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Effect of Pretreatments on the Quality of Grape Wine

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ABSTRACT

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This research paper concentrated on studies on the effect of pretreatments on the quality of grape wine. The changes due to pretreatments on the functional properties are such as total phenols, tannins and catechins. Hence, various thermal treatments such as microwave with a combination of 300 W for 2, 4 and 6 min and conventional thermal pasteurization at 50, 60, 70°C with the time interval of 30 and 60s were applied to the grape must to study the extraction efficiency of polyphenols. The quality parameters of prepared wine from the grape must subjected to conventional thermal pasteurization at 70°C for 60 s revealed increase in total phenolic compound and tannin content with mean values of 210.43 mg/100 ml of wine and 118.27 mg/ 100 ml of wine, respectively whereas microwave treatment for 6 min revealed increase in catechin content with mean value of 68.95 mg/ 100 ml of wine at the end of fermentation.

Introduction

Pasteurization is a thermal process in which heating of liquid food at particular time and successive cooling is performed with the intention of killing pathogens and deactivating enzymes. Grape juice pasteurization even kills the most resistant mold which is detrimental for the production of quality wine (Pederson, 1936).

Hence, pasteurization of grape must prior to fermentation (the inoculation of yeast) may facilitate the population of yeast in the development of quality wine.

Pasteurization can be accomplished by conventional heating, microwave exposure

and high pressure processing (Liu and Casas, 2010). Since high pressure processing is expensive, conventional thermal pasteurization and microwave exposure may be the choice of pretreatment on grape must in preventing mixed culture fermentation and extracting the bioactive compounds from the grapes towards the production of quality grape wine.

The applied pre-treatments will inhibit such kind of problems and maintain the product quality and stability. By introducing the different levels of dosages of pre-treatments like microwave and ultra-violet and blanching, we can perceive the long shelf-life, maintenance of the nutritional content and quality of grape wine.

By analyzing the impacts of pre-treatments on the quality of grape wine, we can provide scientific knowledge on the quality aspects of fermented beverages to food industries those are undergoing fruit processing and preservation. Grapes contains high amount of phenolic compounds that possess antioxidant property which prevents cancer, it reduces the risk of strokes and also it boosts-up the immune system.

Hence, thermal treatments such as conventional thermal pasteurization and microwave pasteurization on grape must were designed to fulfill the targeted objective.

Materials and Methods

In this section, materials and methods used in the preparations of grape wine, pretreatments and quality evaluation are discussed.

Materials

Grapes

The grapes used in the present study were of good grade local variety, acidic and procured from local market.

Yeast culture

To ferment the grape must, commercially available dry granular brewers' yeast procured from Venus Essence, Chennai. The culture was stored at 4°C.

Sugar

Sucrose was added during the process to adjust the Total Soluble Solids (TSS). The sugar content is expressed as per cent soluble solids or °Brix. Initial Sugar content of grape must was measured using a refractometer. The TSS per cent was adjusted to 22°Brix by adding sugar for effective fermentation.

Chemicals

The following chemicals were used for the quality analysis of grape must and wine includes standard sodium hydroxide, phenolphthalein, methanol, sodium carbonate, FCR reagent (Folin Ciocalteu Reagent), gallic acid, cuprous sulfate, sodium potassium tartrate, sodium iodide, sulphuric acid, starch, sodium thiosulfate, methylene blue, Fehling A and B solutions, glucose, lead acetate, potassium chromate.

Glassware

Glass wares *viz.*, burette, pipette, volumetric flask, conical flask, measuring cylinder, test tubes, beaker, petri plates, desiccators, weighing balance, round bottom flask, digestion flask and sterilized jars were used for experimentation.

List of devices or instruments used in experimentation

Magnetic stirrer (Make: Remi equipments), pH meter (Make: SUSIMA Technologies Private Limited, Chennai), Distillation flask, Pycnometer, Hydrometer, Thermometer, Hot plate, Microwave oven (make: Godrej), UV-Visible spectrophotometer (Make: UV mini 1240 shimadzo), Distillation unit (Borosil).

Methods

Selection of fruits

Undamaged, healthy ripened grapes having average pH of 3.0 to 3.6 were selected after manual sorting and discarding of defective bunches.

Preparation of grape must

Undamaged, 20 kg of healthy ripened grape berries were de-stemmed and then washed

with boiled and cooled water. With the help of fruit juicer (available at the department of Food Plant Operation, CFDT) grape must was prepared. Grape skins and seeds were kept in must itself for extraction of color and phenolic compounds. The must was kept overnight under aerobic fermentation.

Analysis of grape must

The grape must was analyzed to estimate the sugar level, pH and amount of phenolic compounds.

Preparation of sugar solution

Commercial sugar was procured from the local market. The stock sugar solution was prepared by dissolving 50g of sugar in 50 ml of water boiled for 10 minutes and then allowed to cool at room temperature and used for supplementation in grape must to adjust the sugar level to 22 °Brix by using hand refractometer.

Pre-fermentation treatments

The extracted grape must was subjected to pretreatments before its fermentation by the yeast to produce ethanol. The grape must was subjected to thermal treatments with conventional pasteurization and microwave radiation to destroy the pathogens (Table 1).

It could help to improve the color intensity and phenolic contents in the final product.

Pasteurization

Pasteurization is the effective heat treatment to destroy all pathogenic organisms which causes spoilage to the product. The temperature is a variable that directly affects the growth rate of the microorganisms (Charoenchai *et al.*, 1998) and the final composition of wine (Torija *et al.*, 2003). Hence, the grape must was

subjected to thermal exposure at 50, 60 and 70°C for 30 and 60 s, respectively (Plate 2.2).

Microwave treatment

The prepared grape must was subjected to radiative treatments under microwave exposure (Plate 2.3) at a power level of 300 W with time intervals of 2, 4 and 6 minutes before addition of starter culture to destroy the harmful microorganisms and extract bioactive compounds from grape skin and seeds in the must.

Preparation of starter culture

The starter culture was prepared by using 2% of brewer's yeast. It was activated by adding 2g of yeast into 100 ml of warm water. Activation of yeast was observed by rise in the volume of yeast in warm water.

Alcoholic fermentation of grape must

The pretreated grape must (500 ml) was taken in 1000 ml Erlenmeyer flask and added with sugar syrup (given in 2.2.4) to adjust the sugar level at 22°Brix. The prepared must was inoculated with 2% starter culture and incubated at 28°C. Steps involved in the alcoholic fermentation are presented in figure 1.

Post fermentation process

The finished wine contains impurities including dead cells, protein hazes and residual sugars and does not have complete quality (sensory) attributes. Hence, it requires post fermentative treatments to make wine potable. In fact, there are five goals of "finishing" a wine: clarity, stability, compositional adjustment, style development and packaging. It is also important to prevent unwanted microbial growth in the wine after primary fermentation (i.e., alcoholic

fermentation) is complete as this will affect the flavor and aroma profile. After alcoholic fermentation, post fermentation processes were carried out.

Siphoning / Racking

The flasks or bottles containing prepared wines were stored in refrigeration and debris called 'lees' was allowed to settle by gravity. No settling or fining agent was added. The clear supernatant was siphoned and bottled separately.

Bottling and Storage

The cleared wine was stored in glass bottles (sterile bottles) under refrigerated conditions. The sequential operations followed in grape wine production is given in plate 2.4.

Results and Discussion

Table 2 shows the total phenol content (mg/100ml) of control and pretreated samples during wine making. Mean values of total phenol content in control and pretreated samples (452.28±0.26, 445.58±0.25, 449.27±0.35, 454.88±0.28, 445.23±0.2, 446.03±0.26, 450.07±0.23, 450.42±0.27, 452.60±0.34 and 458.17±0.22) were obtained during 0th day of wine making. Mean values of total phenol content in control and pretreated samples (175.12±0.12, 176.62±0.17, 178.98±0.12, 190.73±0.22, 176.35±0.19, 178.40±0.17, 182.07±0.22, 190.77±0.3, 195.85±0.3 and 210.43±0.3) were obtained during 20th day.

It was found that there was significant ($P\leq 0.01$) difference in the values of total phenol content of control and pretreated samples during wine making. There were significant changes ($P\leq 0.01$) in the total contents in the treatments during the successive intervals of wine making.

Table 3 shows the tannin content (mg/100 ml) of control and pretreated wine samples. Mean values of the tannin content (mg/100 ml) in control and pretreated wine samples (79.88±.09, 61.50±.35, 76.20±.22, 109.98±.19, 86.18±.26, 94.83±.12, 95.05±.23, 105.70±.12, 100.20±.12 and 118.27±.17) were obtained. It was found that there was significant difference ($P\leq 0.01$) in the values of the tannin content (mg/100ml) of pretreated wine samples with control sample. The relative change in the tanning content of treatments with the control is given in figure 2.

Table 4 shows the catechin content (mg/100 ml) of control and pretreated wine samples. Mean values of the catechin content (mg/100 ml) in control and pretreated wine samples (48.07±.10, 31.03±.20, 44.50±.26, 68.95±.22, 30.07±.22, 30.75±.12, 33.33±.20, 36.20±.70, 35.15±.18 and 37.02±.10) were obtained. It was found that there were significant ($P\leq 0.01$) changes in values of the catechin content of pretreated wine samples with control sample. The relative change in the catechin content of treatments with the control is given in figure 3.

Effect of pretreatments on the quality of grape wine

Effect on total phenols

The effect of microwave and conventional thermal pasteurization on the quality parameters of grape wine such as total phenols, tannin and catechin was studied. It was observed from table 2 that there was significant change in the total phenol content of grape must and wine among the treatments at 1 per cent level. The control recorded the total phenol contents of 452.28mg/100ml of grape must and 175.12mg/100ml for wine, respectively. The treatments exposed at 300 W power levels for 6 min expressed the mean total phenol content of 454.88mg/100ml of

grape must and 190.73 mg/100ml of wine. It was noticed that the microwave exposure for 6 min extracted the phenolic content higher than the control in the grape must and wine and also interpreted that the increase in microwave exposure increases the phenolic extraction in grape must and wine. The results observed were on par with the results of carew *et al.*, (2014).

The intervention of conventional thermal pasteurization on the extraction of total phenol content from grapes was evaluated at 50, 60 and 70°C for 30 and 60s exposure time. The thermal exposure of grape must at 50°C for 30 and 60 s showed a slight change in the total phenolic extraction and the treatments were on par (T₄ and T₅) before fermentation whereas grape wine showed a significant difference at 1 per cent level of significance on the phenolic content. The increased exposure time improved the total phenolic extraction. The thermal exposure of grape must at 60°C for 30 and 60s recorded 450.07 mg/100ml and 450.42 mg/100ml, respectively whereas grape wine recorded 182.07 mg/100ml and 190.77

mg/100ml, respectively in the total phenolic content. The treatments were on par (T₆ and T₇) before fermentation whereas grape wine showed a significant difference at 1 per cent level and the increased exposure time improved the total phenolic extraction.

The thermal exposure of grape must at 70°C for 30 and 60s recorded 452.60 mg/100 ml and 458.17 mg/100 ml, respectively whereas grape wine recorded 195.85 mg/100 ml and 210.43 mg/100 ml, respectively in the total phenolic content and the treatments were on par (T₈ and T₉) before fermentation whereas grape wine showed a significant difference at 1 per cent level of significance.

Among the treatments, higher total phenolic extraction was observed on the treatments that exposed to conventional thermal pasteurization than the treatments exposed to microwave. This may be because of the low power level of the microwave considered in this study. It was also observed that the total phenolic extraction due to the pretreatments was superior to control.

Fig.1 Flowchart for alcoholic fermentation of grape must

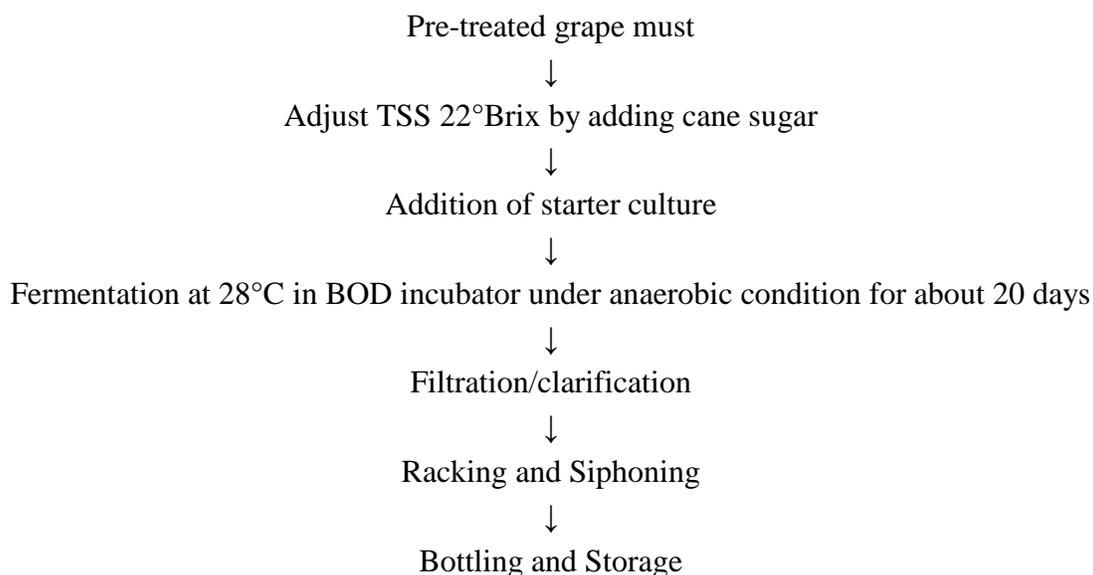


Fig.2 Comparison of tannin content (mg/100 ml) of wine between control and treatments

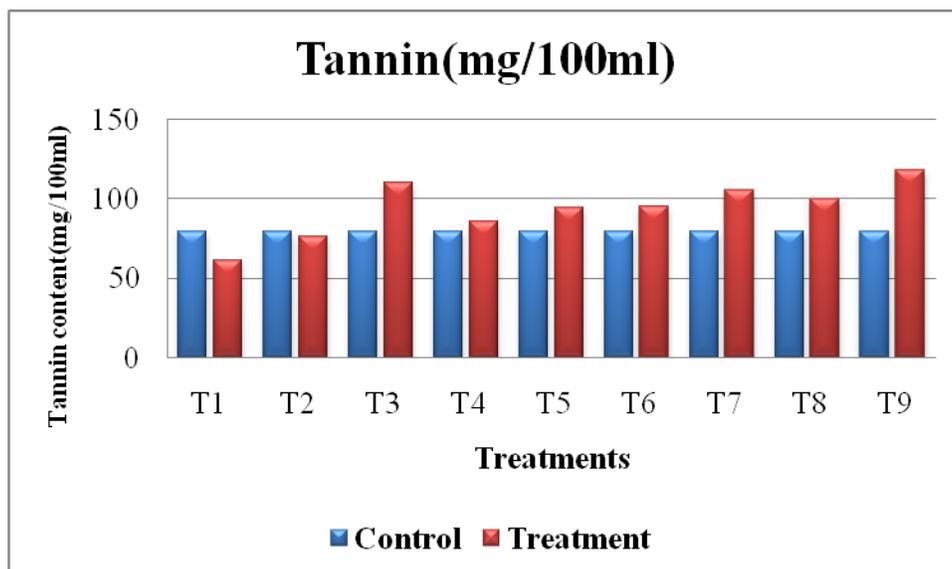


Fig.3 Comparison of catechin content (mg/100 ml) of wine between control and treatments

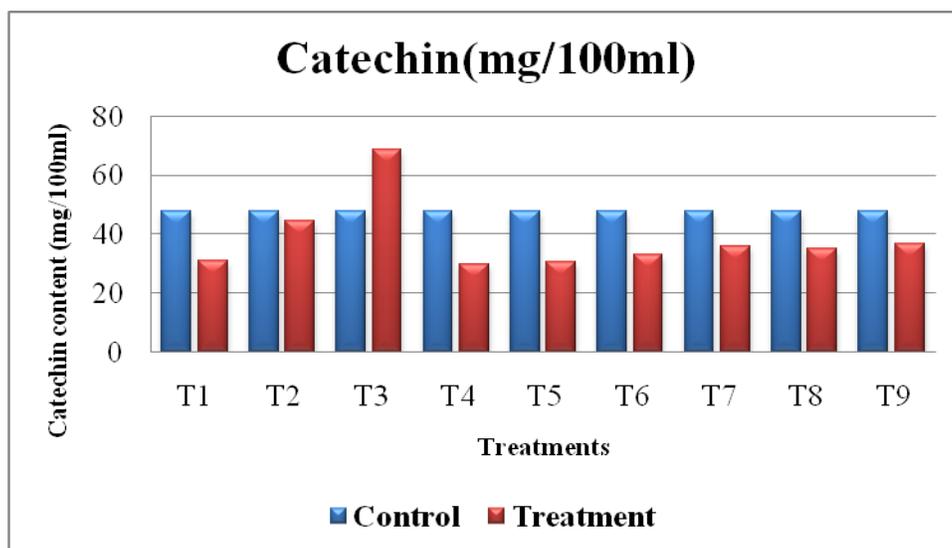


Table.1 Experimental design

Dosages (Independent variables)			Quality parameters (Dependent variables)					
Microwave Treatment	300 W	2 min(T ₁)	1.	Total phenols				
		4 min(T ₂)						
		6 min(T ₃)						
Pasteurization	50 °C	30s(T ₄),60s(T ₅)			2.	Tannins		
		60 °C					30s(T ₆),60s(T ₇)	
		70 °C					30s(T ₈),60s (T ₉)	
							3.	Catechins

Table.2 Total phenol content of pretreated and control samples during wine fermentation (Mean±SE)

Treatment	0 th day	20 th day	F-Value
Control	452.28±0.26 ^d	175.12±0.12 ^a	955710.014**
T ₁	445.58±0.25 ^a	176.62±0.17 ^d	771444.569**
T ₂	449.27±0.35 ^b	178.98±0.12 ^c	558303.391**
T ₃	454.88±0.28 ^e	190.73±0.22 ^f	599834.362**
T ₄	445.23±0.2 ^a	176.35±0.19 ^b	749070.011**
T ₅	446.03±0.26 ^a	178.40±0.17 ^d	984846.176**
T ₆	450.07±0.23 ^c	182.07±0.22 ^d	473784.817**
T ₇	450.42±0.27 ^c	190.77±0.3 ^e	764747.191**
T ₈	452.60±0.34 ^d	195.85±0.3 ^g	371291.804**
T ₉	458.17±0.22 ^f	210.43±0.3 ^h	604723.206**
F-Value	241.999**	264.751**	

**- Highly significant (P≤0.01) at intervals, ^{a, b, c and ABCDE} – superscripts with same letter indicates the treatments are on par

Table.3 Tannin content (mg/100 ml) of wine (Mean±SE)

Treatment	Wine
Control	79.88±.09 ^b
T ₁	61.50±.35 ^a
T ₂	76.20±.22 ^e
T ₃	109.98±.19 ^h
T ₄	86.18±.26 ^c
T ₅	94.83±.12 ^d
T ₆	95.05±.23 ^d
T ₇	105.70±.12 ^g
T ₈	100.20±.12 ^f
T ₉	118.27±.17 ⁱ
F-Value	6317.294**

**Highly significant (P≤0.01) at interval, ^{a, b, c, d, e, f, g, h, i} – superscripts with same letter indicates the treatments are on par.

Table.4 Catechin content (mg/100 ml) of wine (Mean±SE)

Treatment	Wine
Control	48.07±.10 ^h
T ₁	31.03±.20 ^b
T ₂	44.50±.26 ^g
T ₃	68.95±.22 ⁱ
T ₄	30.07±.22 ^a
T ₅	30.75±.12 ^{ab}
T ₆	33.33±.20 ^c
T ₇	36.20±.70 ^c
T ₈	35.15±.18 ^d
T ₉	37.02±.10 ^f
F-Value	1769.973**

**Highly significant (P≤0.01) at intervals, ^{a, b, c, e, f, g, h, i} – superscripts with same letter indicates the treatments are on par

Plate.1 Conventional thermal pasteurization treatment



Plate.2 Microwave treatment



Plate.3 Wine making



a) Grapes, b) Blanching, c) grape must, d) Brewer's Yeast, e) BOD incubator, f) wine

Effect on tannins

It was observed from table 4.9 that there was significant change in the tannin content of grape wine among the treatments at 1 per cent level. The control recorded the tannin contents of 79.88 mg/100ml of grape wine. The treatments exposed at 300 W power levels for 6 min expressed the mean tannin content of 109.98 mg/100ml of grape wine. It

was noticed that the microwave exposure for 6 min extracted the tannin content higher than the control in the grape wine. It was also interpreted that the increase in microwave exposure increases the tannin extraction in grape wine.

The intervention of conventional thermal pasteurization on the extraction of tannin content from grape wine were evaluated at 50,

60 and 70°C for 30 and 60s exposure time. The thermal exposure of grape must at 50°C for 30 and 60 s showed a slight change in the tannin extraction and the treatments (T₆ and T₇) showed a significant difference. The increased exposure time improved the tannin extraction. The thermal exposure of 60°C for 30 and 60 s recorded 95.05 mg/100 ml and 105.70 mg/100 ml of grape wine, respectively and the treatments (T₆ and T₇) showed a significant difference. The thermal exposure of 70°C for 30 and 60 s recorded 100.20 mg/100ml and 118.27mg/100ml of grape wine, respectively in the tannin extraction.

Among the treatments, higher tannin extraction was observed on the treatments that exposed to conventional thermal pasteurization than the treatments exposed to microwave. This may be because of the low power level of the microwave considered in this study. It was also found that increased exposure time and dosage level improved the tannin extraction. It was also observed that the tannin extraction due to the pretreatments was superior to control.

Effect on catechins

It was observed from table 4.10 that there was significant change in the catechin content of grape wine among the treatments at 1 per cent level. The control recorded the catechin contents of 48.07mg/100L of grape wine. The treatments exposed at 300 W power level for 6 min expressed the mean catechin content of 68.95mg/100ml of grape wine whereas treatments exposed at 300 W power level for 2 min and 4 min expressed the mean catechin content of grape wine 31.03mg/100ml and 44.50mg/100ml of grape wine, respectively. It was noticed that the microwave exposure for 6 min extracted the catechin content higher than the control in the grape wine. It was found that the microwave exposure for 2 and 4 min decreased the catechin content than the

control in the grape wine. The increased exposure time of microwave improved the catechin content in wine.

The intervention of conventional thermal pasteurization on the extraction of catechin content from grape wine were evaluated at 50, 60 and 70°C for 30 and 60s exposure time.

The thermal exposure of 50°C for 30 and 60s showed a slight change in the catechin extraction and the treatments (T₄ and T₅) were on par. The increased exposure time decreased the catechin extraction than the control. The thermal exposure of 60°C for 30 and 60s recorded 33.33 mg/100 ml and 36.20 mg/100 ml of grape wine, respectively and the treatments were on par (T₆ and T₇). The increased exposure time improved the catechin extraction.

The thermal exposure of 70°C for 30 and 60s recorded 35.15 mg/100 ml and 37.02 mg/100 ml of grape wine, respectively in the catechin extraction and the treatments (T₈ and T₉) showed a significant difference. Among the pretreatments, microwave treated samples exposed for 6 min exhibited higher catechin extraction whereas among the conventional pasteurization treatments 70°C for 60 s showed maximum extraction than the others. It was also observed that the catechin extraction due to the microwave pretreatments was superior to control and conventional thermal pasteurization. The results were confirmed by Clodoveo *et al.*, (2016).

While considering the quality parameters of wine such as total phenol content, tannin and catechins, the conventional thermal pasteurization recorded maximum values for the total phenols and tannins than the control and microwave samples whereas catechin was found higher in microwave samples than the control and samples exposed to thermal pasteurization.

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