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Utilization of Wine Industry Waste (Red Grapes) as Natural Antioxidant for Development of Functional Mutton Rolls

Apoorva Argade, Ashok Malik, Sanjay Yadav and Satyavir Singh Ahlawat*

Department of Livestock Products Technology, College of Veterinary Science, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, INDIA

**Corresponding author: SS Ahlawat; E-mail: ahlawatss9@gmail.com*

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ABSTRACT

This study was conducted with an objective to utilize the red grapes powder and its extracts as natural antioxidant for development of functional mutton rolls. Incorporation of red grapes powder at 1, 2 and 3 per cent levels, and red grapes aqueous and ethanolic extracts at 5, 10 and 15 per cent levels were compared with control, and selected on the basis of sensory evaluation. The total phenols and TBARS value of selected products were compared with control. The sensory scores including overall acceptability increased with the incorporation levels of red grapes powder and aqueous extracts, but decreased with addition of red grapes ethanolic extract. Addition of 3 per cent red grapes powder and 10 per cent of its aqueous extract were found suitable for incorporation in mutton rolls indicating very good acceptability scores (round 7.00). The addition of red grapes powder and its aqueous extract increased the total phenols while TBARS values were found significantly lower as compared to control and BHT added products. It was concluded that red grapes powder (3%) and red grapes aqueous extracts (10%) can be used for development of very well acceptable functional mutton rolls with improved total phenols and lower TBARS vale.

HIGHLIGHTS

- Wine industry waste (Red grapes) powder and its extracts were used as natural antioxidant.
- Red grapes powder (3%) and its aqueous extracts (10%) can be used in functional mutton rolls.

Keywords: Red grapes, mutton rolls, sensory, TBARS

Synthetic antioxidants BHA (butylated hydroxyl anisole) and BHT (butylated hydroxyl toluene or propyl gallate) have been successfully used in order to prevent oxidation in fresh meat. However, BHA and BHT (Li *et al*., 2014) are suspected to be carcinogens, and consumer concern has led to a decrease in their use in the food. This is one of the reasons for the increased demand of the healthy (natural and functional) foods (Fernandase, *et al*., 2018).

Use of natural preservatives to increase the shelf-life of meat products is a promising technology since many herbs, plants, vegetable and fruits extracts or their powders have antioxidant and antimicrobial properties (Biswas *et al*., 2011). Residues from the wine industries and grape juice processing account for approximately 30% of the total volume of grapes used for wine production. Phenolic antimicrobial compounds are found in grape seeds, skins and stem extracts. A positive correlation was reported between the antioxidant activity and the total phenolic compounds in grape byproducts (Silva *et al*., 2018). Grape contains high amount of dietary fibers and phenolics antioxidants such as phenolic acids, anthocyanidins, proanthocyanidins, catechins and other flavonoids (Gómez-García *et al*., 2012).

Therefore, in this research, using grape pomace as a wine industry and juice processing byproduct, it was aimed not

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only at waste valorization but also being used to obtain functional ingredients to design meat as a functional food.

MATERIALS AND METHODS

Healthy sheep meat (of age 10-12 months) was procured from local market of Hisar city and transferred to department of Livestock Products Technology (in ice box), College of Veterinary Sciences, LUVAS, Hisar. Sheep meat was washed thoroughly and deboned manually after trimming of fat and connective tissue and was frozen for 18-24 hours and then minced in an electrical mincer to use for preparation of meat rolls. Red grapes were also procured from the local market of Hisar city.

The fresh spice ingredients, condiment mix, table salt, binder (egg), sunflower oil and chemicals used in the investigation were procured from the local market through local suppliers from respective companies.

Preparation of red grapes powder and extracts

Red grapes were dried in hot air oven drier at 48±2^{o}C for 36 hrs and ground to fine powder in an electric mixer. The fine powdered red grapes were used to make ethanolic and aqueous extract as per the method prescribed by Khandelwal (2002). Ten per cent ethanolic and aqueous extract of red grapes were made by dissolving 10g of powder in 100 ml of 95% ethyl alcohol and 100 ml of distilled water, respectively. The flask containing the extract was kept on the orbital shaker for 3 hrs, and then incubated at 37^{o}C for 72 hrs. The extract was filtered through Whatman filter paper No. 1. The filtrate was then dried in hot air oven drier for 12-14 hrs till a final concentration of 50±2% was obtained.

Preparation of mutton rolls

Red grapes powders (mixed in chilled water) and extracts (aqueous and ethanolic extracts) were added, independently, at different levels with other additives same as in control (Table 1) meat rolls and mixed in an electric mixer for 2 minutes to prepare stable emulsion.

The prepared emulsion was stuffed in autoclavable beakers manually and uniformly distributed with the help of a glass rod. The beakers were covered with aluminium foil and pressure cooked for 30 minutes at low gas flame. After cooking, rolls were taken out and cooled to room temperature, packaged in polythene bags and stored at refrigerated temperature (4+1^{o}C) for further use.

Analysis

A six member experienced panel of judges consisting of teachers and postgraduate students of College of Veterinary Science, LUVAS, Hisar, evaluated the samples for the sensory attributes using 9-point Hadonic scale), where 9=extremely like and 1=extremely dislike. The test samples were presented to the panelists after assigning the suitable codes. The samples were warmed in a microwave oven for 20 sec before serving to the sensory panelists. The water was served for rinsing the mouth between the samples.

Total phenolic content was estimated by Folin Ciocalteu's method. The data for total phenolic contents of polyherbal formulation were expressed as mg of gallic acid equivalent weight (GAE)/ 100 g of dry mass (Kamtekar *et al*., 2014). The TBARS value was determined according to the method of Zeb and Ullah (2016).

STATISTICAL ANALYSIS

The experiment was repeated thrice in duplicate and the results were analyzed using completely randomized design as per Snedecor and Cochran (Snedecor and Chochran, 1994). The data were subjected the statistical analysis using SPSS MAC, version 22.0, SPSS Chicago (USA).

RESULTS AND DISCUSSION

Sensory evaluation

However, incorporation of red grapes powder at 1 and 2% levels improved colour scores in comparison to control, but being as high level and more colour enhancing effect of 3% red grapes powder was selected (Table 2). This might be due to more desirable colour provided by red grapes in mutton rolls. Similar results of increase in redness values relative to controls in cooked chicken hamburgers were also documented by Sa´yago-Ayerdi *et al*. (2009) with addition of grape pomace.

On addition of red grapes aqueous extract at 10 percent level in mutton rolls, the colour score was comparable with

Table 1: Formulation of control and treated mutton rolls

Treatments	Ingredients (g)							
	Meat	Salt	Egg	Spice mix	Ginger: Garlic (1:1)	Sunflower Oil	Treatments	Total Qty
C_1	76.58	2	10	2	4	5	—	99.58
C_2	76.57	2	10	2	4	5	0.01	99.58
T_1	75.58	2	10	2	4	5	1	99.58
T_2	74.58	2	10	2	4	5	2	99.58
T_3	73.58	2	10	2	4	5	3	99.58
T_4	71.58	2	10	2	4	5	5	99.58
T_5	66.58	2	10	2	4	5	10	99.58
T_6	61.58	2	10	2	4	5	15	99.58
T_7	71.58	2	10	2	4	5	5	99.58
T_8	66.58	2	10	2	4	5	10	99.58
T_9	61.58	2	10	2	4	5	15	99.58

C_1: Control-Meat rolls without BHT and red grapes; C_2: BHT-Meat rolls with 100 ppm BHT as synthetic preservative; T_1, T_2, T_3: Meat rolls incorporated with 1, 2 and 3 % of red grapes Powder; T_4, T_5, T_6: Meat rolls incorporated with 5, 10 and 15% of red grapes Aqueous extract; T_7, T_8, T_9: Meat rolls incorporated with 5, 10 and 15% of red grapes Ethanolic Extract.

Table 2: Sensory evaluation of cooked mutton rolls incorporated with red grapes powder and its extracts (n=6)

Treatments	Parameters					
	Appearance	Flavour	Texture	Tenderness	Juiciness	Overall acceptability
C_1	$7.33^{cd}\pm0.16$	$7.16^{de}\pm0.16$	$7.16^{cd}\pm0.30$	$6.66^{d}\pm0.21$	$6.83^{cde}\pm0.16$	$7.33^{cde}\pm0.21$
C_2	$7.16^{bc}\pm0.16$	$7.16^{de}\pm0.16$	$7.16^{cd}\pm0.30$	$6.66^{d}\pm0.21$	$6.83^{cde}\pm0.16$	$7.00^{cd}\pm0.02$
T_1	$7.00^{cd}\pm0.25$	$6.83^{d}\pm0.16$	$7.00^{cd}\pm0.01$	$7.16^{de}\pm0.16$	$6.66^{cd}\pm0.21$	$6.83^{cd}\pm0.16$
T_2	$7.83^{fg}\pm0.16$	$7.16^{de}\pm0.16$	$7.00^{bcd}\pm0.25$	$7.66^{e}\pm0.21$	$7.50^{ef}\pm0.22$	$7.50^{ef}\pm0.22$
T_3	$8.00^{g}\pm0.25$	$8.00^{f}\pm0.25$	$8.16^{f}\pm0.16$	$7.50^{e}\pm0.22$	$7.66^{f}\pm0.21$	$8.00^{fg}\pm0.25$
T_4	$7.50^{def}\pm0.22$	$7.33^{de}\pm0.21$	$7.33^{de}\pm0.21$	$7.50^{e}\pm0.22$	$7.33^{def}\pm0.21$	$7.50^{ef}\pm0.22$
T_5	$7.33^{cd}\pm0.21$	$7.33^{de}\pm0.21$	$7.16^{cd}\pm0.16$	$7.50^{e}\pm0.22$	$8.00^{f}\pm0.25$	$7.50^{ef}\pm0.22$
T_6	$5.50^{a}\pm0.22$	$4.83^{a}\pm0.16$	$5.16^{a}\pm0.16$	$5.00^{a}\pm0.21$	$5.00^{a}\pm0.25$	$4.66^{a}\pm0.21$
T_7	$5.43^{b}\pm0.16$	$4.33^{b}\pm0.30$	$4.83^{a}\pm0.16$	$5.06^{a}\pm0.16$	$4.18^{b}\pm0.16$	$4.23^{b}\pm0.16$
T_8	$5.50^{bc}\pm0.22$	$4.16^{a}\pm0.25$	$4.20^{a}\pm0.25$	$5.00^{a}\pm0.21$	$4.23^{b}\pm0.21$	$4.20^{b}\pm0.16$
T_9	$5.00^{a}\pm0.21$	$4.16^{a}\pm0.16$	$4.16^{a}\pm0.25$	$5.00^{a}\pm0.25$	$3.50^{a}\pm0.22$	$4.02^{a}\pm0.21$

Mean±SE with different small letter superscripts column wise differ significantly ($p<0.05$); C_1= control, C_2 = 100 ppm BHT; T_1, T_2, T_3: Meat rolls incorporated with 1, 2 and 3 % of red grapes powder; T_4, T_5, T_6: Meat rolls incorporated with 5, 10 and 15% of red grapes aqueous extract; T_7, T_8, T_9: Meat rolls incorporated with 5, 10 and 15% of red grapes ethanolic extract.

control. Further addition of red grapes aqueous extract level declined the colour score significantly with respect to the control. Red grape ethanolic extract treatment at all the tried levels in mutton rolls showed significantly lower colour scores than control products.

Resultant batter colour scores with addition of 3 per cent red grapes powder and 10 per cent red grape aqueous extract in mutton rolls might be due to influence of anthocyanin riched red grapes. Ganhão *et al*. (2010) also investigated that fruit extracts significantly influence the colour of freshly cooked burger patties due to presence of anthocyanins, anthocyanin as major pigment constituents of fruits. Butkhup *et al*. (2010) also reported that anthocyanins impart a distinct purplish colour. The impact of these pigments on mutton rolls led to an intense increase of redness and a decrease of the other colour parameters. Anthocyanins may react with flavanols to produce more stable pigments, either directly or by means of different aldehydes (Pisarra *et al.*, 2003). An intense red brownish colour is accepted and generally preferred by cooked meat consumers (Aaslyng *et al.*, 2007; Berry, 1998).

However, one and 2 per cent levels of red grape powder were acceptable and comparable with control, but the 3 percent addition of red grape powder in mutton rolls enhance flavor score significantly as compare to C_1, C_2, T_1 and T_2. This might be due to high dietary fiber content in the mutton rolls added with 3% red grape powder and it improved stability in flavor score. Sa'yago-Ayerdi *et al.* (2009) also documented similar trends of increased flavor with addition of red grapes pomace in chicken hamburger.

Both red grapes aqueous extract incorporation levels (5 and 10 percent) in rolls were acceptable with similar to control flavor scores. Najeeb *et al.* (2014) also reported that flavor scores of chicken patties incorporated with red grape powder was statistically similar to control and were equally acceptable. However, the flavor scores with addition of red grape ethanolic extract at different levels were not only significantly lowers than control and BHT treated products, but also clearly rejected by the panelists. These findings were also in accordance with the results of Selani *et al*. (2011). Another investigation by Butkhup *et al.* (2010) revealed that Flavan-3-ols (monomeric catechins and proanthocyanidins) are another large family of phenolic compounds of red grapes that are mainly responsible for the astringency, bitterness and undergo partial extraction during the winemaking or ethanolic extraction process.

In case of red grapes powder, the textural property score increased as the incorporation levels increased. The levels of red grape powder at 1 and 2% treated rolls were in acceptable range and comparable with control but the 3 percent addition significantly improve textural property as compare to control. The soluble dietary fiber content in red grape powder might provide good textural property by improved water-holding capacity and avoid the loss of these sensorial attributes (Sa'yago-Ayerdi *et al*., 2009).

However, the trends of texture scores on addition of 10 percent red grape aqueous extract in roll were statistically similar to control but the texture scores of red grape ethanolic extract incorporated mutton rolls at all the tried levels were statistically lower than control. In agreement with Ganhão *et al.* (2010) findings, the addition of most fruit extracts inhibited the texture deterioration seen in the samples by preventing protein oxidation. In accordance to Sa'yago-Ayerdi *et al*. (2009), the soluble dietary fiber content in red grapes pomace might supply texture due to improved water-holding capacity and maintained the textural quality in the products.

On addition of red grapes powder at one, 2 and 3 percent levels in mutton rolls were acceptable and statistically comparable to control. Being as higher level of incorporation of 3% red grape powder in mutton rolls with highest tenderness scores was considered for product development. Najeeb *et al.* (2014) also reported the similar results with addition of grapes powder in meat and meat products.

Tenderness score with incorporation of red grapes aqueous at 5 and 10 percent level treatments were significantly higher as compare to 15 percent level of incorporated rolls and controls. In agreement with Sa'yago-Ayerdi *et al*. (2009), it might be because of the higher soluble dietary fiber content in the extract added rolls which improved stability in tenderness in spite of the phenolic compounds present in the samples. Being

Table 3: Total phenols and TBARS value of raw emulsion and cooked mutton rolls treated with red grapes powder and their extracts (n=6)

Treatments	Total penolic contents (mg gallic acid equivalent /gm)		TBARS Values (mg malondehyde/kg)	
	Raw emulsion	**Cooked rolls**	**Raw emulsion**	**Cooked rolls**
C	$0.38^{aA}\pm0.01$	$0.34^{aA}\pm0.01$	$0.35^{cA}\pm0.010$	$0.44^{cB}\pm0.06$
BHT	$0.78^{bA}\pm0.02$	$0.69^{bB}\pm0.01$	$0.27^{bA}\pm0.003$	$0.36^{bB}\pm0.02$
RGP	$2.38^{cA}\pm0.14$	$2.05^{cB}\pm0.0$	$0.22^{bcA}\pm0.002$	$0.28^{cB}\pm0.01$
RGAE	$2.24^{cA}\pm0.05$	$1.91^{cB}\pm0.08$	$0.23^{cA}\pm0.003$	$0.27^{cB}\pm0.01$

Mean ± SE with different small letter superscripts column wise and capital letters row wise in different parameters differ significantly (p≤0 .05); C =Control, BHT = Butylated Hydroxyl Toulene, RGP = Red grapes powder (3%), RGAE = Red grapes aqueous extract (10%).

higher level of incorporation and having tenderness score near to control, red grapes aqueous extract at 10 percent level treatment was selected. The tenderness scores of red grape ethanolic extract incorporation at different levels (5, 10 and 15 percent) were significantly lower than control. Results for red grape extract were also in accordance to the reports of Carpenter *et al.* (2007).

The juiciness scores increased as the incorporation levels of red grape powder increased and 3 percent level showed the highest juiciness scores.

Addition of 10 percent red grapes aqueous extract in mutton rolls showed significantly higher juiciness scores as compare to controls, 5% and 15% red grape aqueous extract treated rolls. The juiciness scores of red grape ethanolic extract incorporated mutton rolls at 5, 10 and 15 percent levels were very low than control and other treatments and were clearly rejected. These results were in the agreements of Najeeb *et al.* (2014), Brannan *et al*. (2009) and Carpenter *et al*. (2007) reports.

The overall acceptability scores of red grapes powder treated mutton rolls were comparable at all three levels (1 percent, 2 percent and 3 percent) with respect to control. Red grape powder incorporated mutton rolls at 3% level showed highest overall acceptability scores.

In case of red grapes aqueous extract treatment, the overall acceptability scores were statistically similar to treatments at 5 and 10 percent level and further addition of aqueous extract at 15 percent level significantly decreased the overall acceptability scores. The overall acceptability scores with addition of red grape ethanolic extract at all the three levels (5, 10 and 15 percent) were significantly lower than control products and rejected by panelists. The cumulative effect of colour, falvour, tenderness, texture and juiciness scores were reflected in overall acceptability scores also. Similar results with incorporation of red grapes were also reported by various workers. (Selani *et al.*, 2011; Brannan *et al*., 2009; Carpenter *et al*., 2007; Najeeb *et al.*, 2014).

Hence, on the basis of sensory scores, the incorporation of red grape powder (3 percent) and red grape aqueous extract (10 percent) were selected for further studies.

Total phenols and TBARS value

Mutton emulsion treated with RGP and RGAE showed significantly higher total phenols as compared to both control and BHT treatments (Table 3). However, addition of BHT significantly increased the total phenols in raw emulsion as compared to control, but it was significantly lower than red grape treated samples. Amongst all treatments, RGP exhibit highest total phenols followed by RGAE and these values were significantly higher than and control and BHT treatments. Similar trend for total phenols was also observed in cooked mutton rolls. Increase in total phenols in treated raw emulsion and cooked products were due to incorporation of total phenols rich red grapes powder and red grapes aqueous extract.

Cooking significantly decreased the total phenols as compared to raw emulsion and it might be due to some phenol content had been lost during cooking because of exposure of heat and could be due to leaching of phenols during fluid loss.

Results of this study clearly revealed that red grape treated products (mainly RGP treatment) contain high amount of total phenols due to incorporation of phenols rich red grape powder and its aqueous extract. During investigation of this study the total phenolic content in red grapes treated products were found more than that of the results previously documented (Soares *et al.*, 2008; Selani *et al.*, 2011) in the literature. Butkhup *et al.* (2010) also explained that the discrepancies in the phenolic composition of grapes depends on multiple factors including climate, degree of ripeness, berry size and grape vine variety, differences in cultivar, cultivation site, climate, viticultural etc.

An increase in TBARS value (mg malonaldehyde/kg) is an indicator of the development of oxidative rancidity. The TBARS values (mg malonaldehyde/kg) when compared between different treatments, it was found that red grape treated products exhibits slightly a lower TBARS values in mutton rolls than the control and BHT treatment. The possible reason could be that red grape treated mutton rolls have higher total phenolic content than the control and BHT treatment. TBARS number has been found to be correlated to the total phenols of the product (John *et al.*, 2013).

Results of this study were in concurrent with Brannan and Mah (2007), they noticed the antioxidant effect of grape seed extract (GSE) in meat system and stated that, GSE demonstrates the antioxidant activity by reducing the amount of primary lipid oxidation products (e.g. lipid hydroperoxides and hexanal) and secondary lipid oxidation products (e.g. thiobarbituric acid reactive substances TBARS). Lower TBARS of red grapes treated samples were also in agreement with the findings of various workers in restructured mutton slices (Reddy *et al.*, 2013).

Addition of 3 per cent red grapes powder and 10 per cent of its aqueous extract were found suitable for incorporation in mutton rolls indicating very good acceptability scores (round 7.00) and it increased the total phenols content while TBARS values were found significantly lower as compared to control and BHT added products.

CONCLUSION

It was concluded that red grapes powder (3%) and red grapes aqueous extracts (10%) can be used for development of very well acceptable functional mutton rolls with improved total phenols and lower TBARS vale.

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