The Effects of Grape Seed Powder and Extract on Antimicrobial of Fermented Turkish Sausage

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Abstract—In this study, the effects of the grape seed powders and extracts of from two different grape cultivars (Razaki and Siyah Gemre) on the quality characteristics of Turkish sausage were investigated during the ripening period. Some characteristics of Turkish sausage including phenolic content, radical scavenging, hydrogen peroxide scavenging, Fe⁺² chelating activity, Inhibition of Escherichia coli, Staphylococcusaureus, Candida albicans were studied. In fermented sausage, grape seed powders and extracts demonstrated the greatest inhibitory activity against Staphylococcus aureus. Furthermore, the results showed that Siyah Gemre grape seed extract was able to reduce Staphylococcusaureus populations by 42 CFU/g, while the population of Escherichia Coli was reduced by 590 CFU/g. Siyah Gemre grape seed extract was able to reduce Candida albicans populations by 880 CFU/g. Also this study demonstrated that grape seed extracts were more effective than grape seed powders. Our results suggest that the use of grape seed extract is a feasible alternative as antibacterial and antioxidant agents to prevent the deterioration of foods by bacteria and oxidation.

Keywords— Grape seed powder and extract, Razakı and Siyah Gemre antimicrobial, antioxidant, natural preservative.

I. INTRODUCTION

The main changes (microbial, chemical and sensory) affecting the quality and durability of sausage, which is one of the important meat products of the food sector, occur during the maturation process of sausages [1]. Not paying attention to technological and hygienic conditions during the maturation stage of the sausages affects human health negatively. Many synthetic preservatives are currently being used to reduce microbial growth and thereby extend the shelf-life of sausage. However, synthetic preservatives have restricted use in foods as these agents are known to be carcinogenic [2]. The medicinal and nutritional value of grapes (*Vitis vinifera*) has been know for thousands of years. Among other beneficial effects of parts of a grape, grape seeds are believed to have a powerful antioxidant property due to its rich source of polyphenol compounds. The polyphenol compounds are as much as 60-70 % in grape seeds compared only 10 % in the fruit and 28 - 35 % in the peels [3,4]. The aim of this study was to determine the effect of two different grape seeds powder and extracts on antimicrobial of Fermented Turkish sausages.

II. MATERIAL AND METHOD

2.1 Material

The day after slaughter, three-year-olds beef, beef subcutaneous fat and sheep's tail fat were obtained from Biçici sausage ltd., Sti., in Afyonkarahisar (Turkey). Spices used in sausage making was obtained from Soylu spices ltd., Sti., in Afyonkarahisar (Turkey). Razakı and Siyah Gemre grapes cultivars was obtained from public market in Isparta (Turkey). In the study, different concentrations of grape seed powder and extract obtained from Razakı and Black Gemre varieties were added to sausage during the fermentation process.In research was used *Escherichia coli* ATCC Kodu:25922, *Staphylococcus aureus* ATCC kodu:25923 and *Candida albicans* for microbiological analyzes.

2.1.1 Preparation of Grape Seed Powder and Grape Extract

A grape seed obtained from bunches of grape varieties mature Razakı and Black Gemre were washed and cleaned, then dried in the shade. Dried grape seeds were triturated in a laboratory-type mill to obtain powder. The resulting grape seed powder was extracted with petroleum ether at 60 ° C for 6 hours in a Soxhelet extraction apparatus, and then the oil was separated. The remaining oil-free powder was again extruded in a Soxhelet extraction apparatus for 8 hours at 60 ° C with a solution of acetone: water: acetic acid (90: 9.5: 0.5) [5]. The extracts were passed through Whatman 1 filter paper and completely freed from solvent in a rotary evaporator, then freeze-dried in a lyophilizer.

2.1.2 Sausage Production

The meats are first made into the meat cubes in hygienic conditions and spices are added on them. Raw materials and additives used in sausage production are given in Table 1. The mixture was then passed through a mincemeat machine with frozen fat. The dough passed through the mincing machine was allowed to mature at 4°C for 8 hours. Then, grape seed, grape seed extract and bacterium were added into the sausage dough and mixed at a speed of 10-12 rpm for 1 hour and then filled into natural sheaths. Sausages stuffed in natural sheaths are attached to hanging carts by cotton threads. Samples of sausages were taken for microbiological and other analyzes at this stage. The sausages that were filled and put in carts were kept in equilibrium rooms (70-75 % relative humidity at 10-15°C) for 12 hours. Sausages kept in balancing rooms have been subjected to a certain period of submergence. The sausage samples were subjected to drying and maturation for 12 days in the incubation chamber. Sausage samples remaining in the incubation chamber were stored at 25°C and 90 % relative humidity for the first 2 days, 22°C and 80 % relative humidity for 3-4 days, 20°C and 70% relative humidity for 5-7 days, 18°C and 65% relative humidity for 8-10 days, it was maintained at 18°C and 60% relative humidity until the 12th day of maturation. During maturation, the air flow in the environment was set at 0.5 m / s and kept constant. Sausage production was done twice in the same quantity and conditions.

TABLE 1
RAW MATERIALS AND ADDITIVES USED IN FERMENTED TURKISH SAUSAGE

Materials	K	N	RT1/ SGT1	RT2/ SGT2	RT3/ SGT3	RT4/ SGT4	RE1/ SGE1	RE2/ SGE2	RE3/ SGE3	RE4/ SGE4
Meat cubes (kg)	5	5	5	5	5	5	5	5	5	5
Tail fat (g)	500	500	500	500	500	500	500	500	500	500
Salt (g)	5	5	5	5	5	5	5	5	5	5
Garlic (g)	75	75	75	75	75	75	75	75	75	75
Chili pepper (g)	35	35	35	35	35	35	35	35	35	35
Red pepper (g)	20	20	20	20	20	20	20	20	20	20
Black pepper (g)	35	35	35	35	35	35	35	35	35	35
Cumin	35	35	35	35	35	35	35	35	35	35
Pimento (g)	10	10	10	10	10	10	10	10	10	10
Grape seed powder (ppm)			250	500	1000	2000	250	500	1000	2000
Grape seed extract (ppm)			250	500	1000	2000	250	500	1000	2000
NaNO2 (g)		0.5								

RT1/SGT1: Razakı grape seed powder 250 ppm, RT2/SGT2: Razakı grape seed powder 500 ppm, RT3/SGT3: Razakı grape seed powder 1000 ppm, RT4/SGT4: Razakı grape seed powder 2000 ppm, RE1/SGE1: Siyah Gemre grape seed extract 250 ppm, RE2/SGE2: Siyah Gemre grape seed extract 500 ppm, RE3/SGE3: Siyah Gemre grape seed extract 1000 ppm, RE4/SGE4: Siyah Gemre grape seed extract 2000 ppm

2.2 Method

2.2.1 Detection of antioxidant activity values of grape seed extracts

2.2.1.1 Determination of the effect of free radical scavenging

2.5 g of GSE was weighed and extruded by homogenizing in a total of 50 ml of 50% aqueous methanol on an agitator (Ultra Turrax). Extracts from the coarse filter paper were centrifuged at 4000 x g for 3 minutes in a refrigerated centrifuge at $4 ^{\circ}$ C and the blue band (No 589) was filtered through filter paper once the filtrate was complete to 50 ml. To determine the effect of extracts on DPPH added to the medium, Brand-Williams [6] method has been modified and used. For this purpose, dilutions from methanol to 0.4-4 mg/ml were prepared from the obtained extracts. Take 0.1 ml of each dilution and add 3.9

ml of DPPH solution prepared with $6x10^{-5}$ M methanol and mix well. Samples were left in the dark and at room temperature for 60 minutes. Then the absorbance at 515 nm was measured against methanol. The absorbance of DPPH radical is taken as a control

The linear regression equation of the absorbances against concentrations was established by reading the absorbances of the serial solutions prepared at seven different concentrations from the DPPH ($6 \times 10^{-5} \text{ M}$) stock solution at 515 nm:

A
$$(515 \text{ nm})=14,346 \text{ (C DPPH)}-0.0188 \text{ (R}^2=0.981)$$

The residual DPPH concentrations corresponding to the absorbance readings of the sample series prepared at different concentrations were calculated,

DPPH percentage:

% residual DPPH
$$= [DPPH]_{sample} / [DPPH]_{control}$$

A regression equation was established between the percentage of residual DPPH and the amount of sample in the test medium to the amount of DPPH (mg sample / mg DPPH), and sample concentration values were determined which reduced the initial DPPH concentration by 50% for the grape seed extract using this equation. The EC50 values were divided one to one and the antiradical activity (AE) was calculated to be 1 / EC50 value.

2.2.1.2 Detection of chelating activity with Fe⁺²

The methods used to determine the chelating activity of Fe⁺² of extracts were modified by Rival et al. [7]. Extracts prepared at different concentrations ranging from 6-45 mg / ml were taken 1 ml of sample and mixed with 3.7 ml of deionized water. Then 0.1 ml of 2 mM FeCl₂ solution was added, followed by thorough mixing and incubation for 70 minutes in the dark and at room temperature. Subsequently, 0.2 ml of 5 mM ferrozine was added again, and the absorbance of the Fe⁺² - ferrosin complex formed after 10 minutes was measured at 562 nm. 1 ml of water was used instead of the sample in the control reading. The chelating capacity of the samples with Fe⁺² was calculated by the following equation [8]:

% chelating activity = [1- (sample absorbance / control absorbance)] x 100

2.2.1.3 Determination of H₂O₂ disintegration effect

 $\rm H_2O_2$ remove ability of the plant extract can be determined spectrophotometrically [9.] For this, 1 ml (2, 6 and 10 mg / ml) sample was mixed with 3.4 ml of 0.1 M phosphate buffer (pH 7.4) and 0.6 ml of the same buffer prepared with 43 mM $\rm H_2O_2$ and incubated for 60 min. Then the absorbance of the mixture was measured at 230 nm. A control solution without $\rm H_2O_2$ was prepared for each sample concentration. The linear regression equation was used to determine the concentration of unreacted $\rm H_2O_2$ (mM) in the medium. For this, 0.6 ml of 10, 15, 25, 43 and 50 mM $\rm H_2O_2$ solution was added to 3.4 ml of phosphate buffer and the absorbance at 230 nm was measured, and a linear regression equation was drawn by plotting the absorbance versus concentration:

(+) - catechin was used as reference antioxidant. The H₂O₂ removal capacity of the samples was calculated by the following equation:

 H_2O_2 removal capacity (%) = [1- (In the example H_2O_2 conc./Control H_2O_2 conc.)] x 100

2.2.1.4 Determination of Total Phenolic Substance Content

The total phenolic substance contents of the samples were analyzed using the Folin Ciocalteu colorimetric method [10]. Reading was carried out on a spectrophotometer using a wavelength of 765 nm. Values are calculated as mg GA / g. The total phenolic substance content was given as gallic acid (GA) equivalent.

2.2.2 Detection of the effects of the applications on the bacteria Escherichia coli and Staphylococcus aureus on Turkish Fermente Sausage

A sample of 25 g sausage was homogenized with 225 ml dilution fluid (sample volume x 9 diluent fluid). *Escherichia coli* and *Staphylococcus* aureus kit were used to read the nutrient bar code and the broth was opened with 3 ml sterile pure water dispenser. One ml of the sample from the stomacher bag was added to the bottle and the vortex was placed in the filling stand

for a few seconds after mixing. The sample was filled in the card and then removed from the device after being sealed. Cards placed in the incubation stand were left to incubate at 37 °C for 24-27 hours. Cards filled with the last incubation period were placed in the device and read [11].

2.2.3 Detection of the effects of applications Turkish Fermented Sausageon Candida albicans yeast

A sample of 25 g sucuk was homogenized with 225 ml dilution fluid (sample volume x 9 diluent fluid). To the Tempo device, a nutrient bar code was read from the Candida albicans kit and placed in a 3 ml sterile pure water dispenser. One ml of the sample from the stomacher bag was added to the bottle and the vortex was placed in the filling stand for a few seconds after mixing. The sample was filled in the card and then removed from the device after being sealed. Cards placed in the incubation stand were left to incubate for 72-76 hours at 25 °C. Cards filled with the last incubation period were placed in the device and read [11].

2.2.4 Statistical analyzes

The data obtained were evaluated in the SAS statistical program [12]. Variance analysis was used in the General Linear Modeling implementation, and the Tukey test was used when the differences between the groups were determined [13].

III. RESULT AND DISCUSSION

3.1 Antioxidant Activity Values of Grape Varieties

The antioxidant capacities of grape varieties were evaluated in terms of antiradical activity, H_2O_2 inhibition and the total amount of phenolic compounds. Table 2 shows the results of the experiments. DPPH is a free radical compound widely used to determine the free radical scavenging ability of various extracts [3]. When the antiradical activities of the grape samples were compared, the highest value was found in the extracts obtained from Black Gemre grape, which is a black colored variety with 0.88%. The antiradical activity of the extract, which was obtained from Razakı grape seeds (a white variant), was determined to be 0.456%. Among the transition metals, iron is known as the most important lipid oxidation pro-oxidant. 64.15% of the Fe $^{+2}$ chelating activity of the extract obtained from black Gemre seeds and 54.21% of the Fe $^{+2}$ chelating activity of the extract obtained from the Razakı seeds were determined. When the H_2O_2 inhibitory activities of the grape varieties were compared, the inhibition value of the extract obtained from Black Gemre seeds was 78.67% and the inhibition value of the extract obtained from Razakı seeds was 57.71%. Phenolic compounds are extremely important in terms of antioxidant activity value. The total amount of phenolic compounds of the grape varieties in the study was determined as 139,16 mg gallic acid / g in Black Gemre and 123,54 mg gallic acid / g in Razakı variety

TABLE 2
ANTIOXIDANT ACTIVITY VALUES OF THE VARIETIES OF GRAPES

Group Antiradical activity (%)		Fe ⁺² chelating activity (%)	H ₂ O ₂ inhibition (%)	Total amount of phenolic substance (mg gallic acid / g)			
RE	0.456 b*	54.21 ^b	57.71 ^b	123.54 ^b			
SGE	0.88 ^a	64.15 ^a	78.67 ^a	139.16 ^a			

^{*} The difference between the averages with the same lowercase letters in the vertical column is not statistically significant (p>0.05)

3.2 Effects of Staphylococcus aureus applications on Turkish Fermented of Sausage

Staphylococcus aureus values at the fermentation stage of the sausages are shown in Table 3. It was observed that the difference between the values of Staphylococcus aureus was significant (p> 0.05) during the fermentation process of the samples. Staphylococcus aureus according to applications throughout the ripening period in general values. This decrease occurs when N, RE4, SGT2, SGE2, SGE3 and SGE4 administration continued until the 12th day, but 6th day it is not statistically significant after the day. According to control decrease in Staphylococcus aureus values in all treatments has arrived. Grape seed extract and powder have an important effect on Staphylococcus aureus bacteria. In many studies, the results obtained from different applications of grape seed powder and extract are similar to our study. The reason for this is that grape seed extract is rich in polyphenols and therefore has antibacterial activity as a powerful antioxidant [15,16].

TABLE 3
EFFECT OF APPLICATIONS ON SAUSAGE STAPHYLOCOCCUS AUREUS (CFU/G)

	Maturation time (days)					
Sample	0. 1 d	3 d	6 d	9 d	12 d	
N	17916 ^{a*A**}	4150 cbB	2300 bcdeBC	1700 °C	250 °C	
K	18833 ^{aA}	8200 ^{aB}	7150 ^{aB}	7700 ^{aB}	6800 ^{aB}	
RT1	18000 ^{aA}	3450 bcdeB	2500 bcdeB	1400 ^{cB}	940 ^{bB}	
RT2	9600 ^{Ba}	3700 bcdB	2700 bcdC	1301 ^{aD}	910 bD	
RT3	9400 ^{bA}	4350 bB	3350 bBC	3000 bC	925 ^{bD}	
RT4	9050 ^{bA}	1850 ^{fC}	1550 ^{edC}	3000^{bB}	930 ^{bC}	
RE1	9250 ^{bA}	2250 defB	2100^{cdeB}	1300 ^{cBC}	820 ^{cbC}	
RE2	9000 ^{bA}	4100 bcB	2250 bcdeC	1800 ^{cCD}	945 bD	
RE3	8500 bA	$2700^{\text{ cdefB}}$	2200 bcdeBC	1650 ^{cCD}	1100 ^{bD}	
RE4	9050 ^{bA}	1850 fB	1550 deBC	1250 ^{cBC}	935 ^{bC}	
SGT1	9050 ^{bA}	4250 bB	2400 bcdeC	1350 ^{cCD}	940 ^{bD}	
SGT2	8450 bA	2300^{defB}	1800 deBC	1500 ^{cBC}	835 bcC	
SGT3	8250 bA	4050 bcB	1950 ^{deC}	1650 ^{cDC}	975 ^{bD}	
SGT4	8700 bA	4450 bB	3150 bcC	1250 ^{cD}	840 bcD	
SGE1	8600 bA	3050 bcdefB	2150 ^{cdeBC}	1450 ^{cC}	920 ^{bD}	
SGE2	8300 bA	2150 efB	1750^{deBC}	1250 ^{cBC}	830 bcC	
SGE3	8200 bA	$2400^{ m defB}$	1500 eBC	880^{Cc}	420 °C	
SGE4	8800 bA	2200 defB	1400 ^{eBC}	840 ^{Cbc}	455 °C	

^{*} The difference between the averages with the same lowercase letters in the vertical column is not statistically significant (p>0.05) ** The difference between the averages with the same uppercase letters on the horizontal line is not statistically significant (p>0.05)

3.3 Effects of Escherichia coli applications on Turkish Fermented of Sausage

Escherichia coli values in the pipening stages of the sausages are shown in Table 4. It was observed that the applications were significant (p> 0.05) in terms of Escherichia coli values during the fermentation process. During the maturation period, Escherichia coli values decreased. It was observed that the measurements made on different days were statistically significant, and during the 12th day of application, the values of Escherichia coli except K application decreased. Studies conducted to investigate the effect of grape seed extract on Escherichia coli have shown that grape seed extract reduces the number of Escherichia coli. This is due to the anti-bacterial properties of grape seeds. Similar results were obtained in our study [17,18,19].

TABLE 4
EFFECTS OF THE APPLICATIONS ON SAUSAGE ESCHERICHIA COLI (CFU/G)

	Maturation time (days)					
Sample	1. 1 d	3 d	6 d	9 d	12 d	
N	13500 ^{abcd*} A**	9500 bB	3550 bC	1400 ^{bC}	675^{Bc}	
K	16500 ^{aC}	73500 ^{aBC}	130000 ^{aB}	135000 ^{aB}	675000 ^{aA}	
RT1	$13500^{ m abcdA}$	9250 ^{bB}	7500 bB	4700 ^{bC}	1900 ^{bD}	
RT2	12000 ^{abcdeA}	9450 bB	7500 bB	4750 ^{bC}	1750 ^{bD}	
RT3	11500 bcdefA	9550 bab	7250 bB	4550 bC	1650 bD	
RT4	$10500^{ m \ cdefgA}$	9650 bab	7700 ^{bB}	5000 ^{bC}	1600 ^{bD}	
RE1	14500 ^{abcA}	9800 bB	7200 ^{bC}	4500 bCD	2100 bD	
RE2	$12000^{ m abcdeA}$	9667 ^{ьв}	7250 ^{bC}	4200 bCD	2250 ^{bD}	
RE3	$16000^{ m abA}$	9400 bB	6850 ^{bC}	3800 bCD	2000 ^{bD}	
RE4	7700 ^{efgA}	5650 bB	3550 ^{bC}	1900 ^{bD}	1750 bD	
SGT1	9400^{defgA}	7200 bB	3750 ^{bC}	1600 bD	1100 bD	
SGT2	9200 ^{defgA}	7300 bB	4250 bC	1450 ^{bD}	970 ^{bD}	
SGT3	6900^{fgA}	4600 bB	3200 ^{bC}	1500 ^{bD}	1400 ^{bD}	
SGT4	6400 ^{gA}	4200 bB	3050 ^{bC}	1300 ^{bD}	1000 ^{bD}	
SGE1	$7900^{ m efgA}$	6350 ^{bВ}	4200 ^{bC}	1450 ^{bD}	955 ^{bD}	
SGE2	6950 ^{fgA}	5250 bB	2850 ^{bC}	1050 bD	705 bD	
SGE3	6450^{gA}	4100 bB	2400 bC	1100 ^{bD}	890 ^{bD}	
SGE4	6850 ^{fgA}	4650 bB	2250 ^{bC}	955 ^{bD}	590 ^{bD}	

^{*} The difference between the averages with the same lowercase letters in the vertical column is not statistically significant (p > 0.05)

^{**} The difference between the averages with the same uppercase letters on the horizontal line is not statistically significant (p>0.05)

3.4 Effects of Candida albicans applications on Turkish Fermented of Sausage

Candida albicans values in the stages of ripening of sausages are shown in Table 5. It was observed that the difference between Candida albicans values was significant (p> 0.05) during the fermentation process of the applications. The decrease in Candida albicans values during the maturation period was observed to be statistically significant. When compared to two grape varieties, Black Gemre grape varieties became more effective. Sincerely [20], showed compliance with our study by reporting that Grape seed extract inhibited yeast growth in a variety of yeast investigations.

TABLE 5
EFFECTS OF APPLICATIONS ON SAUSAGE CANDIDA ALBICANS(CFU/G)

	Maturation time (days)						
Sample	2. 1 d	3 d	6 d	9 d	12 d		
N	3500 ^{ef*A**}	3050 fghB	2750 efgC	2200 ^{cdeD}	1900 ^{cD}		
K	$3300^{ m efC}$	9200^{Bc}	29500 ^{aBC}	68500 ^{aB}	470000 ^{aA}		
RT1	14000 ^{Ab}	13500 ^{aB}	10517 ^{bВ}	9600 bB	94500 ^{bA}		
RT2	6900 ^{bcdeA}	6900 bcA	6300 ^{cA}	5100 ^{cB}	4800 ^{cB}		
RT3	$3950^{ m defB}$	4700 cdefgA	3950 ^{cdefB}	3250 ^{cdeC}	2900 ^{cC}		
RT4	6400^{bcdefA}	4850 cdefgB	4250 ^{cdefC}	2850 ^{cdeD}	2950 ^{cCD}		
RE1	10000 ^{bA}	5900 ^{cdB}	5250 cdBC	4250 ^{cdC}	4050 °C		
RE2	14000 ^{aA}	4550 cdefgB	3850 ^{cdefB}	3200 ^{cdeB}	2850 ^{cB}		
RE3	$4200^{ m cdefA}$	3750 defghB	$3400^{\text{ defgB}}$	2850 ^{cdeBC}	2450 °C		
RE4	2800 ^{fC}	5150 ^{cdefA}	4600^{cdeAB}	3950 ^{cdeB}	3400 ^{cB}		
SGT1	7650 ^{bcdA}	4850 cdefgB	4050 cdefBC	3050 ^{cdeC}	2700 ^{cD}		
SGT2	9300 ^{bA}	5600 ^{cdeB}	5150 ^{cdeB}	4800 ^{cB}	4250 ^{cB}		
SGT3	$3900^{ m defBC}$	5200 ^{cdefA}	4750 ^{cdeB}	4050 cdeBC	3550 °C		
SGT4	14000^{aA}	12000 ^{aA}	3900 ^{cdefB}	2950 ^{cdeB}	2200 ^{cB}		
SGE1	9300 ^{bA}	3400 efghB	$3000^{ m defgB}$	1650 ^{deC}	1200 °C		
SGE2	$4500^{ m cdefA}$	2100 hB	1350 ^{gC}	985 ^{Ec}	880 ^{eC}		
SGE3	8100 bcA	2550 ghB	2000 ^{fgBC}	1450 ^{edC}	1050 ^{eD}		
SGE4	14000 ^{aA}	3750 defghB	$3000^{ m defgB}$	2050^{cdeB}	1650 ^{cB}		

^{*} The difference between the averages with the same lowercase letters in the vertical column is not statistically significant (p>0.05)

IV. CONCLUSIONS

Antioxidant and antibacterial influences of Black Gemre varieties are more common than Razakı varieties. If the grape kernel is evaluated as antimicrobial and antioxidant, a new alternative product to the food industry will be gained a waste material, which may be a negative effect on the environment, will be evaluated economically. Due to the high antimicrobial and antioxidant properties of grapes, the benefits of heart disease and cancer risk reduction should be exploited on human health. The use of grape seed as a food additive in sausage production is promising.

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^{**} The difference between the averages with the same uppercase letters on the horizontal line is not statistically significant (p>0.05)

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