

Effects of Selected Plant-Derived Nutraceuticals on the Quality and Shelf-Life Stability of Frankfurter Type Sausages during Storage

Kazem Alirezalu, Javad Hesari, Zabihollah Nemati, Boukaga Farmani

Abstract—The application of natural plant extracts which are rich in promising antioxidants and antimicrobial ingredients in the production of frankfurter-type sausages addresses consumer demands for healthier, more functional meat products. The effects of olive leaves, green tea and *Urtica dioica* L. extracts on physicochemical, microbiological and sensory characteristic of frankfurter-type sausage were investigated during 45 days of storage at 4 °C. The results revealed that pH and phenolic compounds decreased significantly ($P < 0.05$) in all samples during storage. Sausages containing 500 ppm green tea extract (1.78 mg/kg) showed the lowest TBARS values compared to olive leaves (2.01 mg/kg), *Urtica dioica* L. (2.26 mg/kg) extracts and control (2.74 mg/kg). Plant extracts significantly ($P < 0.05$) reduced the count of total mesophilic bacteria, yeast and mold by at least 2 log cycles (CFU/g) than those of control samples. Sensory characteristics of texture showed no difference ($P > 0.05$) between sausage samples, but sausage containing *Urtica dioica* L. extract had the highest score regarding flavor, freshness odor, and overall acceptability. Based on the results, sausage containing plant extracts could have a significant impact on antimicrobial activity, antioxidant capacity, sensory score, and shelf life stability of frankfurter-type sausage.

Keywords—Antimicrobial, antioxidant, frankfurter-type sausage, green tea, olive oil, shelf life, *Urtica dioica* L.

I. INTRODUCTION

IN recent years, demand for healthier meat and meat products with reduced content of fat, decreased levels of nitrite and sodium chloride, modified composition of fatty acid profile and incorporated health enhancing ingredients such as plant extracts are rapidly increasing world-wide [1]. Frankfurter-type sausage is popular meat product enjoyed by millions of consumers. However, an increased concern about its shelf life and nutritional quality has led the food technology to develop new formulations meat products [2].

Consumers prefer natural additives instead of synthetic antimicrobials and antioxidants in meat products due to the

health risks involved. Therefore, extensive studies which have been performed to replace of chemical additives with plant-derived nutraceuticals have increased in recent years [3]. Plant extracts are the phenolic compounds which are known to have antioxidative, antimicrobial, anti-inflammatory, and antiviral aspects. It has been proved that plant extracts protect low-density lipoprotein from oxidation, decrease the blood pressure in animal, and inhibit the lipid oxidation [4], [5]. In addition, anti-inflammatory, antiviral, and neuroprotective properties of the olive leaves extract [6], antimicrobial, antioxidant, anti-carcinogenic and anti-arteriosclerotic aspects of the green tea and *Urtica dioica* L. extracts [7], [8] have been reported.

According to Xi et al. [9], green tea extract is capable of inhibiting growth of a number of molds, yeasts, spoilage bacteria and foodborne pathogens. *Urtica dioica* L. is mainly used in the pharmaceutical industry due to its antiviral characteristics. *Urtica dioica* L. leaves extract is an antimicrobial agent and natural phenolic antioxidant and has been shown to significantly reduce lipid oxidation in model systems [10], decrease microbial count the Turkish dry-fermented sausage [11], and improve healthier compounds, essential amino acids, ascorbic acid and other minerals especially Fe in fresh pork sausages [12]. The purpose of the present study was to apply natural antioxidant and antimicrobial in order to improve the functional properties and extended shelf life of frankfurter-type sausage during storage.

II. MATERIALS AND METHODS

A. Preparation of Plant Extracts

Plant extracts were achieved from the grounded olive leaves, green tea and *Urtica dioica* L. according to the method described by Ebrahimzadeh et al. [13], with some modifications as followed. Briefly, leaves were dried (48 h at 40 °C), then ground. 100 g of the plant material was added to Erlenmeyer flask containing the 1000 ml of ethanol 95% solution, and mixed by magnetic stirrer for 48 h. After filtering (Whatman No.1) solution, ethanol was evaporated by rotary evaporator at 40 °C. Plant extracts in 500 ppm concentration were used to produce the frankfurter sausage.

B. Frankfurter-Type Sausage Manufacture and Sampling

Frankfurter-type sausage containing green tea, *Urtica dioica* L. and olive leaves extracts were prepared by a local meat processing plant. Based on our previously obtained results, frankfurter-type sausage incorporated with 500 ppm

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plant extracts concentration had the highest shelf life and sensory characteristics. All TRTs, about 1 kg each, were replicated from a separate beef meat source as three different productions. The following raw materials and ingredients were determined (g per kg) of beef meat (550), sunflower oil (120) ice/water (210), NaCl (15), seasoning (20), starch, soybean flour and other dry materials (81.5), sodium polyphosphate (3.5), sodium ascorbate (0.4) and sodium nitrite (0.012). The beef meat was chopped into cubes approximately 3 mm and ground in a commercial food grinder (Mado, Germany). Afterwards, the minced meat was homogenized with half of the ice/extract (Green tea, *Urtica dioica* L. and olive leaves extracts), NaCl and sodium polyphosphate in a cutter (Kilia EX3000 RS, Germany) for 12 min at 10 °C. Then, starch, soybean flour, seasoning, sodium nitrite and other dried ingredients were slowly added, and the mixture was homogenized for 1 minute. Finally, the remaining half of ice/extract and other remaining ingredients were added and mixed about 2 min until the meat emulsion reached 14 °C. The emulsion was mechanically stuffed (Handtmann VF50, Germany) into polyamide casings. Sausages were cooked by steam at 80-85 °C for 90 min to an internal temperature of 72 °C. After steam cooking, samples were immediately chilled with cold water shower, vacuum packed in polyethylene container and stored at 4 °C for 45 days. Physicochemical, microbiological, and sensory properties of sausage samples were analysed in 1, 15, 30, and 45 days of storage.

C. Proximate Composition

The pH was determined with a pH meter (Hanna, Methrom, Switzerland) according to the method described by Liu et al. [14]. The level of moisture, fat, protein and ash was determined according to the methods of AOAC [15].

D. Thiobarbituric Acid Reactive Substances Assay (TBARS)

TBARS value was measured using a spectrophotometer (UV 2100, Unico Scientific Instruments, New Jersey, USA) according by Faustman et al. [16]. Absorbance was assigned at 532 nm and lipid oxidation was reported as mg malondialdehyde/kg sausage.

E. Total Phenolic Content

The total phenolic content of samples was determined colorimetrically, using the Folin-Ciocalteu reagent described by Liu et al. [14]. Measurements were carried out at 725 nm using a UV-VIS spectrophotometer Hitachi U-3210 (Hitachi, Ltd., Tokyo, Japan). Results were expressed as mg of gallic acid (GA) per 100 gram dry weight.

F. Microbiological Analysis

25 grams of frankfurter-type sausage were homogenized in 225 ml of 0.1% peptone water for 3 min using a sterile bag blender (Neutec; Paddle Lab Blender, USA). Serial dilutions were then made with the same diluent. Plate count agar (PCA, Merck, Darmstadt, Germany) and violet red bile agar (VRB Agar, Merck) and dichloran rose-bengal chloramphenicol agar (DRBC Agar, Merck) were used for enumeration of total mesophilic aerobic (30 °C for 48-72 h), coliform (37 °C for 24

h), mold and yeast (25 °C for 5 days) count, respectively. Microbial count was expressed as log₁₀ colony forming units (CFU) per gr of sausage [17].

G. Sensory Evaluation

The effect of different plant extracts on the organoleptic characteristics of frankfurter-type sausage was evaluated by 12 experienced panelists (eight females, four males). Intensity of appearance color, flavor, odor, texture attributes and overall acceptability were determined on a 5-point scale where '5' corresponded to 'very strong' and '0' corresponded to 'none'. The overall acceptability was obtained as the sum of the attributes scores [18].

H. Statistical Analysis

Data were submitted to an analysis of variance according to a repeated measures experimental design with the MIXED procedure of the statistical analysis software. The groups significantly different from each other were compared by least square means. A $P < 0.05$ was considered to indicate statistical significance. All data were determined in triplicate and are reported as means and standard errors of means.

III. RESULTS AND DISCUSSION

The inclusion of plant extracts did not result in a significant difference ($P > 0.05$) in the proximate compositions of the sausage samples (Table I). The obtained results were consistent with the proximate composition results of the studies carried out with same sausage [19]. Changes in the pH values of frankfurter-type sausage containing plant extracts (500 ppm) during storage period are shown in Table II. The pH of sausage samples was significantly affected ($P < 0.05$) by the addition of plant extracts during storage. The pH of the sausage samples decreased slightly at the first of storage period, due to addition of plant extracts with pH 5-6 [20].

TABLE I
PROXIMATE COMPOSITION OF SAUSAGES CONTAINING PLANT EXTRACTS

Treatment	Properties (%)			
	Moisture	Fat	Protein	Ash
Control	59.67±0.21a	18.28±0.42a	13.74±0.09a	2.58±0.15a
GTE	60.36±0.45a	18.36±0.41a	13.33±0.64a	2.65±0.24a
UDE	61.25±0.36a	17.99±0.53a	13.69±0.23a	2.74±0.18a
OLE	60.83±0.37a	17.48±0.33a	13.96±0.31a	2.79±0.32a

^aNo significance within each column ($P > 0.05$).

The pH decreased significantly ($P < 0.05$) from 6.51 to 5.24 in all sausage samples, which could be due to production of organic acids by acid production microorganisms [21]. The pH of frankfurter-type sausages containing plant extracts and control sample varied with the storage period. In the other words, pH values for sausage containing *Urtica dioica* L. extract had significantly ($P < 0.05$) the highest level of pH at the end of storage.

Data showed that pH for all treatments were within the accepted range during storage period. The obtained results were in agreement with literature that pH of sausage decreased gradually during storage [22]. Hayes et al. [23] showed that

the pH of raw beef patties containing lutein, sesamol, ellagic acid and olive leaf extract decreased from 5.7 to 5.5 over the 12-days storage period but natural antioxidants had no influence on pH ($P > 0.05$) in relation to control.

TABLE II
CHANGES IN pH OF SAUSAGES CONTAINING PLANT EXTRACTS AT 4 °C DURING STORAGE

Treatment	Storage time (Day)			
	1	15	30	45
Control	6.51±0.03Aa	6.02±0.02Cb	5.79±0.04Bc	5.24±0.02Dd
GTE	6.26±0.04Ca	6.18±0.05Bb	5.97±0.03Ab	5.74±0.06Bd
UDE	6.33±0.06Ca	6.25±0.02Ba	6.01±0.03Ab	5.98±0.04Ab
OLE	6.42±0.04Ba	6.37±0.03Aa	5.94±0.05Ab	5.57±0.06Cc

^{a-d} pH level within each row with different letters differ significantly ($P < 0.05$).

^{A-D} pH level within each column with different letters differ significantly ($P < 0.05$).

Lipid oxidation of a meat and meat products could be a useful index to predict oxidative stability [23]. Results on the lipid oxidation of frankfurter-type sausage as affected by addition of green tea, *Urtica dioica* L. and olive leaves extracts as natural antioxidants stored at 4 °C for 45 days are reported in Table III. Lipid oxidation increased significantly ($P < 0.05$) in sausage samples during storage; however, the rate of the increase in the control was higher than in the other samples. Green tea, *Urtica dioica* L. and olive leaves extracts have antioxidant activity dependent on plant extract type and content [24].

Green tea, *Urtica dioica* L. and olive leaves extracts reduced ($P < 0.05$) lipid oxidation in comparison with the control by an average of 40%, 20% and 26%, respectively, at the end of 45 days storage. It had been reported that treated ground beef with 500 ppm *Urtica dioica* L. extract and MAP showed the lowest TBARS values compared to other groups during storage [25]. Antioxidant mechanism of phenolic compounds is mainly due to their capacity in chelating metal ions and trapping reactive oxygen species, which could

generate radicals [26].

The samples involved green tea extract had lowest TBARS values comparing to control sausage and sausage containing olive leaves and *Urtica dioica* L. extracts at 45 days of storage. The high content of phenolic compounds contained in plant extracts may cause its strong antioxidant ability [23]. The same authors observed strong antioxidant effect of *Urtica dioica* L. extract in Turkish dry-fermented sausage [24], olive leaves extract in cooked pork sausages [27] and green tea extract in bologna type sausages [28].

Phenolic content of sausage samples decreased significantly ($P < 0.05$) during storage (Fig. 1). Phenolic compounds can be reduced during heating and storage through oxidative reactions [29]. The highest phenolic compounds were noticed in sausage containing 500 ppm green tea extract compared to other tested samples (Fig. 1).

Plant extracts are rich in phenolic compounds which are secondary plant metabolites in a wide range of specialized functions [30]. The health and technological benefits associated with plant extract application in functional meat products have been attributed to the antioxidant, free radical-scavenging and antimicrobial activity of phenolic compounds [31]. Previously published data showed that phenolic compounds play also a major role in preventing certain chronic human diseases.

TABLE III
CHANGES IN TBARS VALUES OF SAUSAGE CONTAINING PLANT EXTRACTS AT 4 °C DURING STORAGE

Treatment	Storage time (Day)			
	1	15	30	45
Control	0.36±0.04 ^{Bd}	1.41±0.03 ^{Ac}	2.13±0.03 ^{Ab}	2.74±0.06 ^{Aa}
GTE	0.25±0.04 ^{Cc}	1.19±0.06 ^{Bb}	1.25±0.04 ^{Cb}	1.78±0.08 ^{Da}
UDE	0.53±0.07 ^{Ad}	0.98±0.07 ^{Cc}	1.88±0.09 ^{Bb}	2.26±0.07 ^{Ba}
OLE	0.34±0.07 ^{BCd}	0.71±0.07 ^{Dc}	1.36±0.06 ^{Cb}	2.01±0.07 ^{Ca}

^{a-d} TBARS values level within each row with different letters differ significantly ($P < 0.05$).

^{A-D} TBARS values level within each column with different letters differ significantly ($P < 0.05$).

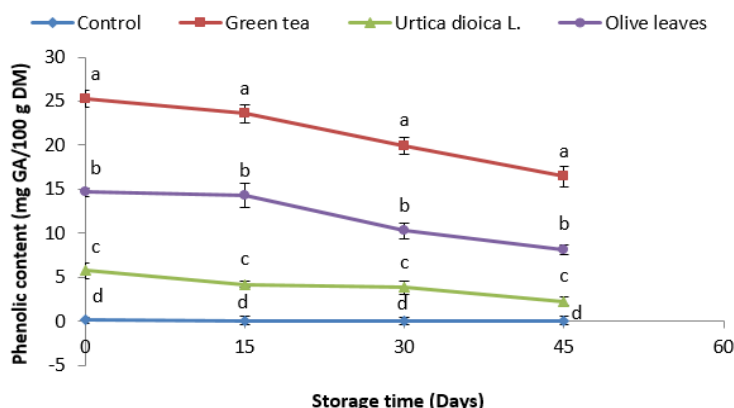


Fig. 1 Changes in phenolic content of sausages containing plant extracts at 4 °C during storage

Total mesophilic aerobic count increased significantly ($P < 0.05$) in sausage samples during storage. Sausage containing *Urtica dioica* L. extract had the lowest total count at the end of

the storage (Fig. 2 (a)). On the other hand, plant extracts can be effect on microbial count and shelf life which concur with Aksu and Kaya [11] on sucuk. Alp and Aksu [25] found that

MA packaging and 500 ppm *Urtica dioica* L. extract had significant effects ($P < 0.05$) on mesophilic, psychrotrophic, pseudomonas and lactic acid bacteria count in sucuk. The

obtained results were paralleled to Paiva-Martins et al. [32] on pork meat and Siripatrawan and Noipha [33] on pork sausage during storage.

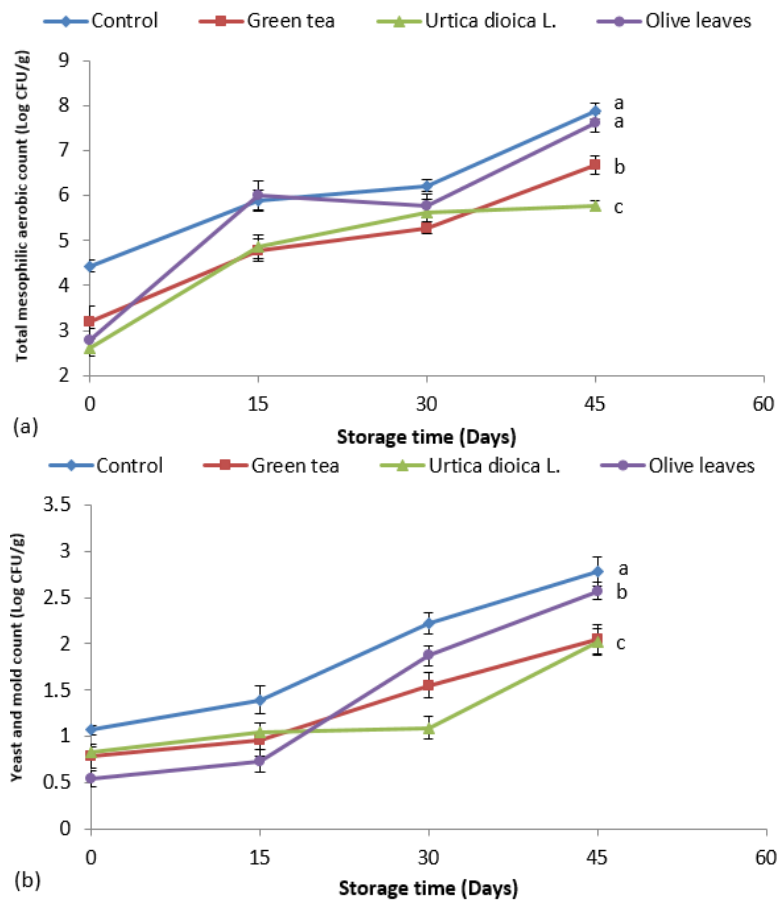


Fig. 2 Changes in total viable count (a), yeast and mold count (b) (log cfu/g) of sausages containing plant extracts at 4 °C during storage

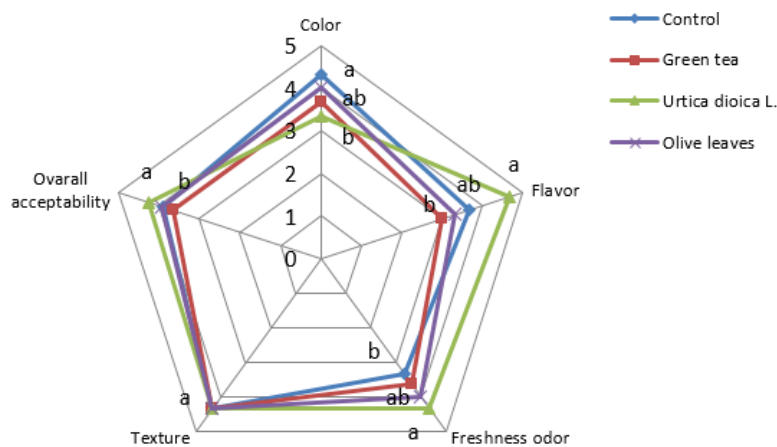


Fig. 3 Changes in sensory scores of sausages containing plant extracts at 4 °C during storage

Plant extracts decreased significantly ($P < 0.05$) mold and yeast count of sausage samples (Fig. 2 (b)). Results showed that there is a significant increase ($P < 0.05$) in mold and yeast count during storage. It was also found that the count of yeast

and mold was the highest in control (2.78 Log CFU/g) and sausage containing olive leaves extract (2.57 Log CFU/g) at the end of storage. However, treated samples containing 500 ppm *Urtica dioica* L. and green tea extracts had significantly

($P < 0.05$) lower count as compared to other treated sausages. Coliform bacteria are commonly used bacterial indicators of hygienic quality of meat and meat products [34]. Coliforms were not detected probably due to primary meat quality and hygienic processing in any treated sausages and control at the 4 °C. Similarly, Aksu and Kaya [11] found that *Urtica dioica* L. extract inhibited Enterobacteriaceae in sucuk.

Fig. 3 shows the effect of green tea, *Urtica dioica* L. and olive leaves extracts on sensory properties of frankfurter-type sausage. The highest color score was observed in control sausage and also flavor, freshness odor and overall acceptability in sausage containing *Urtica dioica* L. extract at the end of storage. The texture scores in all sausage samples were acceptable and there had no any significant difference ($P > 0.05$). Otherwise, plant extracts retarded sausage spoilage and limiting the subsequent formation of off-flavors in the meat products [27].

IV. CONCLUSION

Results of the present study demonstrated the effectiveness of green tea, *Urtica dioica* L. and olive leaves extracts on microbial growth inhibition, sensory characteristics and shelf life extension of frankfurter-type sausage during refrigerated storage. Plant extracts are rich in healthier components, antimicrobial activity and antioxidant capacity and have particular importance in improving the sausage quality. Although the quality of the common sausages is acceptable but incorporating the plant extracts was deemed necessary to obtain value added sausages with. Sausage containing green tea extract had the highest phenolic compounds, antioxidant capacity and shelf life, sausage containing *Urtica dioica* L. extract had the highest microbiological quality and sensory scores and sausage containing olive leaves extract had the highest texture scores. Therefore, the application of mixed extracts is proposed to improve quality and shelf life stability of frankfurter-type sausage.

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