*J. Food and Dairy Sci., Mansoura Univ., Vol. 7 (1): 19 - 25, 2016*

# POLYPHENOLS EXTRACTED FROM GRAPE SEEDS AND ITS EFFECTS AS ANTIOXIDANT AND ANTIMICROBIAL ON BEEF SAUSAGE

**El-Zainy, A.R.M ; A.E. Morsy ; Amal G. Sedki and Noha.M. Mosa**
**Department of Home Economics, Faculty of Specific Education, Mansoura University, Egypt.**
**Corresponding Author: Amal Gamil Sedki, Department of Home Economic, Faculty of Specific Education, Mansoura University, Egypt.**
**E-mail: amalgamil@mans.edu.eg**



## ABSTRACT

This study was carried out to investigate the effect of polyphenols extracted from grape seeds (GPE) on chemical composition, lipid oxidation and microbial growth in raw beef sausage which was made with (0.01%) or without nitrite during frozen storage at -18°C for 3 months. GPE was added by 0.02 and 0.04% to beef sausage during making sausage. Results indicated that GPE is better in terms of lower TBARS value and total bacterial count (TBC) in sausage . In addition, GPE had synergistic antioxidant and antimicrobial effect to nitrite. Therefore, it is suggested that grape seeds, as a natural agro-waste, could be used to prepare polyphenols extract to extend the shelf-life of sausage, providing the consumer with food containing natural additives, which might be seen more healthful than those of synthetic origin.

**Keywords:** *Antimicrobial, Antioxidant, grape seed polyphenols, Nitrite , Sausage.*

## INTRODUCTION

Beef sausage is one of the popular foodstuffs, however, are vulnerable to microbial contamination, lipid oxidation, color changes (Ahn *et al.*, 2007) , Application of suitable agents possessing both antioxidant and antimicrobial activities may be useful for maintaining sausage quality, extending shelf-life and preventing economic loss (Yin and Cheng, 2003). Consequently, several synthetic antioxidants have been added to sausage to prevent undesirable reactions to enhance its shelf-life (Georgantelis *et al.*, 2007). However, the increasing concern over the negative consequences from using synthetic antioxidants has emphasized the value of natural antioxidants. So, interest in natural antioxidants and search on naturally occurring compounds with antioxidant and antimicrobial activities has increased dramatically (Lorenzo *et al.*, 2013).

Agro-industries generate numerous waste materials using these residues as natural antioxidants in the food industry could represent a significant step towards maintaining that should be reduced/eliminated to achieve a sustainable agriculture. In this sense, the possibility of an environment balance. For instance in wine and grape juice production, where residues account for approximately 30% of grape (*Vitis vinifera*) (Rockenbach *et al*., 2008). These by-products of grape, such as seeds and peels, are rich in phenolic compounds, which are known to have high antioxidant activity (Guendez *et al.*, 2005 and Lorenzo *et al*., 2013). Scientific studies have shown that grape seed extract is a more potent scavenger of reactive oxygen species and that it has greater antioxidant power than vitamin C and vitamin E ( Bagchi *et al*., 1997 ; 1998 and Shi *et al*., 2003). Also, Grape seed extract improves lipid stability by reduced the thiobarbituric acid reactive substances (TBARS) in both of raw beef and pork patties vacuum packaged and stored frozen for 4 months, pre-cooked pork patties stored at −18 °C for up to 6 months and pre-cooked, frozen sausage at −18 °C for 4 months. (Monroy, 2007; Sasse *et al*., 2009 and Kulkarni *et al*., 2011). In addition, grape seed extract exhibited antibacterial activity (Baydar *et al*., 2006; Over *et al*., 2009; Sagdic *et al*., 2011 and Perumalla *et al*., 2013).

Grape seed extract (GSE) contains a number of polyphenols, including procyanidins and proanthocyanidins, which have high antioxidant activity (Shi *et al.*, 2003). Grape seed polyphenols extract decreased the TBARs in dry-cured sausages during the ripening period (Li *et al.*, 2013). Since no previous information is available about the antioxidative effects and physicochemical changes of polyphenols grape seeds extract (PGE) when used in fresh sausage during frozen storage. So, this study aimed to determine the effect of grape seeds polyphenols extract on chemical composition, lipid oxidation and microbial growth of sausage in raw beef sausage which was made with or without nitrite during frozen storage at -18°C for 3 months.

## MATERIALS AND METHODS

### Materials

Grape seeds were obtained from Agricultural Research Center, Giza, Egypt.

Meat and fat tissues were purchased from butcher's shop in Mansoura city , Egypt .

Spices: Arab yeast, bay leaf, cardamom cinnamon, clove, corengan, cubeb, nutmeg, rose wood, thyme and white pepper were purchased from the local market of Mansoura city, Egypt.

### Methods

Grape seeds were cleaned to remove strange materials and grounded. The ground seeds were individually pass through 60 mesh sieve and stored in air-tight polyethylene bags and preserved in a deep freezer until use.

Fresh beef meat and fresh fat were purchased from butcher's shop in Mansoura city, Egypt. Meat was obtained from boneless and trimmed from all thick visible connection tissues. Both meat and fat were

separately ground through 4.5 mm plate (twice) in a mincer machine (La Minerva, Bologna, Italy).

**Extraction of polyphenols of grape seeds**

Four hundred grams of grape seeds were finely powdered, mixed with 80 % methanol ( 2 L×4) for 2 days (each extraction) at room temperature, then filtered through Whatman filter paper No. 42, and the 80 % methanolic extract of grape seeds collected . After the 80 % methanolic extract obtained previously was gathered, the solvents were evaporated under vacuum using rotary evaporator at 50°C. The residue was dissolved in water, and the aqueous layer was washed with petroleum ether several times until a clear upper layer of petroleum ether was obtained. The lower layer was then treated with ethyl acetate containing 1% glacial acetic acid. Extraction of polyphenols (PGE) was carried out for 36 h at room temperature, and the combined ethyl acetate was concentrated (Xia *et al.*, 1998). The residue was dried and stored in a deep freezer until use.

**Technological methods**

**Preparation of spices mixture:**

The spices were separately milled then the mixture of the powdered spices was prepared as described in Hassan (2010) as follows: 10.52% arab yeast, 4.74% bay leaf, 1.84% cardamom, 9.91% cinnamon, 7.05 % clove, 8.22% corengan, 25.22% cubeb, 2.69 % nutmeg, 14.61 % rose wood, 4.97 % thyme and 10.22% white pepper.

**Formulations of beef nitrite-free sausages:**

Three formulas from beef nitrite-free sausage were prepared as follows: Control formula (A) was prepared according to the following recipe: minced beef meat (65.66%), animal fat (20%), sodium chloride (1.8%), spices (1.8%), iced water (10.16%), ascorbic acid (0.03%) and fresh garlic (0.55%). Two sausage formulas (B and C) were prepared by replacement iced water with 0.02 and 0.04% of grape seeds polyphenols extract (GPE), respectively.

**Formulations of beef nitrite-sausages:**

Three formulas from beef nitrite- sausage were prepared as follows: Control formula (F) was prepared according to Hassan (2010). Minced beef meat (65.66%), animal fat (20%), sodium chloride (1.8%), sodium nitrite (0.01%), spices (1.8%), ice water (10.15%), ascorbic acid (0.03%) and garlic (0.55%). Two sausage formulas (G and H) were prepared by replacement iced water with 0.02 and 0.04% of polyphenols grape seeds extract (PGE), respectively.

**Analytical methods**

**Chemical and phytochemical composition:**

Gross chemicals composition for beef sausage samples had been done according to AOAC (2005), while carbohydrate was determined by difference as follows :

**Carbohydrates = 100 – (% moisture + % protein + % fat + % ash).**

In addition, phytochemical screening for grape seeds and estimation of polyphenols and tannins in grape seeds and their polyphenols extract were estimated according to Swain and Hillis (1959) .

**Determination of Thiobarbituric acid reactive substances (TBARs):**

TBARs values were determined spectrophotometrically according to the procedure described by Siu and Draper (1978). The TBARs values were expressed as mg / malonaldehyde / kg sausage sample .

**Microbiological examination:**

The total count was performed on nutrient agar media according to Difco Manual (1966). *Coliform* group was detected on MacConkey broth according to method described by EL-Shawaf (1990). *Salmonella* was detected on *Salmonella Shigella* agar medium (Oxoid) according to method described by Bryan (1991). *Staphylococcus aureus* was detected on Baird Parker Agar (BPA, Fluka) supplemented with egg yolk tellurite emulsion (Merck) according to method described by Djenane *et al.* (2012). *E. coli* O157:H7 was detected on Cefixime-Tellurite Sorbitol MacConkey (CT-SMAC) agar (DIFCO Lab, Detroit, MI, U.S.A.) according to method described by Djenane *et al.* (2012). Molds and yeasts count were performed according to the Oxoid Manual (1982). The microbiological results are expressed as $\log_{10}$ numbers of colony forming units/ gram (cfu/g).

**Statistical analysis**

All the obtained data were statistically analyzed by SPSS computer software. The calculated occurred by analysis of variance ANOVA and follow up test LSD by Petrie and Watson (1999).

## RESULTS AND DISCUSSION

The percentage yields of polyphenols' extract in red grape seeds is 2.13%.It is clear that each of grape seeds and their polyphenols' extract contains tannins, flavonoids, sterols, saponins, carbohydrates and alkaloids. On the other hand, they do not contain resins. With respect to cardenolides, grape seeds contain cardenolides, whereas polyphenols extract does not contain cardenolides as illustrated in table (1).

**Table 1. Phytochemical screening of grape seeds and their polyphenols' extract**

| Seeds or extract | Tannins | Flavonoids | Sterols | Saponins | Cardenolides | Carbohydrates | Alkaloids | Resins |
|---|---|---|---|---|---|---|---|---|
| Grape seeds | + | + | + | + | + | + | + | - |
| GPE | + | + | + | + | - | + | + | - |

**- Negative + Positive GPE: Grape seeds polyphenols extract**

From data in table (2), it is clear that polyphenols content of grape seeds and their polyphenols' extract were 0.09 and 3.39 g/100g as pyrogallol, respectively; whereas tannins contents of these seeds their polyphenols' extract was 0.57 and 6.84 g/100g as tannic acid, respectively.

**Table 2. Polyphenols and tannins contents in grape seeds and their polyphenols' extract**

| Seeds or extract | Polyphenols (g/100g as pyrogallol) | Tannins (g/100g as tannic acid) |
|---|---|---|
| Grape seeds | 0.09 ± 0.00 | 0.57± 0.00 |
| GPE | 3.39 ± 0.01 | 6.84 ± 0.04 |

**Results are expressed as a arithmetic mean ± standard error of three determinations.**

Sausage is one of the oldest well-known forms of processed meat products and is very popular in many areas. However, due to the high fat content and low water activity, sausages are generally spoiled faster than other meat products by both lipid oxidation and microbial contamination. Lipid oxidation contributes to the development of unacceptable organoleptic characteristics, whereas microbial growth may cause both spoilage and foodborne diseases (Georgantelis *et al.,* 2007). Therefore, delaying lipid oxidation and preventing bacterial growth are factors that can have a significant contribution towards the extension of shelf life and maintenance of good quality and sensory characteristics of sausage (Georgantelis *et al.,* 2007 and Wenjiao, et al., 2014). In order to achieve these goals, meat product manufacturers in the past few decades had used several synthetic food additives with antioxidative and antimicrobial properties, such as nitrite. Sodium nitrite has been widely used and considered indispensable in many types of meat products for its colouring, flavouring, antioxidative and antimicrobial properties (Honikel, 2008 and Li *et al.*, 2013 ). Despite the beneficial role of nitrite in sausage, nitrite can react with secondary amines to form carcinogenic N-nitrosamines (Honikel, 2008 and Li *et al.*, 2013). So, the problem of using nitrite in meat products is a formation of carcinogenic N-nitrosamines. For this reason, the reduction of nitrite in meat products is desirable (Coutinho de Oliveira *et al.,* 2012 and Li *et al.*, 2013).

Data represented in table (3) showed that beef sausage formulas under study had gradually slightly decrease in moisture, protein and ash contents, but they had gradually increase in crude lipids contents as affected by increasing frozen storage period from zero time to 3 months at -18°C. The addition of nitrite to sausage samples induced a decrease in both the reduction in crude protein and ash contents and also it induced a decrease in the increase in crude lipids content when compared to their corresponding contents for sausage samples which made without nitrite. sausage samples either without or with nitrite were less than the control sample in ash reduction.

**Table 3. Chemical composition of sausage samples supplemented with grape seeds polyphenols extract during frozen storage at -18°C from zero time to after three months. (at fresh basis)**

| Beef sausage | Storage period (months) | Moisture % | Crude protein % | Crude Lipids % | Ash % | Carbohydrates % |
|---|---|---|---|---|---|---|
| A | Zero | 62.99±0.05 | 14.66±0.13 | 18.82±0.03 | 3.01 ±0.06 | 0.52 |
| | 1 | 61.56±0.18 | 14.38±0.05 | 20.88±0.05 | 2.71±0.02 | 0.47 |
| | 2 | 60.99±0.72 | 13.89±0.05 | 22.19±0.04 | 2.42 ±0.01 | 0.51 |
| | 3 | 62.38±0.45 | 13.79±0.00 | 22.83±0.02 | 2.34 ±0.02 | 0.66 |
| B | Zero | 62.22±0.15 | 14.89±0.05 | 19.37±0.05 | 2.99 ±0.03 | 0.50 |
| | 1 | 61.44±0.14 | 14.75±0.05 | 20.41±0.02 | 2.92 ±0.02 | 0.48 |
| | 2 | 60.57±0.30 | 14.31±0.13 | 21.81±0.02 | 2.84 ±0.00 | 0.47 |
| | 3 | 60.02±0.06 | 14.26±0.04 | 22.18±0.05 | 2.74 ±0.02 | 0.50 |
| C | Zero | 61.81±0.23 | 15.17±0.07 | 19.42±0.04 | 3.05 ±0.02 | 0.55 |
| | 1 | 61.95±0.07 | 14.74±0.03 | 20.01±0.02 | 2.92 ±0.02 | 0.38 |
| | 2 | 59.62±0.35 | 14.56±0.07 | 21.60±0.04 | 2.88 ±0.03 | 0.48 |
| | 3 | 59.11±0.52 | 14.47±0.03 | 22.01±0.08 | 2.78 ±0.03 | 0.33 |
| F | Zero | 62.17±0.21 | 14.80±0.07 | 19.41±0.05 | 3.00 ±0.04 | 0.62 |
| | 1 | 61.86±0.07 | 14.70±0.09 | 20.11±0.05 | 2.89±0.03 | 0.44 |
| | 2 | 61.79±0.37 | 14.36±0.03 | 21.00±0.08 | 2.73±0.01 | 0.32 |
| | 3 | 61.49±0.43 | 14.31±0.05 | 20.13±0.01 | 2.65±0.01 | 0.42 |
| G | Zero | 62.04±0.11 | 14.51±0.07 | 19.91±0.09 | 2.91±0.04 | 0.63 |
| | 1 | 61.77±0.08 | 14.58±0.07 | 20.31±0.01 | 2.80±0.03 | 0.54 |
| | 2 | 62.66±0.49 | 14.47±0.03 | 20.71±0.01 | 2.77±0.03 | 0.49 |
| | 3 | 62.65±0.15 | 14.48±0.04 | 20.85±0.06 | 2.72±0.01 | 0.31 |
| H | Zero | 61.75±0.35 | 14.77±0.06 | 20.23±0.11 | 3.06±0.01 | 0.19 |
| | 1 | 61.28±0.04 | 14.45±0.07 | 20.87±0.04 | 2.99±0.03 | 0.41 |
| | 2 | 62.32±0.14 | 14.43±0.05 | 20.99±0.06 | 2.93±0.05 | 0.53 |
| | 3 | 61.88±0.07 | 14.44±0.00 | 21.00±0.08 | 2.89±0.03 | 0.55 |

**A is control sausage formula without any additives, B is nitrite free- sausage formula supplemented with 0.02% GPE, C is nitrite free-sausage formula supplemented with 0.04% GPE, F is control sausage formula containing nitrite without any additives, G is sausage formula that contains nitrite and supplemented with 0.02% GPE, H is sausage formula that contains nitrite and supplemented with 0.04% GPE. Each record is a mean value of three replicates and is followed by the standard error.**

Addition of nitrite protected sausage samples from severe chemical changes. In addition, supplementation with PGE to nitrite sausage improved the chemical composition of sausage during frozen storage period at -18°C. Also, the combination of PGE with nitrite increased the effect of nitrite in reduction the changes of chemical composition of sausage during

frozen storage period at -18°C. This may be due to PGE may has antimicrobial and antioxidant activities.

The results in table (4) showed that the extent of lipid oxidation (TBARs) in all sausage formulas increased nearly over the entire study period. When $NaNO_2$ (0.01%) was added, the TBARs value of the sausage was lower ($P < 0.05$) than that of the sausage without $NaNO_2$ (0 %). Consequently, the $NaNO_2$ addition to beef sausage significantly reduced lipid oxidation. Also, GPE reduced TBARs value in both sausage samples which is made with or without nitrite as compared to control sample .

**Table 4. Thiobarbituric acid values (TBARs) of sausage samples under study from zero time to after three month. (at fresh basis)**

| Beef sausage | Thiobarbituric acid value (TBARs) mg MDA/kg | | | |
|---|---|---|---|---|
| | *Zero time* | *One month* | *Two month* | *Three month* |
| A | 0.2333 | 0.3642 | 0.4175 | 0.6274 |
| B | 0.3424 | 0.4867 | 0.5121 | 0.6989 |
| C | 0.3295 | 0.4578 | 0.4909 | 0.7451 |
| F | 0.1665 | 0.2808 | 0.3521 | 0.5241 |
| G | 0.1188 | 0.1802 | 0.2472 | 0.4985 |
| H | 0.2712 | 0.3970 | 0.4451 | 0.6124 |

**mg MDA/kg : mg malonaldehyde/Kg sausage sample . A is control sausage formula without any additives, B is nitrite free- sausage formula supplemented with 0.02% GPE, C is nitrite free-sausage formula supplemented with 0.04% GPE, F is control sausage formula containing nitrite without any additives, G is sausage formula that contains nitrite and supplemented with 0.02% GPE, H is sausage formula that contains nitrite and supplemented with 0.04% GPE.**

With respect to thiobarbituric acid reactive substances (TBARs) value of beef sausage samples under the study, it is clear that the extent of lipid oxidation in all sausage samples increased nearly over the entire study period. This result agrees with that of Sammet *et al.* (2006). Also, the $NaNO_2$ addition to beef sausage significantly reduced lipid oxidation. When $NaNO_2$ (0.01%) was added, the TBARs value of the sausage was lower ($P < 0.05$) than that of the sausage without $NaNO_2$ (0 %). This result agrees with that of Jo *et al.* (2003) who reported that the $NaNO_2$ addition to pork sausage significantly reduced lipid oxidation. Also, PGE reduced TBARs value in both sausage samples which is made with or without nitrite as compared to control sample. This may be due to the high polyphenols content of PGE which have high antioxidant activity. The presence of these compounds may have retarded ($P < 0.001$) the lipid oxidation during storage process. Thus, the addition of PGE might have reduced lipid deterioration through the inhibition of malonaldehyde formation. This result agrees with that of Li *et al.*(2013) who reported that TBARs increased gradually in dry-cured sausages during ripening, but were significantly reduced with grape seed polyphenols. With respect to the combination effect of nitrite and GPE (0.02%) , it was found that TBARs value for sausage samples which treated with GPE and nitrite was less than its corresponding value for sausage samples which treated with either GPE or nitrite only. So, GPE (0.02%) have synergistic effect to nitrite.

The results in table (5) showed that the increase in frozen storage period caused an increase in total bacterial count (TBC) in all samples. Also, at third month from frozen storage at -18°C, beef sausage sample formulated with both nitrite and GPE (0.04%) had the least TBC among all sausage samples under study (3.301 $\log_{10}$ cfu/ g ), while TBC in control sample which without any additives ( Sample A) (7.477 $\log_{10}$ cfu/ g).

**Table 5. Total bacterial count of sausage samples under study from zero time to after three month. (at fresh basis)**

| *Sausage sample* | Total bacterial count of sausage samples | | | | | |
|---|---|---|---|---|---|---|
| | *Zero time* | | After storage at -18°C | | | |
| | | | *Two month* | | *Three month* | |
| | cfu/g | $\log_{10}$ cfu /g | cfu/g | $\log_{10}$ cfu /g | cfu/g | $\log_{10}$ cfu /g |
| A | $2.5 \times 10^2$ | 2.398 | $2.2 \times 10^5$ | 5.342 | $3.0 \times 10^7$ | 7.477 |
| B | $1.8 \times 10^2$ | 2.255 | $3.0 \times 10^4$ | 4.477 | $1.0 \times 10^5$ | 5.000 |
| C | $1.5 \times 10^2$ | 2.176 | $2.0 \times 10^3$ | 3.301 | $3.0 \times 10^4$ | 4.477 |
| F | $8.1 \times 10$ | 1.908 | $1.8 \times 10^3$ | 3.255 | $1.0 \times 10^5$ | 5.000 |
| G | $6.0 \times 10$ | 1.778 | $1.6 \times 10^3$ | 3.204 | $1.0 \times 10^5$ | 5.000 |
| H | $3.7 \times 10$ | 1.568 | $8.0 \times 10^2$ | 2.903 | $2.0 \times 10^3$ | 3.301 |

**cfu : Colony forming unit. A is control sausage formula without any additives, B is nitrite free- sausage formula supplemented with 0.02% GPE, C is nitrite free-sausage formula supplemented with 0.04% GPE, F is control sausage formula containing nitrite without any additives, G is sausage formula that contains nitrite and supplemented with 0.02% GPE, H is sausage formula that contains nitrite and supplemented with 0.04% GPE.**

With regard to total bacterial counts (TBC) of sausage samples, it is clear that TBC of all samples except control sample (sample A which was made without any additives) remained below 6 $\log_{10}$ cfu/g which is the maximal permissible limit for fresh sausages (Triki *et al.*, 2013), which indicated the spoiling. Also, it is clear that nitrite had a noticeable effect on reduction of total bacterial count. This result agrees with that obtained by Hospital *et al.* (2012) and Sannino and Bolzoni (2013) who found that nitrite

produces a growth inhibition of spoilage and pathogenic bacteria such as *Clostridium botulinum*. The anti-bacterial effect of nitrite is due to compounds such as $HNO_2$ and NO, deriving from the reduction of nitrite could be responsible for the antimicrobial effect through a variety of different mechanisms including the inhibition of oxygen uptake, oxidative phosphorylation and proton-dependent transport (Davidson *et al.,* 2004 and Weiss *et al.,* 2010). Nitrite was also found to inhibit a number of enzymes that are essential to the metabolism of bacteria such as aldolase. Moreover, nitrite generally causes a breakdown of the proton gradient in bacteria needed to generate ATP (Weiss *et al.,* 2010). In addition, GPE improved the microbial status of beef sausage relative to the controls. This result agrees with that of Ahn *et al*. (2007) who reported that grape seed polyphenols functioned as bactericidal, which caused disruption of the bacterial cell wall. Also, GPE had synergistic antimicrobial effect to nitrite. At third month from frozen storage at -18°C, beef sausage samples formulated with nitrite and GPE (0.04%) together had the least TBC among all sausage samples under study ($3.301\log_{10}$ cfu/g).

With respect to pathogenic bacteria detection, it is clear that *Coliform* group, *Salmonella typhimurium*, *Staphylococcus aureus* and Enterohaemorrhagic *E. coli* (*E. coli* O157:H7) were not detected in all sausage samples at zero time and during frozen storage at -18 °C. This may be due to that these pathogenic bacteria were not in raw materials used in sausage preparation and also, the cleaning of the equipments and packing materials used during processing and frozen storage period

Coliform group, *Salmonella spp*., *Staphylococcus aureus* and Enterohaemorrhagic E. coli (*E. coli O157:H7*) were not detected in all sausage samples at zero time and during frozen storage at -18 °C.

## CONCLUSION

Generally, supplementation of raw beef sausage with grape seed polyphenols extract during freeze storage (-18°C) processing proved to be advantageous with regard to lipid stability. The efficiency of the grape seed polyphenols extract increased with increasing its concentration in the studied range. This study suggested that grape seeds, as a natural agro-waste, could be used to extract polyphenols (GPE) that extend the shelf-life of sausage, providing the consumer with food containing natural additives, which might be seen more healthful than those of synthetic origin.

## REFERENCES


AOAC (2005). Official Methods of Analysis,18th edn. Washington, DC: Association of Official Analytical Chemists.

Ahn, J.; Grün, I. U.and Mustapha, A. (2007). Effects of Plant Extracts on Microbial Growth, Color Change, and Lipid Oxidation in Cooked Beef. Food Microbiology 24: 7-14.

Bagchi, D.;Garg, A.; Krohn, R.L.; Bagchi, M.; Tran, M.X. and Stohs, S.J. (1997). Oxygen Free Radical Scavenging Abilities of Vitamins C and E, and a Grape Seed Proanthocyanidin Extract *in Vitro*. Res Commun Mol Pathol Pharmacol 95: 179-189.

Bagchi, D.; Garg, A.; Krohn, R.L.; Bagchi, M.; Bagchi, D.J.and Balmoori, J. (1998). Protective Effects of Grape Seed Proanthocyanidins and Selected Antioxidants against TPA-Induced Hepatic and Brain Lipid Peroxidation and DNA Fragmentation, and Peritoneal Macrophage Activation in Mice. Gen Pharmacol 30: 771–776.

Baydar, N.G. ; Sagdic, O. ; Özkan, G.and Cetin, S. (2006). Determination of Antibacterial Effects and Total Phenolic Contents of Grape (*Vitis vinifera L.*) Seed Extracts. International Journal of Food Science and Technology, 41:799–804.

Bryan, F.L. (1991). Teaching HACCP Techniques to Food Processors and Regulatory Officials. Dairy, Food and Environmental Sanitation 11: 562-568.

Coutinho de Oliveira, T.L.; Malfitano de Carvalho, S.; Soares, R.A.; Andrade, M.A.; Cardoso, M. G.and Ramos, E. M. (2012). Antioxidant Effects of *Satureja montana L.* Essential Oil on TBARs and Color of Mortadella-type Sausages Formulated with Different Levels of Sodium Nitrite. LWT - Food Science and Technology 45: 204 – 212.

Davidson, P. M. ; Sofos, J. N.and Branen, A.L.(2004). Antimicrobials in Food. Boca Raton: Taylor and Francis.

Difco Manual (1966). Dehydrated Culture Media and Reagents Procedures,9th edn. USA: Difco Laboratories.

Djenane, D.; Aïder, M. ; Yangüela, J. I.; dir, L. ; Gómez, D.and Roncalés, P. (2012). Antioxidant and Antibacterial Effects of Lavandula and Mentha Essential Oils in Minced Beef Inoculated with *E. Coli* O157:H7 and *S. aureus* during Storage at Abuse Refrigeration Temperature. Meat Science 92: 667–674.

EL-Shawaf, A. M .(1990). Microbial Studies on Aflatoxins in Meat Products. PhD Thesis, Faculty of Agriculture, Mansoura University.

Georgantelis, D.; Ambrosiadis, I.; Katikou, P. ; Blekas, G.and Georgakis, S.A. (2007). Effect of Rosemary Extract, Chitosan and α-Tocopherol on Microbiological Parameters and Lipid Oxidation of Fresh Pork Sausages Stored at 4 °C. Meat Science 76: 172–181.

Guendez, R.; Kallithraka, S.; Makris, D.P.and Kefalas, P. (2005). Determination of Low Molecular Weight Polyphenolic Constituents in Grape (*Vitis vinifera sp*.) Seed Extracts: Correlation with Antiradical Activity. Food Chemistry 89: 1–9.

Hassan, F. (2010). Studies on Microbiological and Chemical Characteristics of Some Meat Products. PhD Thesis, Faculty of Agriculture, Mansoura University, Egypt.

Honikel, K. O. (2008). The Use and Control of Nitrate and Nitrite for the Processing of Meat Products. Meat Science 78: 68–76.

Hospital, X. F.; Hierro, E.and Fernández, M. (2012). Survival of *Listeria innocua* in Dry Fermented Sausages and Changes in the Typical Microbiota and Volatile Profile as Affected by the Concentration of Nitrate and Nitrite. International Journal of Food Microbiology 53: 395–401.

Jo, C.; Ahn, H. J. ; Son, J. H. ; Lee, J.W.and Byun, M. W. (2003). Packaging and Irradiation Effect on Lipid Oxidation, Color, Residual Nitrite Content, and Nitrosamine Formation in Cooked Pork Sausage. Food Control 14: 7–12.

Kulkarni, S.; DeSantos, F. A. ; Kattamuri, S. ; Rossi, S.J. and Brewer, M. S. (2011). Effect of Grape Seed Extract on Oxidative, Color and Sensory Stability of a Pre-Cooked, Frozen, Re-Heated Beef Sausage Model System. Meat Science 88: 139–144.

Li, L. ; Shao, J. ; Zhu, X.; Zhou, G.and Xu, X. (2013). Effect of Plant Polyphenols and Ascorbic Acid on Lipid Oxidation, Residual Nitrite and N-Nitrosamines Formation in Dry-Cured Sausage. International Journal of Food Science and Technology 48: 1157–1164.

Lorenzo, J. M. ; González-Rodríguez, R. M. ; Sánchez, M. ; Amado, I. R.and Franco, D. (2013). Effects of Natural (Grape Seed and Chestnut Extract) and Synthetic Antioxidants (Butylatedhydroxyltoluene, BHT) on the Physical, Chemical, Microbiological and Sensory Characteristics of Dry Cured Sausage "Chorizo". Food Research International 54: 611–620.

Monroy, M. C. (2007). Effect of Grape Seed Extract on Oxidative Stability of Meat Systems and a Model System. PhD Thesis, Graduate College of the University of Illinois at Urbana-Champaign.

Over, K.F. ; Hettiarachchy, N. ; Johnson, M. G.and Davis, B. (2009). Effect of Organic Acids and Plant Extracts on *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella typhimurium* in Broth Culture Model and Chicken Meat Systems. Journal of Food Science, 74(9): M515- M521.

Oxoid Manual. (1982). Oxoid Manual of Culture Media Ingredient and Other Laboratory Services,5th edn. Oxoid Limited.

Perumalla, A. V. S. ; Hettiarachchy, N. S. ; Over, K. ; Ricke, S. C. ; Slavik, M. F. ; Gbur, E. ; Davis, B.and Acosta, S. (2013). Effect of Partial Replacement of Potassium Lactate and Sodium Diacetate by Natural Green Tea and Grape Seed Extracts and Postpackaging Thermal Treatment on the Growth of *Listeria monocytogenes* in Hotdog Model System. International Journal of Food Science and Technology 48: 918–926.

Petrie, A.and Watson, A. (1999). Statistics for Veterinary and Animal Science,1st edn. United Kingdom: The Blackwell Science Ltd; pp. 90-99.

Rockenbach, I. I. ; Silva, G. L. ; Rodrigues, E. ; Kuskoski, E. M.and Fett, R. (2008). Influência do Solvente no Conteúdo Total de Polifenóis, Antocianinas E Atividade Antioxidante de Extratos de Bagaço de Uva (*Vitis vinifera*) Variedades Tannat e Ancelota. Ciência e Tecnologia de Alimentos 28: 238–244.

Sagdic, O. ; Ozturk, I. ; Yilmaz, M.T. and Yetim, H. (2011). Effect of Grape Pomace Extracts Obtained from Different Grape Varieties on Microbial Quality of Beef Patty. Journal of Food Science 76: M515 −M521.

Sammet, K. ; Duehlmeier, R. ; Sallmann, H. ; von Canstein, C. ; von Mueffling, T.and Nowak, B. (2006). Assessment of the Antioxidative Potential of Dietary Supplementation with α-Tocopherol in Low-Nitrite Salami-Type Sausages. Meat Science 72: 270–279.

Sannino, A.and Bolzoni, L. (2013). GC/CI–MS/MS Method for the Identification and Quantification of Volatile N-Nitrosamines in Meat Products. Food Chemistry 141: 3925–3930.

Sasse, A. ; Colinderes, P.and Brewer, M.S. (2009). Effect of Natural and Synthetic Antioxidants on the Oxidative Stability of Cooked, Frozen Pork Patties. Journal of Food Science 74: S30-S35.

Shi, J. ; Yu, J. ; Pohorly, J.E. and Kakuda, Y. (2003). Polyphenolics in Grape Seeds. Biochemistry and Functionality. J Med Food 6: 291–299.

Siu, G.M.and Draper, H. H. (1978). A Survey of the Malonaldehyde Content of Retail Meats and Fish. Journal of Food Science 43: 1147–1149.

Swain, T.and Hillis, W. E. (1959). The Qualitative Analysis of Phenolic Constituents. Journal of Food Agriculture 10: 63-70.

Triki, M. ; Herrero, A.M. ; Jiménez-Colmenero, F.and Ruiz-Capillas, C. (2013). Effect of Preformed Konjac Gels, with and without Olive Oil, on the Technological Attributes and Storage Stability of Merguez Sausage. Meat Science 93: 351–360.

Wenjiao, F. ; Yunchuan, C. ; Junxiu, S.and Yongkui, Z. (2014). Effects of Tea Polyphenol on Quality and Shelf Life of Pork Sausages. J Food Sci Technol 51: 191–195.

Weiss, J. ; Gibis, M. ; Schuh, V.and Salminen, H. (2010). Advances in Ingredient and Processing Systems for Meat and Meat Products. Meat Science 86: 196–213.

Xia, J. ; Allenbrand, B.and Sun, G.Y. (1998). Dietary Supplementation of Grape Polyphenols and Chronic Ethanol Administration on LDL Oxidation and Platelet Function in Rats. Life Science 63: 383-390.

Yin, M. C.and Cheng, W. S. (2003). Antioxidant and Antimicrobial Effects of Four Garlic-Derived Organosulfur Compounds in Ground Beef. Meat Science 63: 23–28.

# إستخلاص البولي فينول من بذور العنب وتأثيره كمضاد للأكسدة والنمو الميكروبي في سجق اللحم البقري

**أشرف رفعت الزيني ، علاء الدين مرسي ، أمل جميل صدقى و نهى ماهر موسى .**
**قسم الإقتصاد المنزلي ـ كلية التربيه النوعيه ـ جامعة المنصورة**

تهدف هذه الدراسة إلي فحص تأثير البولي فينول المستخلص من بذور العنب علي التركيب الكميائي وأكسدة الدهون والنمو الميكروبي في السجق البقرى سواء المصنع بإضافة نيتريت بنسبة ٠.١ % او بدونه ، والمخزن على درجة حرارة -١٨°م لمدة ٣ شهور حيث تم إضافة مستخلص البولي فينول بنسبة ٠.٠٢ % و ٠.٠٤ % إلي السجق البقري وقد أوضحت النتائج مايلي :

مستخلص البولي فينول خفض بدرجه ملحوظه أكسدة الدهون في عينات السجق البقري . وكذلك إنخفاض العد الكلي البكتيري في العينات .

من النتائج يتضح ان مستخلص البولي فينول ومستخلص المتبقي اضافوا مزايا مضادة للأكسدة وللميكروبات في السجق النيء اثناء فترة التخزين بالتجميد على درجة حرارة -١٨°م. لذا ، فانه يقترح ان بذور العنب " وهي من المخلفات الزراعية الطبيعية" يمكن ان تستخدم لإطالة فترة صلاحية السجق وكذلك لتزويد المستهلك بطعام محتوي على مواد مضافة طبيعية والتي ربما تكون صحية اكثر من المواد المضافة الصناعية.