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THE EFFECT OF POLYPHENOLS FROM *POLYGONUM MULTIFLORUM* THUNB. ROOT EXTRACT ON THE STORAGE OF GROUND BEEF

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Abstract: *The goal of this research is to evaluate the influence of the polyphenols extract of Polygonum multiflorum Thunb. root on the lipid oxidation, chemical properties and sensory characteristics of ground beef during frozen storage. Beef was ground in aqueous solutions of polyphenols extract at different concentrations: 830, 415, 277, 208 and 166 mg GAE/L, polyphenols solution/sample ratio is 1/20 (v/w). Then, the ground beef was stored for up to 100 days at -20±2^{o}C. The best oxidation inhibitor for ground beef was at the highest polyphenols concentration of 830 mg GAE/L. All quality parameters (pH, PoV, MDA, color parameter and sensory evaluation) of the treated sample and of the control sample display significant differences ($p<0.05$) during storage period. For this reason, it was concluded that the polyphenols extract of Polygonum multiflorum Thunb. root could be used as an alternative source of natural antioxidant in beef processing.*

Key words: *Extract, ground beef, lipid oxidation, polyphenols.*

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1. Introduction

As the third most consumed meat worldwide, beef accounts for about 25% of all meat after pork and poultry [15]. In Vietnam, the demand for beef has increased sharply over recent years and the government has to import a large amount of beef (about 15 000 tons in 2014) [2]. Beef is the main source of proteins for the human metabolic processes [4] and has many microminerals such as selenium, copper, zinc, manganese, and iron. These minerals are necessary because they have major roles in the metabolic pathways and in the antioxidative enzymatic system. In addition, beef also has the amount of lipid which contributes to its palatability, cooking characteristics and overall organoleptic properties [29]. There are many products made from beef such as sausage, beef pie, beef ball, etc., especially ground beef. However, the saturated fatty acid or cholesterol in beef can affect the level of acceptance of meat by consumers, and condition its nutritional value in accordance with the usual dietary recommendations [24] and shelf-life of products. Hence, using additives, which can inhibit the lipid oxidation, is very important. Currently, synthetic additives can harm the consumer's health, thus we need to replace them by an alternative source of natural antioxidant. However, because each polyphenol has a different mechanism involved in the antioxidant effectiveness, the activity of these compounds is quite difficult to predict.

Polygonum multiflorum Thunb. is a member of the *Polygonaceae* family. The plant is known as "Ha Thu O Do" (HTOD) in Vietnam. *Polygonum multiflorum* Thunb. is a precious plant and is one of the most important natural herbal sources. Its root has been used as medicine since thousands of years ago in Asia. The root of this plant is considered to be a valuable source of medicine because it contains a high level of bioactive compounds, and especially phenolic compounds such as flavonoids, tannins and anthraquinones. Besides, the phenolic compounds of the plant's root can be used widely for treatment, hair-blackening, liver and kidney detoxification, anti-aging effects [10], tonic tension [9], some kinds of cancer [28] and antioxidant activity [26]. In the food industry, many reports show that polyphenols in plants have positive effects on food storage or food processing, for instance, polyphenols of date seed extract [11], red grape pomace (peels and seeds) [18], green tea and onion extract [19]. Until now, no research has studied the influences of polyphenols from *Polygonum multiflorum* Thunb. root on frozen storage methods to preserve ground beef.

The main aim of this research is to determine the effects of polyphenols extract from *Polygonum multiflorum* Thunb. root on the physicochemical and sensory parameters of ground beef. A better understanding of the reactions that occur during the storage process enables higher quality products.

2. Materials and Methods

2.1. Extract Preparation

Polygonum multiflorum Thunb. roots were harvested from Cao Bang province (Vietnam). The roots were then cleaned with tap water, sliced and dried at 60°C

until the moisture level was less than 12%. The slices were then ground into fine powder (diameter less than 0.5 mm) and vacuum-packed. Polyphenols from dried powder of *Polygonum multiflorum* Thunb. roots were extracted by microwave system with an acetone concentration of 57.35%, a solid/solvent ratio of 1/39.98, an extraction time of 289 seconds and a microwave power of 127 W [16] because the total phenolic content and antioxidant capacity achieved the best results in these conditions. The crude extract was filtered by means of Whatman filter paper. The filtered extract was evaporated at 45^{o}C until the solvent evaporated completely. Then, the extract was used for the preparation of 830, 415, 277, 208 and 166 mg GAE/L solution in distilled water.

2.2. Preparation of the Ground Beef

Beef samples were purchased from Go Vap market in Ho Chi Minh City 3 hours after slaughter. Each sample was cleaned, chopped and ground with various extract concentrations, using an extract solution/sample ratio of 1/20 (v/w). In addition, there were the control samples (untreated samples). Ground samples were placed in polyethylene bags and stored in the freezer at -50^{o}C. After that, all samples were moved to a freezer, which was kept at -20^{o}C during the storage time. These samples were analyzed after every 20 days up to 100 days of storage.

2.3. Chemicals and Reagents

Folin-Ciocâlteu and DPPH (2.2-diphenyl-1-picrylhydrazyl) reagents were purchased from Merck (Germany). TBA (2-Thiobarbituric acid) and TMP (Tetramethoxypropane) was supplied by Sigma-Aldrich (USA). All other chemicals and organic solvents were of analytical grade.

2.4. Determination of the Total Phenolic Content (TPC) and Antioxidant Capacity (AC) of the Extract

The TPC in the extract was slightly modified and determined by using the Folin-Ciocâlteu colorimetric method [22]. The results were based on a standard curve obtained with gallic acid. TPC was expressed as mg of gallic acid equivalent per gram of dry weight (mg GAE/g DW).

The AC of the extract was determined by DPPH assay which was adapted and modified according to a study of Soto et al. [23]. Trolox was used as the standard. AC was expressed in TEAC (Trolox equivalent antioxidant capacity) determined as μmol of Trolox per gram of dry weight (μmol TE/g DW).

2.5. Chemical Analysis

2.5.1. pH

According to Shim et al. [21], the pH value of 5 g samples blended with 20 mL distilled water for 60 seconds in a homogenizer (Panasonic 1L MX-AC400WRA, Japan) was determined with a pH meter (Trans Instruments BP3001, Singapore).

2.5.2. Peroxide Values (PoV)

The PoV was described by Seo et al. [20] with slight modifications. The lipids from minced samples (5 g) were homogenized with 50 mL of acetic acid-isooctane for 5 minutes. Then, samples were filtered by means of Whatman filter paper. The filtrate

was then added to 1 mL saturated potassium iodine solution and shook gently for 1 minute. 100 mL of distilled water and 0.5 mL of 0.1% starch solution were subsequently added to the mixture. The final solution was titrated with a 0.01 N sodium thiosulfate ($Na_2S_2O_3$) solution until the violet color disappeared. The results were expressed as meq oxygen/kg sample.

2.5.3. Thiobarbituric Acid Reactive Substances (TBARS)

The TBARS values were determined through the Vyncke [25] method with some slight modifications. Firstly, 5 g of samples were homogenized in 20 mL of 10% trichloroacetic acid (TCA) solution and 0.5 mL of BHT. Then, samples were filtered by means of Whatman filter paper and made up to 100 mL of 10% TCA solution. The filtrate (5 mL) was mixed with 5 mL of 0.02 M 2-thiobarbituric acid (TBA) solution, heated in a boiling water bath for 35 min at 100^{o}C to develop the rose-pink color from the reaction between malondialdehyde and TBA, then cooled by tap water for 10 min. Absorbance was measured at 532 nm against a blank prepared with 5 mL of 0.02 M TBA solution and 5 mL of 10% TCA solution, using a spectrophotometer. TBARS values were described as µg of malondialdehyde (MDA)/kg of sample.

2.5.4. Color Parameters

Color parameters consist of L^* (lightness), a^* (from redness to greenness), b^* (from yellowness to blueness) values were recorded and were carried out by using a Chroma Meter CR-400 (Minolta, Japan). The instrument was calibrated with a standard light plate and three different positions on the surface of each sample were measured for each storage time.

2.5.5. Sensory Evaluations

Samples were prepared according to Masniyom et al. [12] with some slight modifications. The ground frozen samples were cut into 30×20×20 mm cubes, thawed, wrapped with aluminum foil and steamed for 15 min at 70^{o}C. The 60 non-trained panelists evaluated the steamed ground beef by color, odor, taste, texture and overall acceptability; using 9-point hedonic scales from 1 (dislike extremely) to 9 (like extremely) [13].

2.6. Data Analysis

The experimental data were analyzed by using the one-way analysis of variance (ANOVA) method and significant differences among the means from triplicate analyses at ($p<0.05$) were determined by Fisher's least significant difference (LSD) procedure using the Statgraphics software (Centurion XV). The values obtained were expressed in the form of a mean±standard deviation (SD).

3. Results and Discussion

3.1. Total Polyphenol Content and Antioxidant Capacity Of Extract

The TPC and AC values of the extract achieve 47.53±0.79 mg GAE/g DW and 334.07±3.04 µmol TE/g DW, respectively. TPC and TEAC of samples from MAE method were higher than samples from China which were extracted through decoction methods using distilled water as solvent (33.91±0.62 mg GAE/g DW; 257.9±3.7 µmol TE/g DW) and maceration

methods with 50% ethanol as solvent (40.42±0.63 mg GAE/g DW; 256.7±0.7 µmol TE/g DW) [8]. The results showed that the differences in extraction methods, land and gender, etc. which cause changes in TPC and AC values. The crude extract was filtered and evaporated at 45°C until the solvent evaporated completely. The extract was then used for the preparation of 830, 415, 277, 208 and 166 mg GAE/L solution in distilled water to mince with beef.

3.2. Changes in the PoV

Figure 1 shows that the PoV of all samples increased steadily during storage time and they were significantly different ($p<0.05$). Initial samples have the PoV of 0.4 meq/kg. After 100 days of storage, the PoV of the control samples and treated samples (208 and 166 mg GAE/L) reached the highest value of 1.77, 1.97 and 1.9 meq/kg, respectively, whereas the PoV of treated samples at the concentration of 830 mg GAE/mL is only 1.53 meq/kg.

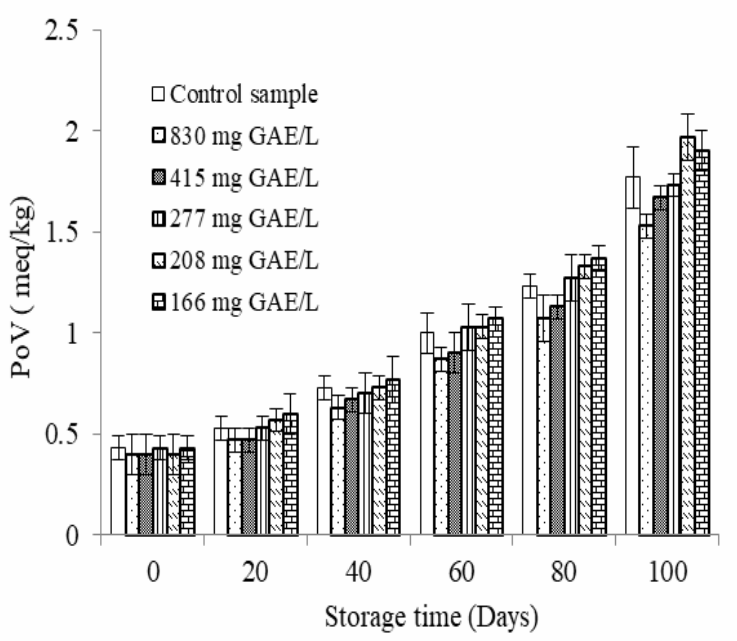

Fig. 1. *PoV (meq/kg) of ground beef during the storage*

The autoxidation is a spontaneous reaction of molecular oxygen with lipids, leading to oxidative deterioration and proceeds by a free radical chain mechanism in meat [27]. The results above show that the addition of *Polygonum multiflorum* Thunb. extract at a high concentration significantly inhibits the increase of the PoV. Therefore, the presence of the polyphenols extract may retard lipid oxidation by preventing the formation of free radicals or by interrupting the propagation of the free radical by several mechanisms such as scavenging species that initiate peroxidation, quenching $^{*}O_2$ preventing formation of peroxides, breaking the autoxidative chain reaction, reducing localized O_2 concentrations and chelating metal ions so that they are unable to

generate reactive species or decompose lipid peroxides [14].

The result of this study is similar to that of the research of Lee et al. [7], in which the authors used the mustard leaf (*Brassica juncea*) kim chi extract to inhibit an increase of PoV in ground pork meat or of Yu et al. [30] who stored cooked and raw ground beef with peanut skin phenolic extract as antioxidative and antibacterial agent against the lipid oxidation.

3.3. Changes in the pH Value

Figure 2 shows that the pH values have significant differences ($p<0.05$) throughout the frozen storage time and decreased slightly after 100 days of frozen storage. None of the samples showed any signs of damage such as a rancid odour. The pH values ranged from 5.6 to 5.9 and the pH values of the control sample are lower than those of other treated samples during storage time. The above results proved that the extracted polyphenols affected strongly the pH values and could change some chemical properties of the samples. In addition, there are many causes which can change the pH value such as initial raw material, stress levels of animal, storage methods and the kind of polyphenols in the extract, etc. In this case, the decrease of the pH value also helps to limit the growth of microorganisms.

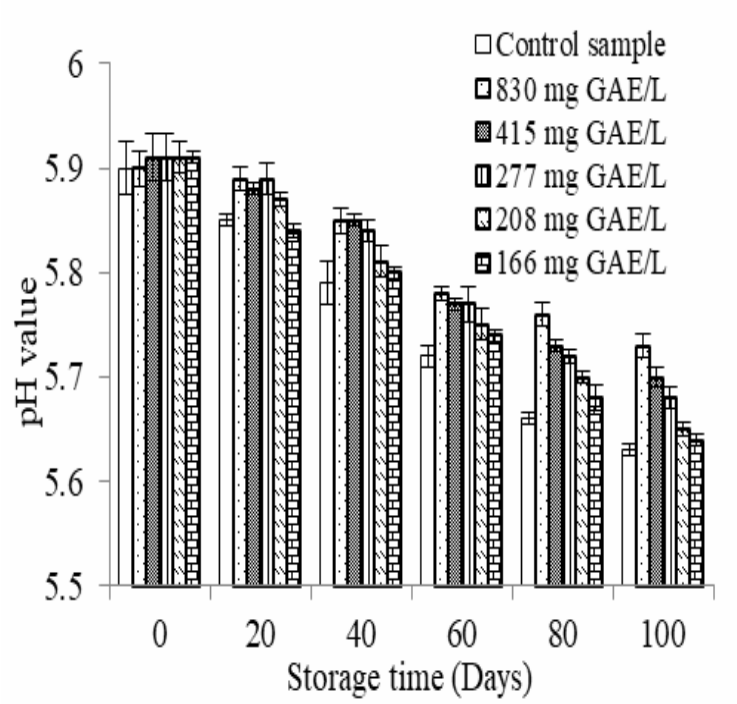

Fig. 2. *pH values of ground beef during the storage*

Meat can experience a degradation of qualities during storage because of the microbial enzyme; the protein produces non-proteinic nitrogen, which leads to the

increase of pH value [5]. However, the result contrasts with that of other studies, for instance, Aubourg et al. [3] who preserved horse mackerel (*Trachurus trachurus* Linnaeus) by the freezing method with citric and ascorbic acid and the pH did not change significantly. In addition, this result is also similar to the study of Shim et al. [21] who preserved raw ground pork at 4°C by adding onion peel extract with a slight decrease of pH values.

3.4. Changes in the Color Parameters

The color of the meat is an important factor for consumers in evaluating the quality of the product. According to Table 1, the color parameters (L^*, a^*, b^*) of initial ground beef changed significantly during storage time ($p<0.05$). The L^* values decrease slightly, the a^* values decline sharply whereas the b^* values increase slowly. Changes in color parameters are quite complex.

Color parameters of ground beef during the storage Table 1

Storage time (Days)	Polyphenols concentrations of extract [mg GAE/L]					
	Control sample	830	415	277	208	166
			L^*			
0	50.55 ± 0.69^{Ab}	50.66 ± 0.37^{Aab}	50.12 ± 0.4^{Abc}	50.64 ± 0.66^{Abc}	50.46 ± 0.53^{Ac}	50.17 ± 0.23^{Ab}
20	46.81 ± 0.74^{Aa}	52.53 ± 0.28^{Db}	51.23 ± 0.65^{CDcd}	49.44 ± 1.16^{BCab}	48.32 ± 0.81^{ABb}	46.66 ± 0.61^{Aa}
40	45.92 ± 0.26^{Aa}	52.59 ± 2.36^{Bb}	51.74 ± 1.21^{Bd}	51.94 ± 0.89^{Bc}	48.53 ± 1.02^{Ab}	46.8 ± 2.06^{Aa}
60	45.97 ± 2.03^{Aa}	52.01 ± 1.7^{Dab}	49.94 ± 1.17^{CDbc}	48.22 ± 1.17^{ABCa}	48.69 ± 1.32^{BCb}	47.39 ± 0.23^{Aba}
80	46.05 ± 0.32^{Aa}	51.19 ± 0.17^{Bab}	49.66 ± 0.54^{Bab}	49.84 ± 1.26^{Bab}	46.26 ± 1.01^{Aa}	46.94 ± 1.41^{Aa}
100	47.24 ± 0.61^{ABa}	49.46 ± 0.6^{Ca}	48.26 ± 0.36^{BCa}	48.53 ± 0.36^{BCa}	48.49 ± 0.98^{BCb}	45.72 ± 1.7^{Aa}
			a^*			
0	22.2 ± 0.64^{Dd}	17.68 ± 0.21^{Ac}	17.71 ± 0.51^{Ad}	19.39 ± 0.17^{Be}	18.11 ± 0.95^{Ac}	20.93 ± 0.56^{Ce}
20	18.59 ± 0.5^{Ec}	9.18 ± 0.3^{Aa}	14.54 ± 0.6^{Cb}	12.67 ± 0.49^{Bb}	17.24 ± 1.29^{Dc}	15.57 ± 0.47^{Cd}
40	16.17 ± 0.91^{CDb}	12.36 ± 1.13^{Ab}	16.16 ± 0.52^{CDc}	15.59 ± 0.09^{BCd}	14.81 ± 0.26^{Bb}	17.13 ± 0.68^{Dc}
60	12.7 ± 0.3^{ABa}	12.13 ± 0.58^{Ab}	13.45 ± 1.28^{ABb}	14.3 ± 1.32^{Bcd}	14.19 ± 1.44^{Bb}	13.79 ± 1.19^{ABb}
80	12.38 ± 0.2^{Ba}	8.63 ± 0.44^{Aa}	11.97 ± 0.81^{Ba}	13.26 ± 1.15^{Bbc}	12.42 ± 0.79^{Ba}	12.46 ± 0.96^{Bab}
100	11.73 ± 0.53^{Ca}	8.95 ± 0.58^{Aa}	10.76 ± 0.56^{Ba}	10.74 ± 0.44^{Ba}	11.49 ± 0.59^{BCa}	11.85 ± 0.52^{Ca}
			b^*			
0	14.96 ± 0.52^{Ca}	13.56 ± 0.53^{Ba}	12.13 ± 0.12^{Aa}	13.28 ± 0.43^{Ba}	13.53 ± 0.19^{Ba}	14.48 ± 0.49^{Ca}
20	15.14 ± 0.86^{ABa}	15.03 ± 0.3^{ABa}	15.55 ± 0.43^{Bbc}	15.04 ± 0.5^{ABb}	16.72 ± 0.18^{Ccd}	14.42 ± 0.32^{Ba}
40	15.01 ± 0.42^{Aa}	14.95 ± 1.07^{Ab}	14.83 ± 2.2^{Abc}	16.66 ± 0.5^{Ac}	15.85 ± 0.5^{Abc}	16.76 ± 0.83^{Ab}
60	15.27 ± 0.48^{ABab}	14.26 ± 0.47^{Aab}	16.65 ± 1.2^{BCc}	16.69 ± 0.81^{BCc}	17.34 ± 1.33^{Cd}	16.07 ± 0.65^{BCb}
80	16.53 ± 0.34^{Bbc}	14.82 ± 0.36^{Ab}	14.6 ± 0.17^{Ab}	14.61 ± 0.86^{Ab}	15.19 ± 0.86^{Ab}	14.78 ± 0.42^{Aa}
100	17.01 ± 1.32^{Ca}	14.67 ± 0.77^{Aab}	14.99 ± 0.59^{ABbc}	16.42 ± 0.95^{BCc}	15.92 ± 0.3^{ABCbc}	16.23 ± 0.52^{BCb}

Different superscript lowercase letters in the same column denote significant differences ($p<0.05$).
Different superscript capital letters in the same row denote significant differences ($p<0.05$).

Keeping a red or pink color of the ground beef is a difficult challenge because meat color is influenced by oxidative deterioration (lipid oxidation and protein oxidation in meat) [6]. In particular, ground beef oxidizes and degrades faster than other meat product because its large surface can easily be exposed to oxygen in the air and bacteria, which can penetrate inside the meat [1].

Furthermore, color changes depend on several factors including raw materials, storage methods and process methods. Therefore, antioxidant usage in meat processing is necessary and could restrict the color changes in the products, especially antioxidants from herbs and spices. In this study, *Polygonum multiflorum* Thunb. roots extract strongly affect L^*, a^*, b^* values. In general, the color of the treated sample is more attractive than the control sample.

3.5. Changes in the TBARS Value

There are significant differences between the TBARS values of all samples during storage as these values increase steadily from day 0 to day 100 (Figure 3). The highest TBARS values were found in control samples because they did not contain polyphenols extract. After the 100 day storage, samples with the extract concentrations of 830 GAE/L had the lowest TBARS value of 0.245 mg MDA/kg while that of control samples was of 0.294 mg MDA/kg. In general, the TBARS values of meat constantly increased during storage time because aldehydes (for instance MDA, malondialdehyde) were formed. It is one important factor which causes the product to go rancid.

The above results show that the combination of the frozen storage method and polyphenols in *Polygonum multiflorum* Thunb. roots extract positively affect the inhibition of lipid oxidation. In this study, the polyphenols content in the extract was tested as a natural antioxidant to retard or prevent the increase of oxidative rancidity in ground beef. These results are in agreement with Alp and Aksu [1] who preserved ground beef by using the modified atmosphere packaging with water extract from *Urtica dioica* L. or Shim et al. [21] who preserved raw ground pork through refrigeration storage with onion peel extracts or Reihani et al. [17] who preserved beef patties by frozen storage with extracts from Ulam raja leaves (*Cosmos caudatus* Kunth).

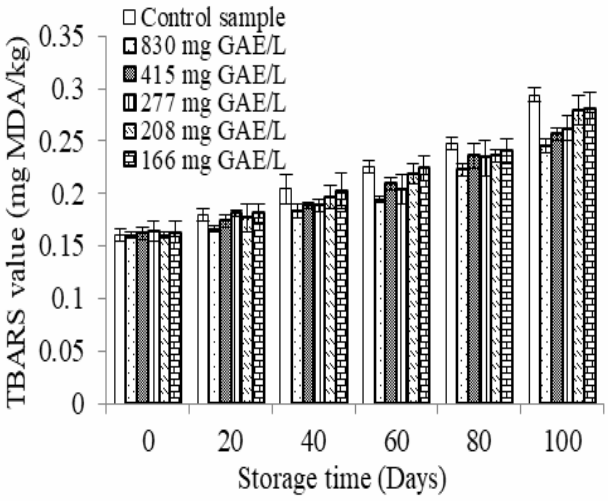

Fig. 3. *TBARS values (mg/kg) of ground beef during the storage*

3.6. Changes in Sensory Attributes

Phenolic compounds in *Polygonum multiflorum* Thunb. roots extract can change the qualities of the product including its sensory attributes, especially with high polyphenols concentrations of 830 mg GAE/L, which could inhibit significantly the increase of PoV, TBARS values and improve product color. Although the dark color of the botanical extracts could alter the visual aspect of the food products, phenolic compounds in *Polygonum multiflorum* Thunb. roots extract show superior effects in this study.

Samples with 830 mg GAE/L were chosen for sensory attribute evaluation and compared with control samples after 100 days of storage regarding their odor, color, taste, texture and overall acceptability. Figure 4 shows that there were significant differences in all sensory attributes ($p<0.05$) between control and treated samples. The scores of sensory attributes of treated samples are higher than those of control samples. Therefore, adding phenolic compounds from the extract can improve the sensory attributes of the product.

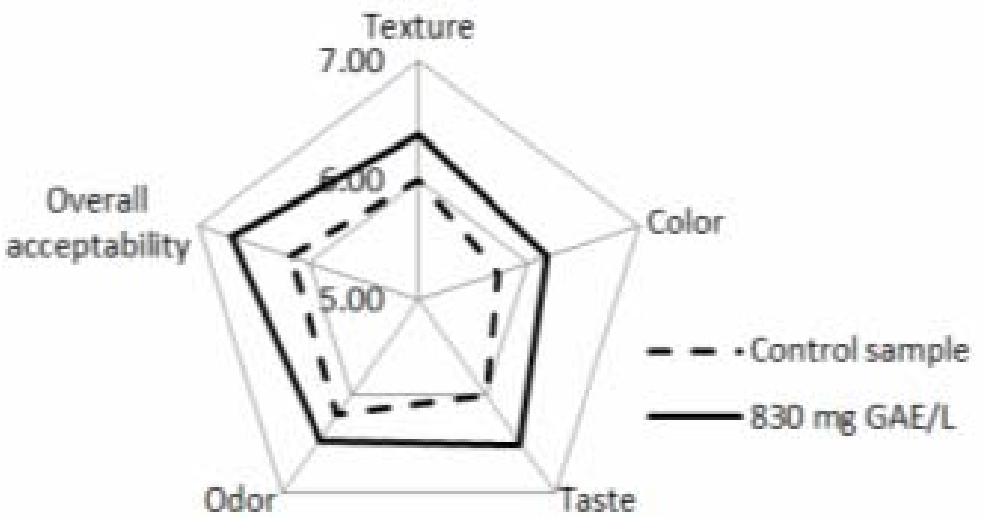

Fig. 4. *Sensory evaluation of steamed ground beef*

4. Conclusions

On the basis of the obtained results, polyphenols in *Polygonum multiflorum* Thunb. roots extract strongly affect the chemical properties of ground beef. It can inhibit the oxidation process of this kind of meat during storage time. Using polyphenols extract had many advantages such as improving the chemical-physical qualities indexes and the sensory indexes of the treated ground beef.

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