ± 0.21	6.83 ± 0.67	6.40 ± 0.43	5.45 ± 0.20	4.22 ± 0.45^{c}
Olive leaves	MB + OLE	7.13 ± 0.21	6.76 ± 0.21	6.36 ± 0.10	4.29 ± 0.41	3.84 ± 0.45^{d}
Mix-1	Mix (BSE+ ME+ OSE+ BE)	7.13 ± 0.21	6.93 ± 0.45	6.63 ± 0.47	6.34 ± 0.32	5.70 ± 0.21^{a}
Mix-2	Mix (CE+ PPE+ FE+ OLE)	7.13 ± 0.21	7.02 ± 0.37	6.65 ± 0.44	6.39 ± 0.31	5.91 ± 0.49^{a}

[*] Each value represents mean of ten panelists ±SD. Means with various superscript letters in the same column are different significantly at $p \leq 0.05$.

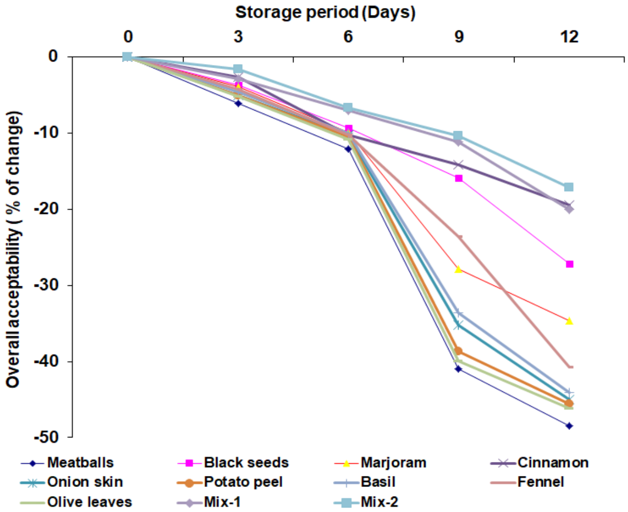
3.6. Evolution of Sensory Scores for Perception of Overall Acceptability of Meatballs with Added Natural Products Extracts During Storage Time

Sensory scores for perception of overall acceptability of meatballs with added natural products extracts during storage time are shown in Table (5) and Figure (7). The analysis of variance for the score of overall acceptability data indicates that these values in meatball samples were significantly affected ($P \leq 0.05$) by both natural product treatments and the storage period. The highest ($P \leq 0.05$) scores for overall acceptability were obtained for black seeds, cinnamon, marjoram and fennel treatments, the lowest for control samples and there were not differences in overall acceptability perception between basil, onion skin, potato peel and olive leaves treatments. As for mixing the extracts to form Mix-1 and Mix-2 and treating the meatballs with them, it was more effective in recording the high score for overall acceptability during the storage period until its end. Therefore, the results of this study prove that the volatile components responsible for the odor and flavor, specific to each extract, greatly affect the overall acceptance scores of the products beside the other biologically active compounds contained in these extracts, which have many biological roles (antioxidant, inhibition of fat oxidation, antimicrobial activity), which naturally prevents the deterioration of the sensory qualities responsible for the total acceptance of products. Perhaps this confirms the results of the samples/products in which the mixtures of natural products (Mix-1 and Mix-2) were applied, which recorded the highest scores for the overall acceptability, as a result of the interactive effects caused by the components of those mixtures together.



Each value represents mean of three replicates. Mix-1, meatballs with black seeds, marjoram, onion skin and basil aqueous extracts, Mix-2, meatballs with cinnamon, potato peel, fennel and olive leaves

Figure 6. Lactic acid bacteria (LAB) counts (% of change) in meatballs with added natural products extracts, during storage time (12 days at 4°C)



Each value represents mean of three replicates. Mix-1, meatballs with black seeds, marjoram, onion skin and basil aqueous extracts, Mix-2, meatballs with cinnamon, potato peel, fennel and olive leaves

Figure 7. Evolution of sensory scores for perception of overall acceptability (as a % of change)of meatballs with added natural extracts during storage time

4. Conclusion

The results of the current study demonstrated the effectiveness of using aqueous extracts of some spices, herbs and plant parts, including biologically active compounds such as phenolic compounds, as natural antioxidants in cooked meatballs. The application of these extracts was beneficial to control the degradation of protein molecules and the development of rancidity and off flavors, thus improving product acceptability and prolonging shelf life. In addition to the anti-bacterial activity shown by these extracts. The data also showed that the use of mixtures of these extracts gave more effective results compared to the use of extracts in a single way, which proves the interactional effects of the different groups of bioactive compounds contained in those extracts in the case of mixing. Finally, the results of the study are an important step in the search for compounds that may allow a shift from synthetic antioxidants to natural ones, especially after it has been proven that this trend is justified. Where, it was suspected of the possibility of cancer as a result of the use of synthetic antioxidants, which prompted some countries to develop food regulations, laws and legislation that limit or prevent their use in food applications.

Ethical Clearance

This study was carried out after ethical approval from the Scientific Research Ethics Committee (SREC), Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt (Approval no. 17- SREC- 12-2021).

Conflicts of Interest

No potential conflict of interest was reported by the authors.

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