THE EFFECT OF GRAPE SEED AND SKIN EXTRACTS ON OXIDATIVE AND COLOR STABILITY OF MINCED PORK MEAT

Yavor Ivanov[1], Tzonka Godjevargova[1]

Department Biotechnology
University "Prof. d-r A. Zlatarov"
1 Prof. Yakimov Blvd., Burgas, Bulgaria
E-mail: godjevargova@yahoo.com

Received 13 September 2023
Accepted 20 December 2023 *DOI: 10.59957/jctm.v59.i4.2024.6*

ABSTRACT

The aim of this study was to determine and compare the antilipid potential of seed (GSE) and skin extracts (SE) of Pinot Noir red grape. The preparation of extracts and the evaluation of their color were performed. The antilipid potential of GSE and SE was investigated in the minced pork meat samples. Four experimental samples were prepared: with grape seed and skin extracts, with synthetic antioxidant and without additives. The samples were stored at 4°C for 10 days. During this period product color and formed thiobarbituric acid-reactive substances were determined. It was found that the GSE at concentration 1000 μg g^{-1} limited the oxidative processes more effectively (55.32 %) than the SE (34.04 %) with the same concentration. These results were correlated very well with the TPC and the phenolic composition of the two extracts. The obtained results with GSE (500 and 1000 μg g^{-1}) were like those obtained with the synthetic antioxidant (200 μg g^{-1}). The effect of added GSE and SE onto the minced meat color change during its storage was determined. The results are optimistic because they indicate that grape seed extract can be used as a natural antioxidant in the meat products.

Keywords: grape seed extract, skin extract, antilipid potential, minced meat.

INTRODUCTION

One of the main strategies to prevent lipid and protein oxidation during food storage is the addition of antioxidants [1]. Antioxidants have a great ability to prevent or reduce lipid oxidation in foods. In this way, they protect the consumer from several diseases: cancer, arteriosclerosis, heart disease, etc. [2]. Extracts from grapes, grape seeds and skins have a high potential for application as an anti-lipid agent in the meat industry and can replace the usage of synthetic antioxidants known to exhibit cytotoxic effects at high concentrations [3, 4]. However, the application of grape extracts as antilipid agents is still limited, due to insufficient research on their composition, their antioxidant properties and their antilipid potential in various meat products. This is one of the most promising applications of grape extracts and deserves to be studied in detail. Even more, considering that grape seeds and skins are waste products from winemaking, this makes it possible to obtain a valuable economically advantageous bioactive products that can be used as an antilipid agent in meat products.

Grape seeds and skins are a rich source of polyphenol compounds with high antioxidant capacity [5]. Some authors reported that they can be used with success as a source of biologically active compounds in the technology of innovative meat products: sausages [6], minced beef [7], raw and cooked chicken meat [8] and many other meats [9]. Proof of the relevance of this problem are the published reviews concerning the application of natural oxidants in meat products as antilipid agents [3, 10].

The phenol compounds in skins and seeds are qualitatively and quantitatively different and depend on the grape varieties [11]. Previous studies have shown

that the content of polyphenols in grape skins can be up to 25 - 30 %, and polyphenols in seeds are as high as 60 - 70 % [12]. Flavanols and anthocyanins are the most abundant phenolic compounds in red grape skins, while grape seeds are rich in flavan-3-ols - (+)-catechin, (-)-epicatechin, procyanidins [4].

Also, there are many studies proving that the red grape seed and skin extracts have a higher content of polyphenols and antioxidant capacity compared to those of white grapes [13]. But the usage of grape seed extract (GSE) and skin extract (SE) of red grapes as an anti-lipid and antimicrobial agent in food products results in color change of the product [14, 15]. Pinot Noir grapes belong to the red varieties, but the wine produced from it has a faint red color. Extracts obtained from its seeds are expected to be almost colorless and not to affect the color of food products. For this reason, the present study was focused on three aims: 1. To prepare seed and skin extracts of Pinot Noir grape variety and determined their color intensity. 2. To estimate the antilipid potential of extracts in minced pork meat model systems. 3. To observe the color change of treated meat samples with the extracts. The obtained data may contribute to the right selection of suitable antilipid additive in meat products on the base of GSE or SE.

EXPERIMENTAL

Materials and chemicals

The materials used in this study were the wastes (pomaces) from the vinification of red wine *Vitis vinifera* L. cv. Pinot Noir (Pink Pelikan Winery). These grape sorts are grown in the Danube region, near the city of Ruse, Bulgaria.

The chemicals used for the experiments: trichloroacetic acid, butylated hydroxytoluene (BHT), thiobarbituric acid were purchased from Sigma-Aldrich Co, Germany. For the extraction of polyphenols was used ethanol 99.9 % v/v, purchased from Valerus, Bulgaria. Deionized water purified by ELGA system (UK) was used throughout the experiments.

Preparation of grape seed and skin extracts

The preparation of grape seed and skin extract was described in our previous work [16]. Briefly, the treatment of grape seeds and skins was carried out separately. Processing included separately washing, drying at 40^{o}C for 14 h, and storage at 4^{o}C. For each experiment, a certain amount of dry grape seeds or skins were grinded to powder with a diameter of 2.5 - 22.5 μm. The mixture of grape seed or skin powder (5 g) with 25 mL 70 % aqueous ethanol was stirred on a magnetic stirrer MMS-3000 (Boeco, Germany) - 500 rpm, room temperature, for 3 h. The upper liquid was separated. Then the process was repeated with another amount of 70 % aqueous ethanol. The resulting mixture was centrifuged at 4830 x g for 10 min. The supernatant was separated and concentrated to 1 mL in a vacuum evaporator (Rotavapor R-215, Buchi, Flawil, Switzerland).

Color evaluation of GSE and SE

The color of Pinot Noir grape seed and skin extracts was determined using a CR-410 Chroma Meter (Minolta Co, Osaka Japan). The conditions were 13 mm port size, illuminant D65 and 10^{o} standard observer. The X-Rite's white and black standards were used to calibrate the spectrophotometer. Color results were determined in the CIE L*a*b* scale. The L* value represented lightness, and a* and b* values represented redness and yellowness, respectively.

Determination of antilipid potential of GSE and SE added to raw minced pork meat

The lipid oxidation of raw minced pork meat was determined on the base of thiobarbituric acid-reactive substances (TBARS), [7]. The fresh raw minced pork meat was purchased from local market in Burgas city and was transferred aseptically to the laboratory. Then the minced meat was divided into six samples of 100 g. Pinot Noir GSE with concentrations 10, 50 and 100 mg was added to three of the samples, SE with concentration 100 mg to the fourth sample and 20 mg butylated hydroxytoluene (BHT) to the fifth sample, as they were preliminary dissolved in 1 mL sunflower oil. Control sample without antioxidant was prepared also (only the sunflower oil was added to minced meat). Then the six samples were separately well mixed and packaged in sterile polyethylene bags, labeled, and stored at 4^{o}C for 10 days. On day 0, 4, 7 and 10, the sample (5 g) of each package was taken and lipid oxidation was evaluated. Each ground pork sample (5 g) was homogenized (15000 rpm, 30 s, 25°C) together with

30 mL of a 7.5 % aqueous trichloroacetic acid (TCA) solution. After filtration, 5 ml of the supernatant was mixed with 5 ml of 0.02 mol L^{-1} aqueous thiobarbituric acid (TBA) in a stoppered tube. The sample was incubated at 100°C for 35 min in a water bath and then cooled for 10 min with cold water. The absorbance at 532 nm against a blank containing 5 mL of distilled water and 5 mL of TBA reagent was measured. The result was presented as mg of malondialdehyde (MDA) per kg of minced meat, using standards with different concentrations of malondialdehyde (from 6 to100 µg mL^{-1}). The experiment was performed in triplicate.

Color stability of raw minced pork meat containing added GSE and SE

The color stability of raw minced pork meat with added GSE and SE was determined on the same device determining the color of obtained GSE and SE. The treatment minced meat samples were homogenized before measurement. The three parameters were determined - lightness (L*), redness (a*) and yellowness (b*). The color evaluation of raw minced pork meat samples with added GSE or SE was performed on days 0, 4, 7 and 10.

Statistical Analysis

Experimental results were means ± SD of three parallel measurements. Analysis of variance was performed by ANOVA procedures (DPS 7.55 for Windows).

RESULTS AND DISCUSSION

Preparation of grape seed and skin extracts and evaluation of their color

The main aim in this study was to evaluate and compare the antilipid potential of seed and skin extracts of Pinot Noir red grape. The first task was the preparation of the extracts. The extraction of grape seeds and skins was carried out by magnetic stirring at 500 rpm, at room temperature, for 3 h. Aqueous mixture of ethanol (70 %, v/v) was chosen for conducting the grape seed and skin extraction. For the medical and food purpose, ethanol is preferred as a solvent, as it is harmless for health. The extract yield and total phenolic content (TPC) of the obtained GSE and SE were determined in our previous work [16]. The extract yield of GSE (12 %) was 2.2 times higher than those of SE (5.5 %). The value of TPC of GSE was 111.22 mg GAE g dw^{-1} matter and it was 2.5 times higher than TPC value of SE.

The color evaluation of the GSE and SE was performed (Table 1). The value of GSE redness (a* value) was 4 times lower than the redness (a* value) of SE. On the contrary, for the other two parameters, GSE lightness and yellowness values were respectively 1.5 and 3.2 times higher than those values of the SE. The obtained results show that the grape seed extract is almost colorless and it will be very suitable for food additive.

Determination of antilipid potential of GSE and SE added to raw minced pork meat

The antioxidants in low quantities, act by preventing or greatly inhibiting the oxidation of the easily oxidizable components such as fats. It is known, that minced pork meat has a high fat content and frequently can be spoiled by lipid oxidation of fat [17]. The effect of Pinot Noir GSE and SE as an antilipid agents in fresh minced pork meat were studied (Table 2). The main objective was to track the possible storage time of untreated and treated with grape extract minced meat, in refrigerator (4°C).

The extend of lipid oxidation was determined by the formation of thiobarbituric acid-reactive substances (TBARS) during storage of the minced pork meat samples, treated with GSE, SE and commercial synthetic antioxidant BHT. The products of the primary

Table 1. Color evaluation of seed and skin extract of Pinot Noir grape.

Extracts	Lightness (L* value)	Redness (a* value)	Yellowness (b* value)
seed	41.84	4.21	21.78
skin	27.14	16.25	6.78

The values represent Mean ± SD of three experiments.

oxidation are hydro peroxides, which rapidly degrade to several substances, mainly malondialdehyde (MAD), and they react with thiobarbituric acid. A control sample without extract is also made in parallel. The samples were stored in refrigerator, at 4°C for 10 days. Analyzes were performed on days 0, 4, 7 and on 10-th day. The lipid oxidation of the samples was measured in mg MAD kg^{-1} minced meat.

Table 2 shows that the control sample storage time has a significant effect on the lipid oxidation development in the fresh raw mince, since already on 4th day of storage at 4°C, TBARS values increases 2.0 times on the 4th day, 2.4 times on the 7th day, and 3.0 times on 10th day. The treatment of the samples with the GSE and SE leads to a reduction of the fresh minced meat lipid oxidation. The results of the treated samples with 100 μg of GSE per gram of fresh minced meat showed a slight inhibition of the sample lipid oxidation over time. When the samples were treated with 500 and 1000 μg GSE per gram of fresh minced meat, there was a stronger inhibition of lipid oxidation and the TBARS values increased slowly. It is evident that the greater amount of GSE introduced into the samples, reduces lipid oxidation to a greater extent. The experiments were also conducted with the addition of SE at a concentration of 1000 μg g^{-1} of fresh minced meat. The experiment was done with only one concentration of SE, since the results described in our previous paper [16] showed that SE has weaker antioxidant activity and polyphenol content compared to GSE. When comparing the parallel results obtained with the addition of 1000 μg g^{-1} GSE and 1000 μg g^{-1} SE, it was found that the antilipid effect of GSE was 1.6 time greater than that of SE. The GSE has limited the oxidative processes (55.32 %), measured at 10-th day of sample storage at 4°C, more effectively than SE (34.04 %). These results were correlated very well with the TPC and the phenolic composition of the two extracts [16]. The greater antilipid activity of GSE correlates with its high TPC values and rich phenolic profile. The influence of the widely used synthetic antilipid agent BHT (0.02 %) for meat products was also monitored. This BHT concentration was chosen because it is used at the production of meat products. The antilipid effect of BHT was similar to the antilipid effect of 500 and 1000 μg g^{-1} GSE. In our previous work [16] it was found that the Pinot Noir GSE was the richer in catechins, epicatechin and dimeric procyanidins in comparison to SE. The ABTS and DPPT antioxidant capacity of GSE was 20 and 7,5 times higher, respectively than the same of SE [16]. Xia et al. found that the most abundant compounds in GSE were procyanidin dimers, catechin and epicatechin, which exert the strongest radical scavenging activity among the majority of polyphenols [18]. The mechanism of the protective effect on lipid oxidation may be because Pinot Noir grape seed extract was rich in flavonoids and procyanidins which have ability to sequestrate radicals [16]. The obtained results agree with findings reported by Carpenter et al. [19]. They found that a high concentration of GSE (1000 μg g^{-1}) in pork meat was four times more effective reducing TBARS values than a low concentration (50 μg g^{-1}). Many studies indicated that the GSE can successfully reduce lipid oxidation of meat [4, 20, 21].

Table 2. Effect of different antioxidant (AO) treatments on TBARS values (mg MAD/kg meat) in minced pork meat during refrigerated storage at 4°C (n = 3).

Treatments	μg AO g^{-1} minced meat	Storage period (days)			
		0	4	7	10
Control	0	0.32 ± 0.12	0.65 ± 0.16	0.76 ± 0.22	0.94 ± 0.28
Seed extract	100	0.31 ± 0.12	0.33 ± 0.14	0.45 ± 0.17	0.67 ± 0.20
Seed extract	500	0.30 ± 0.11	0.29 ± 0.14	0.33 ± 0.14	0.55 ± 0.13
Seed extract	1000	0.29 ± 0.11	0.27 ± 0.13	0.31 ± 0.13	0.42 ± 0.14
Skin extract	1000	0.32 ± 0.12	0.31 ± 0.14	0.40 ± 0.17	0.62 ± 0.19
BHT	200	0.31 ± 0.11	0.29 ± 0.13	0.33 ± 0.17	0.45 ± 0.13

The values represent Mean ± SD of three experiments.

Color stability of raw minced pork meat containing GSE and SE

Lipid oxidation can promote myoglobin oxidation, leading to meat discoloration during storage. The influence of the added extracts of grape seeds and skins and 0.02 % BHT on the lightness (L* values), redness (a* values) and yellowness (b* values) of the minced pork meat stored in a refrigerator at 4°C for 10 days was investigated (Table 3). Samples containing 500 and 1000 μg g^{-1} GSE and 0.02 % BHT showed no change in L* values on day 4. A slight decrease in L* values was observed on days 7 and 10. The change of these values was the least in the samples with added 1000 mg GSE and 0.02 % BHT. The latter additives also showed the least decrease in the a* values of the minced meat, probably due to a weaker oxidation of the myoglobulin. Apparently, because of the synergistic effect of the polyphenolic compounds contained in GSE, the oxidation of myoglobulin is inhibited [4, 22]. In the storage process, it was found that the effect of SE on the preservation of a* values of the samples was weaker compared to the effect of GSE. This is probably due to the lower content of polyphenols in the SE extract. The polyphenol-rich GSE showed relatively good preservation of minced meat redness, analogous to the action of BHT. The b* values (yellowing) of the minced pork samples after addition of the extracts were also measured. During storage of the samples at 4°C, a gradual decrease in the b* values of the samples was observed from 0 to 10 days, but the lowest change was observed when the samples were treated with 1000 μg g^{-1} GSE and 0.02 % BHT.

CONCLUSIONS

The color intensity of seed and skin extracts of Pinot Noir grape was compared. The grape seed extract is almost colorless and its redness (a* value) was 4 times

Table 3. Effect of different antioxidant (AO) treatments on the lightness (L* values), redness (a* value) and yellowness (b* values) of minced meat stored in refrigerator at 4°C (n = 3).

Treatments (n = 3)	μg AO g^{-1} minced meat	Storage period (days)				Treatments Mean ± SD (n = 15)
		0	4	7	10	
Lightness (L* values)						
Control	0	36.75	35.22	33.95	33.05	34.74 ± 0.52
Seed extract	100	38.07	37.14	36.65	36.35	37.18 ± 0.75
	500	38.16	37.95	37.15	36.86	37.53 ± 0.78
	1000	40.05	39.86	39.02	38.92	39.46 ± 0.85
Skin extract	1000	31.02	30.12	29.14	28.83	29.78 ± 0.45
BHT	200	38.49	38.17	37.74	37.35	37.94 ± 0.72
Redness (a* value)						
Control	0	11.46	6.89	4.82	3.75	6.73 ± 0.32
Seed extract	100	10.95	8.39	6.05	4.21	7.40 ± 0.35
	500	10.75	9.98	8.55	6.55	8.96 ± 0.41
	1000	10.23	9.85	8.48	6.45	8.70 ± 0.37
Skin extract	1000	12.55	8.29	5.52	4.25	7.65 ± 0.36
BHT	200	10.59	9.63	8.53	6.45	8.80 ± 0.38
Yellowness (b* values)						
Control	0	6.05	5.53	4.65	4.21	5.11 ± 0.21
Seed extract	100	7.05	6.73	6.15	5.95	6.47 ± 0.23
	500	7.37	7.21	6.98	6.88	7.11 ± 0.28
	1000	7.93	7.87	7.83	7.78	7.85 ± 0.31
Skin extract	1000	7.23	7.01	6.35	6.21	6.70 ± 0.26
BHT	200	7.51	7.31	7.24	7.05	7.28 ± 0.28

lower than those of the skin extract. The polyphenol-rich GSE in amounts of 500 and 1000 μg g^{-1} successfully reduce lipid oxidation of minced meat. GSE limited the oxidative processes more effectively than the SE. The greater antilipid activity of GSE compared to activity of SE correlates very well with its higher total phenolic content and richer phenolic composition. The polyphenol-rich GSE showed relatively good preservation of minced meat redness, analogous to the action of BHT. The high antilipid oxidation capacity, and lack of color make the Pinot Noir GSE a valuable additive of meat products.

Acknowledgements

This study was funded by Bulgarian National Scientific Research Fund, Grant number KP-06-H57/9.

REFERENCES

1. L.D. Shirahigue, C.J. Contreras-Castillo, M.M. Selani, A.P. Nadai, G.B. Mourão, C.R. Gallo, Winery Grape-residue Extract: Effects on Quality and Sensory Attributes of Cooked Chicken Meat, Food Sci. Biotechnol., 20, 5, 2011, 1257-1264.
2. L. Sochorova, B. Prusova, M. Cebova, T. Jurikova, J. Mlcek, A. Adamkova, S. Nedomova, M. Baron, J. Sochor, Health effects of grape seed and skin extracts and their influence on biochemical markers, Molecules, 25, 22, 2020, 5311.
3. R. Amin and S. Edris, Grape seed extract as natural antioxidant and antibacterial in minced beef, PSM Biol. Res., 2, 2, 2017, 89-96.
4. S.Y. Lee, D.Y. Lee, O.Y. Kim, H.J. Kang, H.S. Kim, S.J. Hur, Overview of Studies on the Use of Natural Antioxidative Materials in Meat Products. Food Sci. Anim. Resour., 40, 6, 2020, 863-880.
5. A.B. Mora-Garrido, M.J. Cejudo-Bastante, F.J. Heredia, M.L. Escudero-Gilete, Revalorization of residues from the industrial exhaustion of grape by-products, LWT-Food Sci. Technol., 156, 2022, 113057.
6. G-Y. Tang, C-N. Zhao, Q. Liu, X-L. Feng, X-Y. Xu, S-Y. Cao, X. Meng, S. Li, R-Y. Gan, H-B. Li, Potential of grape wastes as a natural source of bioactive compounds. Molecules, 23, 2018, 2598.
7. J.M. Lorenzo, R.M. Gonzalez-Rodriquez, M. Sanchez, I.R. Amado, D. Franco, Efects of natural (grape seed and chestnut extract) and synthetic antioxidants (buthylated hydroxytoluene, BHT) on physical, chemical, microbiological and sensory characteristic of dry cured sausage “chorizo”, Food Res. Int., 54, 2013, 611-620.
8. M.M. Selani, C.J. Contreras-Castillo, L.D. Shirahigue, C.R. Gallo, M. Plata-Oviedo, N.D. Montes-Villanueva, Wine industry residues extracts as natural antioxidants in raw and cooked chicken meat during frozen storage, Meat Sci., 88, 3, 2011, 397-403.
9. J. Garcia-Lomillo, M.L. Gonzalez-San Jose, Applications of wine pomace in the food industry: Approaches and functions, Compr. Rev. Food Sci. Food Saf., 16, 2017, 3-22.
10. P.E.S. Munekata, B. Gullón, M. Pateiro, I. Tomasevic, R. Domínguez, J.M. Lorenzo, Natural Antioxidants from Seeds and Their Application in Meat Products, Antioxidants, 9, 2020, 815.
11. L.P. Santos, D.R. Morais, N.E. Souza, S.M. Cottica, M. Boroski, J.V. Visentainer, Phenolic compounds and fatty acids in different parts of *Vitis labrusca* and *V. vinifera* grapes, Food Res. Int., 44, 5, 2011,1414-1418.
12. V. Di Stefano, C. Buzzanca, M.G. Melilli, S. Indelicato, M. Mauro, M. Vazzana, V. Arizza, M. Lucarini, A. Durazzo, D. Bongiorno, Polyphenol Characterization and Antioxidant Activity of Grape Seeds and Skins from Sicily: A Preliminary Study, Sustainability, 14, 2022, 6702.
13. I. Rockenbach, V. Gonzaga, M. Rizelio, A. De Souza, S. Gonçalves, M. Genovese, R. Fett, Phenolic compounds and antioxidant activity of seed and skin extracts of red grape (*Vitis vinifera* and *Vitis labrusca*) pomace from Brazilian winemaking, Food Res. Int., 44, 4, 2011, 897-901.
14. R.G. Brannan, Effect of grape seed extract on descriptive sensory analysis of ground chicken during refrigerated storage, Meat Sci., 81, 4, 2009, 589-595.
15. O. Tita, E. Lengyel, D.I. Stegarus, P. Savescu, A.B. Ciubara, M.A. Constantinescu, M.A. Tita, D. Rata, A. Ciubara, Identification and Quantification of Valuable Compounds in red grape seeds, Appl. Sci., 11, 2021, 5124.
16. Z. Chengolova, Y. Ivanov, T. Godjevargova,

Comparison of identification and quantification of polyphenolic compounds in skins and seeds of four grape varieties, Molecules, 28, 10, 2023, 4061.
17. E-Q Xia, G-F Deng, Y-J Guo, H.B. Li, Biological Activities of Polyphenols from Grapes, Int. J. Mol. Sci., 11, 2, 2010, 622-646.
18. X. Yang, B. Xu, H. Lei, X. Luo, L. Zhu, Y. Zhang, Y. Mao, Yan-wei, R. Liang, Effects of grape seed extract on meat color and premature browning of meat patties in high-oxygen packaging, J. Integr. Agric., 21, 8, 2022, 2445–2455.
19. R. Carpenter, M.N. O'Grady, Y.C. O'Callaghan, N.M. O'Brien, J.P. Kerry, Evaluation of the antioxidant potential of grape seed and bearberry extracts in raw and cooked pork, Meat Sci., 76, 2007, 604-610.
20. G.V. B. Reddy, A.R. Sen, P.N. Nair, K.S. Reddy, K.K. Reddy, N. Kondaiah, Effects of grape seed extract on the oxidative and microbial stability of restructured mutton slices, Meat Sci., 95, 2013, 288-294.
21. P. Klinhom, S. Methawiwat, W. Singkon, Effect of Grape Seed and Green Tea Extracts on Lipid Oxidation and Quality of Ground Buffalo Meat during Storage, Food Appl. Biosci. J., 6, 1, 2018, 28-38.
22. J. Libera, A. Latoch, K.M. Wojciak, Utilization of Grape Seed Extract as a Natural Antioxidant in the Technology of Meat Products Inoculated with a Probiotic Strain of LAB, Foods, 9, 1, 2020,103.