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Effect of adding natural extracts on quality properties of meat products subjected to refrigeration process

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Abstract: The present study is being in the development and application of natural products with both antioxidants and antibacterial activities in meat products may be necessary and useful to prolong their storage shelf life and potential for preventing food diseases. The analysis of variance for the malonaldehyde content (MDA) data indicates that the MDA values were significantly affected ($P < 0.05$) by both the extract treatments and the storage period. Initial MDA values for all extract samples were significantly lower than those for the control ($P < 0.05$). For the control samples, the MDA was recorded 0.29 mg/kg sample which increased to 4.73 mg/kg sample (1531.03%) at the end of storage period (12 days at 4 ^{0}C). All the extract treatment leads to decreasing in the formation of MDA in meatball samples after storage periods. The highest decreasing effect was recorded for thyme, marjoram and coriander extracts (231.03, 337.93 and 275.86 % as a percent of change, respectively), while the lowest one for dill seeds (1072.41%). For the mixing of some such herbs/spices by equal quantities [Mix I (Thyme + Rosemary + Carnation) and Mix II (Coriander + Cumin + Dill)], they were exhibited high effects which recorded 462.07 and 834.48%, respectively. The same trend was recorded for the bacteriological examination, lactic acid bacteria (LAB) of beef meatballs with different natural extracts added during storage time. Data of the present study confirmed that there is a trend to search for compounds that may allow a shift from synthetic to natural antioxidants. This trend is justified because a carcinogenic potential from the use of synthetic antioxidants has been suspected.

Keywords: Meatballs, natural antioxidants malonaldehyde**,** lactic acid bacteria.

Introduction

Refrigeration is a process in which work is done to move heat from one location to another. The work of heat transport is traditionally driven by mechanical work, but can also be driven by heat, magnetism, electricity, laser, or other means. The life of many foods may be increased by storage at temperatures below 4° C (40° F). Commonly refrigerated foods include fresh fruits and vegetables, eggs, dairy products, and meats. Some foods, such as tropical fruits (*e.g.*, bananas), are damaged if exposed to low temperatures. Also, refrigeration cannot improve the quality of decayed food; it can only retard deterioration.

Meat constitutes a substantial portion of protein in the present day diets, hence the concern to market a safe, high quality product. The appearance of foods is one of the major determinants of its appeal to consumers and consequently, sales of the product. Bacterial contamination and lipid oxidation are the main factors that determine food quality loss and shelf-life reduction. Therefore, preventing bacterial cross-contamination and delaying lipid oxidation are highly relevant to food processors. The growth of microorganisms in meat products may cause spoilage or food-borne diseases. Oxidative processes in meat lead to the degradation of lipids and proteins which, in turn, contribute to the deterioration in flavour, texture and color of displayed meat products (Decker *et al.*, 1995).

Fresh meat products undergo major undesirable changes during storage. Although synthetic additives have been widely used in the meat industry to inhibit both, the process of lipid oxidation and microbial growth, the trend is to decrease their use because of the growing concern among consumers about such chemical additives (Chastain *et al.*, 1984). Consequently, search for natural additives, especially of plant origin, has notably increased in last year's (Loliger, 1991). Compounds obtained from natural sources such as grains, oilseeds, spices, fruit and vegetables have been investigated (Chen *et al.*, 1996). Therefore, the aim of the present is being in the development and application of natural products with both antioxidants and antibacterial activities in meat products may be necessary and useful to prolong their storage shelf life and potential for preventing food diseases.

Materials and methods

Materials

Thyme (Thymus vulgaris), marjoram (Origanum majorana L.), coriander (Coriandrum sativum L.), rosemary (Rosmarinus officinalis L.), basil (Ocimum basilicum L.), carnation (Dianthus caryophyllus), cumin seed (Cuminum cyminum L.) and dill seed (Anethum graveolens L.) powders were obtained from Bab El-Khalek market, Cairo, Egypt.

Meat samples

Rose meat samples were obtained from the Egyptian local markets, transported to the lab, cutted into small pieces using sharp knife and mincing using electrical mixer (Moulinex Egypt, Al-Araby Co., Egypt) and used for meatballs processing.

Methods

Natural extracts preparation

Thyme water extract (TWE), marjoram water extract (MWE), coriander water extract (CWE), rosemary water extract (RWE), basil water extract (BWE), carnation water extract (CrWE), cumin seed water extract (CuWE)) and dill seed water extract (DWE) powders were prepared as follow: ground samples (100 g) were extracted with 100% deionized water on an orbital shaker for 120 min at 70 ^{0}C. The mixture was subsequently filtered (Whatman No. 5) on a Buchner funnel, and the filtrates were assayed for antioxidant activity and used in beef meatballs manufactures.

Meatball manufacture

Product formulation

Swedish-style meatballs were manufactured according to Fernandez-Lopez *et al.,* (2005): 78% minced beef (20% fat content), 14.5% flake potatoes, 5% water and 2.5% salt. A set of 5 treatment samples differing only by the plant parts powder added were prepared as follow:

- Minced meat (control samples)
- Minced meat + 0.25 % (w/w) thyme water extract (TWE).
- Minced meat + 0.25 % (w/w) marjoram water extract (MWE).
- Minced meat + 0.25 % (w/w) coriander water extract (CWE).

- Minced meat + 0.25 % (w/w) rosemary water extract (RWE).
- Minced meat + 0.25 % (w/w) basil water extract (BWE).
- Minced meat + 0.25 % (w/w) carnation water extract (CrWE).
- Minced meat + 0.25 % (w/w) cumin seed water extract (CuWE).
- Minced meat + 0.25 % (w/w) dill seed water extract (DWE).
- Minced meat + 0.25 % (w/w) mixture TWE + RWE + CrWE by equal parts.
- Minced meat + 0.25 % (w/w) mixture CWE + CuWE + DWE by equal parts.

Plant parts were used at the concentrations suggested by Fernandez-Lopez *et al.,* (2005).

Product processing

The products were prepared in a pilot plant resembling to commercial processing conditions. All ingredients were homogenized in a bowl mixer with a spiral dough hook (Moulinex Egypt, Al-Araby Co., Egypt) during 5 min. For each treatment, the corresponding plant part powder was added at the concentrations suggested, and then mixed again for 5 min. Meatballs were formed by hand (15 g, 20-25 mm in diameter) and then subjected to a two stage cooking process. First, the meatballs were flash fried into sunflower oil at 190 ^{0}C for 30 seconds to seal the surface of the ball and produce the characteristic browned look. They were then thoroughly cooked in a forced draught oven (Zanussi, Italy) at 250 ^{0}C during 4 min to reach an internal temperature of 72 ^{0}C in the center of the meatball. The temperature was monitored using an Omega digital thermometer (Omega Engineering, Inc., Stamford, CT) with a chromel–alumel (Omega K) thermocouple probe positioned in the geometric center of the product samples. When the endpoint temperature was achieved, the samples were immediately placed in a chiller (2-5 ^{0}C) to reach a product temperature below 12 ^{0}C. Three replications of this experiment were made.

Malonaldehyde content (TBA values)

Lipid oxidation was assessed as malonaldehyde content in triplicate by the 2-thiobarbituric acid (TBA) method of Tarladgis *et al.,* (1960) with minor modifications. A 10 g sample was blended with 50 ml distilled

water for 2 min and then transferred to a distillation tube. The cup used for blending was washed with additional 47.5 ml distilled water, which was added to the same distillation tube with 2.5 ml 4N HCl and a few drops of antifoam agent silicone o/w (Fisher Scientific, Loughborough, UK). The mixture was distilled and 50 ml distillate was collected. Five ml of 0.02 M 2-thiobarbituric acid in 90% acetic acid (TBA reagent) was added to a vial containing 5 ml of the distillate and mixed well. The vials were capped and heated in a boiling water bath for 30 min to develop the chromogen and cooled to room temperature. The absorbance was measured at 538 nm, against a blank prepared with 5 ml distilled water and 5 ml TBA-reagent, using a PV 8625 spectrophotometer (Philips, UK). Thiobarbituric acid-reactive substances (TBARS) were calculated from a standard curve (8–50 nmol) of malondialdehyde (MA), freshly prepared by acidification of TEP (1,1,3,3-tetraethoxy propane). Reagents were obtained from Sigma (UK). The TBA numbers were calculated as mg MA/kg sample.

Microbiological analysis

Lactic acid bacteria (LAB)

A composite sample (10 g) was formed with portions of at least 3 meatballs and homogeneized with sterile 1.5% peptone water, in a Stomacher 400 (Colworth, London, UK) for 1 min. Aliquotes were serial diluted in peptone water and plated out following standard methodologies (Gerhardt *et al.,* 1994). Lactic acid bacteria (LAB) counts were determined on MRS Agar (pH 5.6), with the plates incubated under anaerobic conditions (Gas generating kit anaerobic system, Oxoid Unipath Ltd., Basingtoke, Hampshire, UK) at 30 ^{0}C for 2 days. Culture media were from Oxoid (Oxoid Unipath Ltd., Basingtoke, Hampshire, UK). Results were expressed as $\log_{10}$ cfu/ml.

Statistical analysis

Statistical analysis was performed with the Student *t*-test and MINITAB program (Minitab Inc., State College, PA).

Results and Methods

Rancidity evolution of beef meatballs with different natural extracts added during storage time

The effect of the natural extracts on lipid oxidation of cooked meatballs during storage is shown in Table (1) and Figure (1). The analysis of variance for the malonaldehyde content (MDA) data indicates that the MDA values were significantly affected ($P < 0.05$) by both the extract treatments and the storage period. Initial MDA values for all extract samples were significantly lower than those for the control ($P < 0.05$). For the control samples, the MDA was recorded 0.29 mg/kg sample which increased to 4.73 mg/kg sample (1531.03%) at the end of storage period (12 days at 4 ^{0}C). All the extract treatment leads to decreasing in the formation of MDA in meatball samples after storage periods. The highest decreasing effects was recorded for thyme, marjoram and coriander extracts (231.03, 337.93 and 275.86 % as a percent of change, respectively), while the lowest one for dill seeds (1072.41%). For the mixing of some such herbs/spices by equal quantities [Mix I (Thyme + Rosemary + Carnation) and Mix II (Coriander + Cumin + Dill)], they were exhibited high effects which recorded 462.07 and 834.48%, respectively.

These results suggest that these natural antioxidants retarded lipid oxidation during and immediately after cooking. These results agree with that reported by Ahn *et al.,* (2002) and Fernandez-Lopez *et al.,* (2003) for other natural antioxidants applied to cooked beef. Sato and Hegarty (1971) reported that non-heme iron was the active catalyst in cooked meats. Chen *et al.,* (1984) also 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. At the end of storage time (day 12) all treatments resulted in significantly lower ($P < 0.05$) TBA values when compared to the control, which indicates that all the tested natural extracts added to meatballs showed antioxidant properties. The product samples with rosemary extracts (OR, WR, OWR) showed the lowest ($P < 0.05$) TBA values at each time of storage. Only treatments with orange extracts (OF) and rosemary water miscible

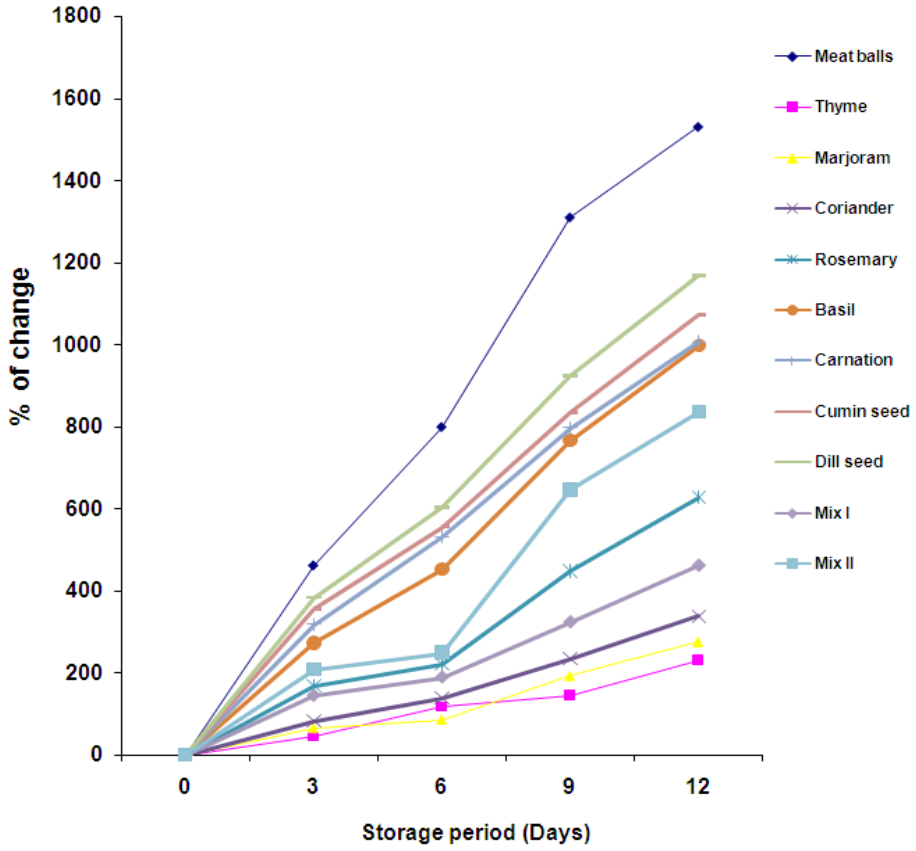

Figure 1. The effect of adding spices/herbs extracts and their mixtures on malonaldehyde content (% of change) of meat samples storage at 4 ^{0}C for 12 days *

* Mix I: Mixture of Thyme + Rosemary + Carnation; Mix II: Mixture of Coriander + Cumin + Dill

extracts (WR) maintained ($P > 0.05$) the initial TBA values during the 12 day storage period, and no differences were found between them ($P < 0.05$). Samples treated with rosemary oil and water miscible extracts (OWR) had slightly increased TBA values only during the first 6 days of storage and became stable after that period. The products with added lemon extracts (LF) reached higher ($P < 0.05$) TBA values than those with

orange extracts (OF) by the end of storage. Consistently, this difference in antioxidant properties between orange and lemon extracts (OF and LF) has also been detected by using the Rancimat method, as explained before. Finally, the present data are in accordance with that obtained by Hegazy, (2015) who reported that the addition of different plant parts to meatball including herbs/spices leads to decrease in TBA values during the storage process. Also, adding the gum Arabic (GA) with the plant parts extracts to the meatball samples induced more decreasing in TBA when compared with the similar samples without GA addition.

Recently, there is an increasing international concern about the presence and the adverse effects of some toxic compounds such as malonaldehyde (See Figure 20). 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 (Reviewed in Gray and Morton, 1981). The effect of malonaldehyde on human health has been reported by many authors that is mutagenic and carcinogenic (Shamberger *et al.,* 1974, Mukia and Goldstein, 1976 and Tawfik *et al.,* 2003). The mutagenucity of malonaldehyde has been demonstrated by the Ames salmonella revertant procedure (Shamberger *et al.,* 1979) while its carcinogenicity was observed when painted on the skin of mice (Shamberger *et al.,* 1974). The significance for human health of any of these reported concentrations of MDA in meatball samples are unknown, but reports that this substance is mutagenic and carcinogenic emphasizes the desirability of minimizing their occurrence during marketing, storage and processing.

Bacteriological examination of beef meatballs with different natural extracts added during storage time.

The effect of the natural extracts on Lactic acid bacteria (LAB) of meatballs during storage is shown in Table (2) and Figure (2). The analysis of variance for the LAB data indicates that the LAB levels were significantly affected ($P < 0.05$) by both the extract treatments and the storage period. Initial LAB values for all extract samples were

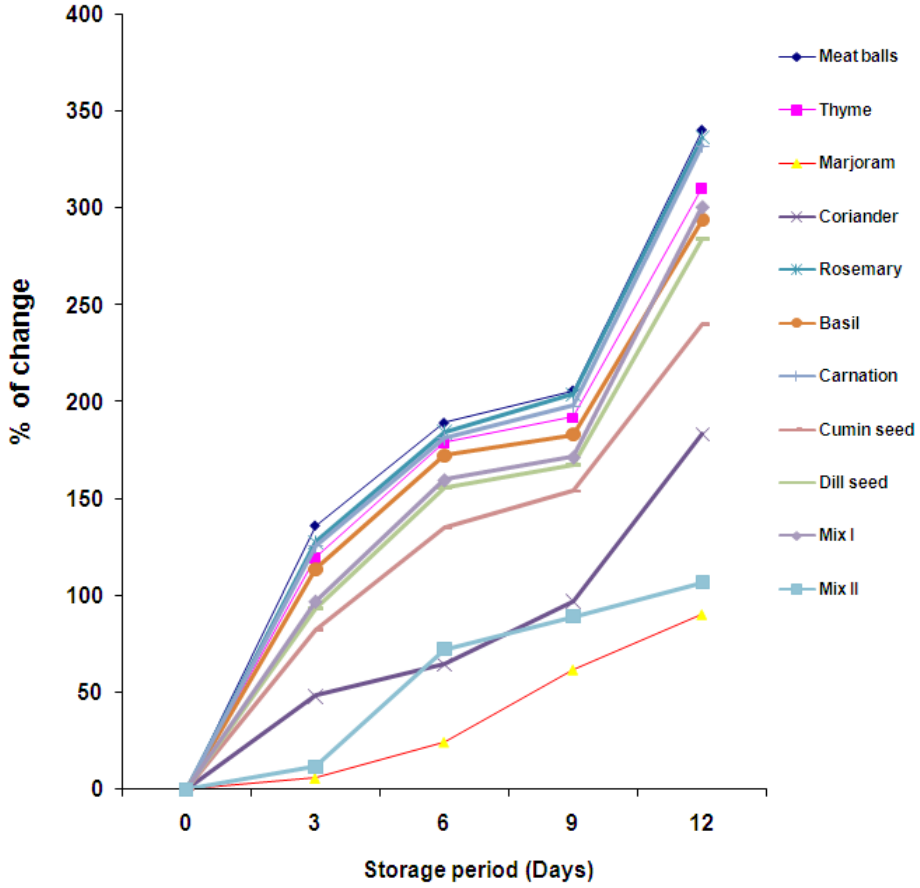

Figure 2. The effect of adding spices/herbs extracts and their mixtures on total lactic acid bacteria (% of change) of meat samples storage at 4 ^{0}C for 12 days *

* Mix I: Mixture of Thyme + Rosemary + Carnation; Mix II: Mixture of Coriander + Cumin + Dill

significantly lower than those for the control ($P < 0.05$). For the control samples, the LAB was recorded 1.02 $\log_{10}$cfu/g which increased to 4.49 $\log_{10}$cfu/g (340.20%) at the end of storage period (12 days at 4 ^{0}C). All the extract treatment leads to decreasing in the formation of LAB in meatball samples after storage periods. The highest decreasing effects was recorded for marjoram and coriander extracts (90.20 and 183.33% as

a percent of change, respectively), while the lowest one for rosemary (336.27%). For the mixing of some such herbs/spices by equal quantities [Mix II (Coriander + Cumin + Dill)], was exhibited high effects which recorded 106.86% as a percent of change, while Mix I (Thyme + Rosemary + Carnation) was exhibited slight effect, 300.98% as a percent of change, respectively.

Some spore formers and heat resistant strains which have been linked with spoilage of meats are likely to contribute to LAB counts (Borch *et al.,* 1996 and Kuri, 1998). After 12 days storage, the growth of LAB to levels of 103 log cfu/g was similar ($P > 0.05$) for control samples and treatments with rosemary extracts. These bacterial groups were not detected in samples from any treatment with citrus extracts during storage time. Despite the presence of lactic acid bacteria, there was no evidence of strong lactic fermentation in any product, as confirmed by very low (<15 mM) lactic acid as determined by HPLC, 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. While there was *in vitro* antilisterial activity when the rosemary extracts were tested using the agar diffusion *t*-est, this was not clear when used in the meatballs at the concentrations tested. Pandit and Shelef (1994) found rosemary extracts to have an effect against *L. monocytogenes* growth in a pork liver sausage. As can be seen, the antibacterial activity obtained for rosemary extracts have not been manifested during the storage of cooked meatballs with added extracts. This could be explained by the dilution of the rosemary extracts necessary for its use in meat products, but there is also a possibility of reduction of the effectiveness of the antimicrobial extract due to physical interactions with the food matrix, which has been observed before for nisin in meat (Davies *et al.,* 1999 and Kuri, 1998). Doyle (1999) notes a reduced effect of plant extracts related to fat in meat. Also, it is important to observe that citrus extracts which showed the lowest antibacterial activity, appear to be more effective than the others to control LAB growth during the storage of meatballs. This is likely to be related with the lowered water activity within the product that would be the result of adding the citrus fruit preparations as a dry powder which contains fibre with high water absorption, as reported by Lario *et al.,* (2003). Finally, the present data are in accordance with that obtained by Hegazy, (2015) who reported that the

addition of different plant parts to meatball including herbs/spices leads to decrease in LAB values during the storage process. Also, adding the gum Arabic (GA) with the plant parts extracts to the meatball samples induced more decreasing in LAB when compared with the similar samples without GA addition.

Conclusion

The data of the present work with that carried out by the others could be represent the mile stone towards the extension of using plant parts i.e. herbs/spices extracts as natural antioxidants in many different food technological applications particularly in the field of food preservations. Data of the present study confirmed that there is a trend to search for compounds that may allow a shift from synthetic to natural antioxidants. This trend is justified because a carcinogenic potential from the use of synthetic antioxidants has been suspected.

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تأثير إضافة المستخلصات الطبيعية على خواص الجودة فى منتجات اللحوم التى تعرضت للحفظ بالتبريد

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تهدف الدراسة الحالية إلى إستكشاف مدى تأثير إضافة بعض المستخلصات النباتية الطبيعيه المضاده للأكسده والنمو الميكروبى على بعض منتجات اللحوم والتى تنعكس بدورها على مدة تخزينها وصلاحينها للإستهلاك الآدمى. تشير النتائج الى تاثر معنوى لمحتوى اللحوم من مركب المالنولدهايد (MDA) بكلا من النباتات المضافه وفترة التخزين. القيم الأوليه لمركب الـ MDA للعينات المستخلصه اقل معنويا من العينه الكنترول. بالنسبه للعينه الكنترول كان لمركب الـ MDA فى بداية التجربه 0.29 mg/kg وزاد حتى وصل بعد تخزين لمدة 12 يوم على درجة حراره 4سليزيس الى 4.73 mg/kg. انخفض معدل تكون مركب الـ MDA بكل عينات اللحوم المعامله بالنباتات وكان اعلى انخفاض بالعينات المعامله بالزعتر والبردقوش والكزبره (231.03, 337.93 and 275.86 %) على التوالى. وكان اقل انخفاض لبذور الشبت بنسبة (172.41%). وبالنسبه لخلطات الأعشاب { مزيج 1 (روزمارى+ زعتر+ قرنفل) ومزيج2 (كزبره+ كمون+ شبت)} فكان لها تاثير عالى فى خفض تكوين مركب الـ MDA بنسبة 462.07 و ,834.48% على التوالى. كما ظهر نفس السلوك بالنسبة لتاثير المستخلصات الطبيعيه على المحتوى الميكروبى (مستوى بكتريا حامض اللآكتيك) للحوم المخزنه. لذلك تؤكد نتائج الدراسة على استخدام مستخلصات الأعشاب والتوابل كمضات أكسدة طبيعية بديلا عن مثيلاتها الصناعيه التى أثبتت بعض الدراسات الحديثة إحتمالية تصنيفها كمواد مسببة للسرطان.

الكلمات المفتاحية: كرات اللحم- المالونالدهيد- بكتريا حمض اللآكتيك- مضادات الأكسدة الطبيعية.