



## Prolongation Minced Meat Shelf Life Using Eggplant Leaves Ethanolic Extract

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Article History	Abstract
<p>Received: 23 June 2023 Revised: 13 Sept 2023 Accepted: 03 October 2023</p> <p>CC License CC-BY-NC-SA 4.0</p>	<p><b>Background:</b> This research aimed to extend the storage period of minced meat by adding eggplant leaves (<i>Solanum melongena</i>) ethanolic extract (ELEE) under the regular refrigeration conditions. Eggplant leaves were collected, washed, dried, crushed, soaked in 100% absolute ethanol, filtered and then concentrated. Ethanolic thick extract was added to a minced beef meat 1.5% w/w and stored at -5°C for 4 weeks. The DPPH of the ethanolic thick extract and phenolic content were determined. The pH, peroxide value, thiobarbituric acid and microbial content at zero time and after 30 days of freeze storage of two tested samples were assayed. Control and 1.5% samples were sensory evaluated by trained panelists (n=20) after storage period. Results revealed that 1.5%ELEE recorded highly DPPH radical scavenging activity and has good amount of total phenolic compounds. In addition, 1.5% of the tested extract caused an improvement in all estimated values like pH, peroxide value and thiobarbituric acid. This addition also preserved the minced meat from microbial growth after a period of 30 days of freezing. Also, the general acceptability of the sensory evaluation that was carried out on the minced meat sample supplemented with 1.5% of the extract and compared to the control sample indicated that it was acceptable from panelists. Therefore, according to the obtained results, eggplant leaves ethanolic extract may be beneficial for extending the storage period of some meat products and more researches should be done on using eggplant leaves with other industrial meat products.</p> <p><b>Key words:</b> Eggplant leaves, Ethanolic extract, Beef meat, Storage period</p>

### 1. Introduction

Humans can use the high levels of nutritious ingredients in meat to meet their daily nutritional needs. Protein, vitamin B, iron, and fat are nutrients found in meat that help to improve human health. Fresh meat is very conducive to the microbial growth because of its high-water content, optimal pH and abundant nutrients (Liang et al., 2021), which will lead to the spoilage of fresh meat. Since fat has an impact on the flavour, softness, and juiciness of meat products, it plays a crucial role in the manufacturing process. However, during manufacturing and storage, meat products were microbial contaminated and easily oxidized, which led to serious damage to the digestive tract Sembring and Chin, (2021). Meat is not only highly susceptible to spoilage, but also frequently implicated in the spread of food borne illness Podpecan et al. (2007).

To minimize oxidative reactions, prevent microbiological growth, and ultimately lengthen the shelf life of meat products, several synthetic preservatives are used. The necessity of investigating the usefulness of natural preservatives in extending the shelf life of stored meat products while keeping their safety and quality has been brought on by a growing concern of human health and the appropriate fear of

artificial substances. In recent years, special attention has been paid to some plants that could be used as potential sources of antioxidants for meat and meat products preservation and nutritional quality improvement Ghoneim et al., (2023).

Phenolic metabolites are common components of fruits and vegetables and have high antioxidant activity. The antioxidant properties of phenolic acids and flavonoids depend on their redox properties and chemical structure, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. Additionally, some compounds have chelating activity, which prevents transition metals to act as oxidation promoters (Kumar et al., 2015).

Flavonoid, which are often present in many plants and contain phenolic compounds, have a variety of functions, including antioxidant and antibacterial activities. In oxidative stress, which can lead to many degenerative diseases, antioxidants can stop the overproduction of free radicals. Fruits and vegetables include phenolic and flavonoid chemicals that have antioxidant potential, therefore eating them can provide natural antioxidants. Previous studies suggested that the antioxidant properties of phenolic and flavonoid compounds could be linked. The antioxidant-active compounds flavonoid and tannin were abundant in eggplant (*Solanum melongena*) Fidrianny et al., (2017). Therefore, this research aimed to extend the storage period of minced beef meat using 1.5% eggplant leaves ethanolic extract (ELEE).

## **2. Materials and Methods**

### **Materials:**

#### **Eggplant leaves extract**

Eggplant (*Solanum melongena*) were collected from Agriculture Research Center Ministry of Agriculture, Giza, Egypt. Leaves were washed carefully with tap water, followed by distilled water and air dried for 10 days. Dried Leaves were crushed into fine powder by using electrical blender. Eggplant fine powder was soaked in 100% absolute ethanol at 7.5 times of powder weight for three days. The extract was left for one day to stand to form the sediment; then, the extract was filtered. A rotary evaporator concentrated the filtered extract at a temperature of 45 °C to obtain a thick ethanolic eggplant leaves extract (ELEE) Hassanien et al., (2022). The obtained extract was added to minced beef at 1.5% concentration (w/w).

#### **Minced beef preparation**

A two-kilograms of minced beef meat were purchased from butcher shop in Agriculture Research Center, Ministry of Agriculture, Giza, Egypt, packed in polyethylene bags Rahman et al., (2015). Minced meat was divided into two samples one of them served as control and the other mixed carefully in electrical blender with 1.5% ELEE (w/w). All samples packaged in polyethylene bags and stored for one month at -5 °C. Each sample was analyzed in the first and 30th day of storage.

### **Methods:**

#### **Chemical analysis**

The 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) assay for the ELEE was determined according to the method described by Brand-Williams et al., (1995). Total phenolic content of the extract was determined according to Ainsworth and Gillespie, (2007). The pH, peroxide value and thiobarbituric acid were determined according to Barriuso et al., (2013), Raharjo et al., (1992) and Zhang et al., (2019), respectively, in the first and the end of the experiment period (one month).

#### **Microbial analysis**

Total viable fungi and total yeast count were demonstrated by the method described by Dave and Ledwani (2012). *Staphylococcus aureus* count was assayed by the method described by Anawar et al., (2004).

### Panel test

After one month of freezing, control and 1.5% ELEE sampled were cooked without any additives and sensory evaluated. Twenty trained panellists evaluated the smell, taste, texture, appearance and overall acceptability of two samples Stone and Sidel, (1993).

### Statistical analysis

Obtained results were analysed using Statistical Package for the Social Sciences (SPSS) for Windows. Collected data were presented as mean  $\pm$  standard error (SE) Armitage and Berry, (1987). All differences were considered significant if P-values were ( $P < 0.05$ ).

## 3. Results and Discussion

The Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity was assayed to investigate the antioxidant potential of ELEE. Data in Table (1) indicated that ELEE recorded higher DPPH radical scavenging activity with 69.31% in the high tested level 0.4% of sample as compared with 0.2% and 0.1% of sample that recorded 47.19% and 20.79% of antioxidant activity, respectively. DPPH is a relatively stable organic-free radical which has been widely used to test the free radical scavenging ability of various samples containing antioxidant molecules.

These results are in agreement with those reported by Mbah et al., (2018), also reported that DPPH concentration increases, the scavenging activity increases. This reveals that *S. melongena* could be a potent in-vitro antioxidant. Moreover, Gradiappan and Rangasamy, (2012), reported a similar finding on antioxidant activity of different species of *S. melongena*. This suggests that *Solanum melongena* Linn fruit is capable of donating hydrogen to a free radical in order to remove odd electrons that lead to free radical reactivity. This is the main mechanism of determining DPPH radical scavenging activity as noticed by the change of colour that was measured spectrophotometrically. This could imply that pathological health challenges could be managed with ethanolic extract of *Solanum melongena* Linn fruit at high concentration Ghosal and Mandal, (2012). Furthermore, Sharma and Kaushik, (2021), study revealed that the flavonoid content in the leaves was much higher than that in the fruits with mean average values 15.6 vs. 0.9  $\mu\text{g}/\text{mg}$ , respectively, which suggested that eggplant leaves are a potential source of naturally occurring antioxidants due to the presence of high flavonoid concentrations.

Regarding to total phenolic content of the ELEE, the extract recorded  $3.87 \pm 0.11$  as gallic acid equivalent/100g. Total phenolic content is known to possess strong antioxidant (Idriss et al., 2021) and consistent with the fact that antioxidant activity of plant products is generally attributed to radical scavenging activity of phenolic compounds (Deli et al., 2019). Indeed, the phenolic compounds such as flavonoids, polyphenols and tannins are the main bioactive molecules that contribute to antioxidant activity of plant extracts (Becker et al., 2017). Similarly, Tiwari et al., (2009) carried out a phytochemical screening of *S. melongena* fruit, finding the presence of alkaloids, saponins, tannins, phenolic compounds, and flavonoids in methanolic and aqueous extracts. Moreover, Contreras-Angulo et al., (2022) proved that the leaf and fruit presented the highest concentration of phenolic compounds, flavonoids, anthocyanins, and alkaloids, accompanied by the highest antioxidant capacity.

The results of pH analysis are presented in Table (2). The PH of control sample at the 30th day significant decrease compared with the 1st day of freeze storage with mean values  $5.07 \pm 0.12$  vs  $5.72 \pm 0.23$ , respectively. Whereas, 1.5% ELEE minced meat sample at the 30th day of storage non-significant decrease compared with the 1st day of freeze storage with mean values  $5.61 \pm 0.15$  vs  $5.74 \pm 0.09$ , respectively. In the other hand, 1.5% ELEE and control samples non-significant different at the beginning of the experiment but at 30th day 1.5% ELEE sample significant increase compared to control sample. These data revealed that the reduction of pH rate was faster in control samples compared to ELEE meat sample. These differences between control and ELEE sample may be attributed to the antimicrobial properties due to the total phenolic content of the eggplant leaves ethanolic extract. pH decreases with the microbial spoilage and increase of organic acids Pamuk et al., (2022), so this is in line with the results of that study. Furthermore, Sam et al., (2021) reported a slow alteration in the pH during storage for frankfurter-type sausage prepared with *Daucus carota* paste compared with control sample.

The peroxide value of control and treated minced meat samples was illustrated in Table (3). Mean values of control sample in the 30th day significant increase compared with 1st day with mean values  $14.50 \pm 1.07$  vs  $10.00 \pm 0.98$  mEq/Kg, respectively. therefore, minced meat containing 1.5% ELEE non-significant increase in the 30th day compared with the 1st day of the experiment with mean values  $10.52 \pm 1.09$  vs  $09.32 \pm 0.76$  mEq/Kg, respectively. From another side, all two samples non-significant different at the beginning of the experiment but after 30th day of freeze storage 1.5% ELEE sample significant decrease compared to control sample.

The oxidation of lipids leading to rancidity is one of the most important changes during food storage and production. Lipid oxidation leads to the formation of free radicals and hydroperoxides. Control and monitoring of lipid oxidation during meat processing or storage are important due to increased demand for precooked convenient meat products for home, fast food and institutional uses. Endogenous antioxidant could potentially delay the onset of oxidative rancidity in stored meat. Lipid peroxidation is one of the primary mechanisms of quality deterioration in stored foods, especially in muscle tissues Gheisari, (2011). Pereira and Abreu, (2018) reported that for reduction of lipid peroxidation in meat and meat products, the antioxidant compounds have been added to products derived directly as was done in this experiment by adding 1.5% ELEE as containing phenolic compounds.

Table (4) illustrates the thiobarbituric acid of control and treated minced meat samples. Mean values of control samples in the 30th day significant increase compared to 1st day of storage with mean values  $1.94 \pm 0.012$  vs  $0.54 \pm 0.003$ , respectively. Therefore, minced meat sample which contain 1.5% ELEE after 30th day of freeze storage non-significant increase compared to 1st day with mean values  $0.71 \pm 0.002$  vs  $0.52 \pm 0.001$ , respectively. Whereas, all two samples non-significant different at the beginning of the experiment but at the end of freeze storage 1.5% ELEE sample significant decrease compared to control sample.

The thiobarbituric acid value was determined in order to evaluate the lipid oxidation of meat samples Zwolan et al., (2020). Values of this parameter was in agreement with Choe (2020), reported that polyphenols containing one or more aromatic rings and carrying at least two hydroxyl groups in their structures can readily donate hydrogens to radicals. So, polyphenolic content delayed the lipid oxidation in minced meat and controlled the lipid oxidation better than the control sample. There are also reports of the successful use of extracts rich in phenolics for the preservation of different meat products. For instance, *Nigella sativa* L. seed, water, and ethanol extracts, which are high in quercetin derivatives, delayed lipid oxidation in chicken meatballs.

Total viable fungi, total yeast and staphylococcus aureus count of control and treated minced meat samples mean values were presented in Table (5). Regarding to Total viable fungi (TVF), there no noticeable changes in TVF count between minced meat samples in both control and 1.5% ELEE after and before 30 days of freeze storage with mean values  $<1.00 \pm 0.02$  vs  $<1.00 \pm 0.04$  and  $1.10 \pm 0.02$  vs  $<1.00 \pm 0.02$  CFU/g, respectively. Whereas, total yeast as well as staphylococcus aureus recorded significant increase in both control and 1.5% ELEE sampled after and before 30 days of freeze storage with mean values  $1.01 * 10^2 \pm 0.02$  vs  $0.81 * 10^2 \pm 0.02$  and  $1.50 * 10^2 \pm 0.02$  vs  $1.00 * 10^2 \pm 0.03$  for total yeast and  $8.70 * 10^2 \pm 0.03$  vs  $3.83 * 10^2 \pm 0.02$  and  $4.99 * 10^2 \pm 0.02$  vs  $1.20 * 10^2 \pm 0.03$  CFU/g of staphylococcus aureus, respectively. Staphylococcus aureus mean values of minced meat which contain 1.5% ELEE refers to slight significant difference after 30 days of freeze storage compared to control sample.

Similar findings were proved by Mbah et al., (2018), which reported that the ethanolic extract of *S. melongena* Linn fruit exhibited varying degree of antibacterial and antifungal activities on most of the tested pathogens (*Escherichia coli*, *Staphalococcus auerus*, *Pseudomonas aeroginosa*, *Shigella dysenteriae*, *Salmonella typhi*, *Candida albican*, *Rhizopus stolonifer* and *Aspergillus flavus*). This is an indication that as the concentration of the extract increases the inhibition of these microbes would increase. This could indicate its broad-spectrum activities as an antimicrobial agent and with a potent pharmacological activity. The spectra of antimicrobial activity shown by the extract could be as a result of the presence of flavonoid, tannins and saponin.

Table (6) shows the smell, taste, Texture, appearance and overall acceptability of the panel test of control minced meat and another tested sample which contain 1.5% ELEE after 30 days of freeze storage. Regarding to smell and taste, 1.5% ELEE sample was significant increased compared to control

sample with mean values  $4.75 \pm 0.21$  vs.  $3.94 \pm 0.07$  and  $3.65 \pm 0.05$  vs.  $2.99 \pm 0.93$ , respectively. With regard to texture, appearance and overall acceptability, 1.5% ELEE sample was significant increase in texture and overall acceptability values compared to control sample with mean values  $3.97 \pm 0.22$  vs.  $3.38 \pm 0.15$  and  $4.02 \pm 0.60$  vs.  $3.95 \pm 0.35$ , respectively. The appearance of 1.5% ELEE sample was nonsignificant decreased compared to control sample with mean values  $4.54 \pm 0.21$  vs.  $4.89 \pm 0.37$ , respectively.

It is clear from the sensory evaluation of the control and minced meat containing 1.5% ELEE that there are no remarkable differences between these two samples, which indicates that the effect of the extract in this percentage did not significantly affect the general acceptance of the sample. These results are in a line with Rubén et al., (2021) which reported that among the sensory indicators, the addition of extracts from eggplant improved the colour properties of the product due to increased anthocyanin content. The results of this study may provide a basis for studies on the preparation of more meat or other products containing eggplant ethanolic extract.

**Table (1):** The DPPH radical scavenging activity and total phenolic content of control and treated minced meat samples.

ELEE	DPPH Radical Scavenging Activity			*Total Phenolic content
	0.1 %	0.2 %	0.4 %	Gallic acid equivalent /100 g
	20.79	47.19	69.31	3.87±0.11

\*Three trials and mean values presented as mean ± SE.

**Table (2):** The Ph of control and treated minced meat samples

Storage period	Control	1.5% ELEE
1st day	5.72±0.23aA	5.74±0.09aA
30th day	5.07±0.12bB	5.61±0.15aA

All result for 3 trials expressed as mean ± SE. Values in the same column, which have similar small superscript letters, are non-significantly different at  $P < 0.05$ . Mean values carrying different capital superscript letters in the same row are significant at  $P < 0.05$ .

**Table (3):** The peroxide value of control and treated minced meat samples

Storage period	Control	1.5% ELEE
1st day (mEq/Kg)	10.00±0.98bA	09.32±0.76aA
30th day(mEq/Kg)	14.50±1.07aA	10.52±1.09aB

All result for 3 trials expressed as mean ± SE. Values in the same column, which have similar small superscript letters, are non-significantly different at  $P < 0.05$ . Mean values carrying different capital superscript letters in the same row are significant at  $P < 0.05$ .

**Table (4):** The thiobarbituric acid of control and treated minced meat samples

All result for 3 trials expressed as mean ± SE. Values in the same column, which have similar small superscript letters, are non-significantly different at  $P < 0.05$ . Mean values carrying different capital superscript letters in the same row are significant at  $P < 0.05$ .

Storage period	Control	1.5% ELEE
1st day (mg MDA/Kg)	0.54±0.003bA	0.52±0.001aA
30th day (mg MDA/Kg)	1.94±0.012aA	0.71±0.002aB

superscript letters in the same row are significant at  $P < 0.05$ .

**Table (5):** Total Viable Fungi, Total Yeast and Staphylococcus aureus count of control and treated minced meat samples



Storage period	Control		1.5% ELEE	
	Zero Time	After 30days	Zero Time	After 30days
	CFU/g			
Total Viable Fungi	<1.00±0.04a	<1.00±0.02a	<1.00±0.02a	1.10±0.02a
Total Yeast	0.81*102±0.02a	1.01*102±0.02b	1.00*102±0.03a	1.50*102±0.02a
Staphylococcus aureus	3.83*102±0.02a	8.70*102±0.03b	1.20*102±0.03a	4.99*102±0.02b

All result for 3 trials expressed as mean ± SE. Values in the same row, which have similar superscript letters, are non-significantly different at P< 0.05.

**Table (6):** Smell, Taste, Texture, Appearance and Overall Acceptability of control and treated minced meat samples

Storage period	Control	1.5% ELEE
Smell	3.94±0.07b	4.75±0.21a
Taste	2.99±0.93b	3.65±0.05a
Texture	3.38±0.15a	3.97±0.22a
Appearance	4.89±0.37a	4.54±0.21a
Overall Acceptability	3.95±0.35a	4.02±0.60a

All result expressed as mean ± SE. Values in the same row, which have similar superscript letters, are non-significantly different at P< 0.05.

#### 4. Conclusion

Extending the storage period of minced meat using ethanolic extract of eggplant leaves. This research aims to prolong the storage period of minced meat by adding ethanolic extract of eggplant leaves under normal freezing conditions. Eggplant leaves were collected, washed, dried, crushed, soaked in 100% ethanol, filtered, and then concentrated. Concentrated ethanol extract was added to ground beef at a ratio of 1.5% w/w and stored at -5 °C for 4 weeks. DPPH and phenolic content were determined. The pH, peroxide value, thiobarbituric acid, and microbial content were estimated at the beginning of the experiment and after 30 days of storage. Freezer for my meat samples. At the end of the experiment, a sensory evaluation was conducted for the control sample and the sample to which the extract was added by trained judges (n=20). The results revealed that adding 1.5% of the extract recorded a high degree of DPPH and contained a good number of phenolic compounds. In addition, addition of 1.5% of the tested extract caused an improvement in all estimated values such as pH, peroxide value and thiobarbituric acid. This addition also preserved the minced meat from the growth of microbes after a period of 30 days of freezing. Also, the overall acceptability of the sensory evaluation performed on the ground beef sample fortified with 1.5% extract and comparing it to the control sample indicated that it was acceptable to the judges. Therefore, according to the results obtained, ethanolic eggplant leaf extract may be useful for prolonging the storage period of some meat products and so more research should be conducted on the use of eggplant leaves with other industrial products.

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