DEVELOPMENT OF FERMENTED BEVERAGE FROM FRUITS AND VEGETABLE POMACE

Abstract

Pomace can be defined as anything unused or not used to full advantage or simple term for any solid material – such as pulp, skins and seeds. The objective of this current study was development of value added fermented beverage, evaluating its physic-chemical properties and decreasing production costs. Fruit and vegetable pomaces were segregated, blanched and 3 variants were prepared. Prepared must was fermented for 7 days at 25˚C using activated yeast *(Saccharomyces cerevisia*). Clarification, filteration, pasteurization of the product was carried out followed by filling in labelled glass bottles. The range of anti-oxidant activity and vitamin C of the variants resulted between 45-55% and 0.105mg/100ml- 0.200mg/100ml. Alcohol content was observed to be 5% at 25˚C and pH of the variants resulted to be 3.8-4.0. Ash content was highest in variant A i.e.,0.14% .Acidity of variant A and B was observed to be as 0.015% and for variant C, 0.03% respectively. Phenolic content of variant resulted in the range of 10.5-10.7 mg of gallic acid eq. wt./100ml and maximum tannin content was observed in sample A i.e., 6.5mg/1ml whereas for B and C value resulted in 5.5-5.7mg/1ml. The present manuscript demonstrates the quality parameters of a fermented beverage which is rich in antioxidant content and phenolics. The beverage is a ready to serve drink which is prepared using pomace of different fruits and vegetable. The outcome of this study may have greater potential for beverage industry in order to utilize the waste and present consumers with a healthy pomace based fermented drink.

Keywords : Anti-oxidant activity, fermentation, fermented beverage, pomace, *Saccharomyces cerevisiae*

.

I INTRODUCTION

India, one of the rapidly growing developing country comprising of 1.34 billion population ranks second in production of fruits and vegetable. It contributes to 14% of total production of the world. Around 18% of fruits and vegetables of worth Rs. 44,000 crores is going to pomace annually in India. Serious environmental and health problems are caused due to solid pomace disposal.

Vegetable pomace has 80-90% moisture content (Dasa and Mondal, 2013).

According to FAO, about 1.3 billion tons of food has been pomaced worldwide per year, which represents one-third of the total food industry production. Severe fruits and vegetable losses are at agricultural stage accounting

25% losses of total production in developing countries like India. Conventionally, fruits and vegetables pomace (FVP) are used as animal feed or organic fertilizer. Although, these pomace contain a large amount of bioactive molecules and biopolymers but results in a considerable nutritional losses (Ferreira et. al., 2013). Pomace can be stated as anything unused or not used to full advantage or general term for any solid material – such as skins, pulp and seeds. Food processing pomaces are those end products of various food processing industries that have not been recycled or used for other purposes. It includes rotten organic matter, cellulosic pomace like peels. Indian fruits and vegetable industries produce more than 1, 50,000 tonnes of products annually in which FVW include peels, seeds and stones produced by the fruit and vegetable processing can be successfully used as a source of phytochemicals and antioxidants (Kumar, 2004 ; Rudra et. al., 2015). Utilization of these pomaces as by-products on the production of food additives or supplements with high nutritional value products will help in economic growth of country. The by-products represent an important source of sugars, minerals, organic acid, dietary fibre, etc. As per epidemiologic studies, diet rich in fruits and vegetables play a relevant role in reducing the risk of developing chronic diseases like cancer , cardiovascular disease, diabetes, etc. India is 9th largest producer of apples around the globe contributing about 1/3rd of total apple production of the world. Apple pomace typically contains 66.4–78.2% (wet basis) moisture and 9.5–22.0% carbohydrates. Apple pomace contains 26.4% dry matter (DM), 4.0% proteins, 3.6% sugars, 6.8% cellulose, 0.38% ash, 0.42% acid and calcium, 8.7 mg/100 g of wet apple pomace (Gulhane et. al., 2015). By products of citrus processing industry are the solid by-products and constitute of 50% of fresh fruit weight. After extraction of juice from the fruit, the remaining residues comprises of peel (flavedo and albedo), pulp (juice sac residue), rag (membranes and cores) and seeds (Rachana and Gupta, 2010). These citrus by-product contains 600-650g/kg peel, 500-700g/kg sugar, 300-350g/kg pulp. The peels are a good source of natural flavonoids, and contain higher amount of phenolics compared to the edible portions. It has been reported that total phenolic content in peels of oranges, lemons and grapefruit were 15% higher in comparative to peeled fruits.

Pineapple pomace is a potential source of vitamins, sugars and growth factors. Bromelain is a valuable and a component of research interest from the pineapple waste. It is a crude extract of pineapple that contains proteinases. Fruit phenolic content was found as 40.4 mg/100g as gallic acid equivalent with the highest ethyl acetate bound phenolic. It has been speculated that dietary fibre powder prepared from pineapple shell contains 70.6% total dietary fiber with better sensory properties. Beetroot ranks 10th most powerful vegetable with respect to its antioxidant activity. It is an important source of water-soluble pigment, called betalains, that has two important groups, red betacyanins and the yellow beta-xanthins. The pulp wastes are good sources of calcium and phosphorus. Soluble dietary fibre is high in beetroot pulp waste i.e., (21g/100g). Fermentation leads to alteration of the must by altering the conjugation of organic acids and phenolics (polyphenols and phenolic acids)

by extraction and formation of co-pigments. Polyphenolic compunds present in wine contribute to astringency and color. Taste and mouthfeel sensations are due to few compounds (water, ethanol, sugars, glycerols, organic acids). The main principle involved for the fermentation is the oxidation of carbohydrates and related derivatives to produce a number of end products like alcohol, acids and carbon dioxide. Food spoiling micro-organisms get inhibited by these end products and also because of partial oxidation the food still retains sufficient energy to be of nutritionally benefit for the consumers (Many et. al., 2014). It has been widely reported that cardio protective effect can be achieved by moderate consumption of some alcoholic fermented beverages as the consequence of their content of phenolic compounds and ethanol . Fermented beverages have high potential value in the market among beverages as is high in demand in terms of their characteristic aroma and flavor of the material that rises to them after processing.

Phenolic compounds greatly affect the appearance, colour, taste, mouthfeel, etc of the fermented beverages. They are complexed compounds which help the fermented products to maintain their quality (Friaz, 2002). Due to the high content of phenolic compounds in citrus products, these can be exploited for pharmaceutical and food industries for functional foods production. The acidity is characterized by citric acid, mallic acid and tartaric acid. Acidity influences the taste of fermented beverage. Acidity refers to freshness, sourness and tartness of a given fermented product. Ash content is considered as the mineral content of the product. Amount of mineral present in the present is important due to its health impact and their role in the stability of the fermented products (Ignat and Volf , 2011).

Vitamin C and carotenoids of the peels and fruits by products are considered as an active ingredient in production of functional foods when designing a healthy product or a synthetic preservative substitute, (Ozsoy et. al., 2008). Antioxidant activity of the product is measured by DPPH assay. Antioxidant of the product can be defined as the ability of the product to protect it from degradation due to free radical induced oxidative stress, (Sharma et. al., 2016). Antioxidant capacity has been linked to the prevention of diseases associated with oxidative stress (Caplice, 1999). DPPH is very stable free radical. DPPH remains unaffected by certain side reactions i.e., metal ion chelation and enzyme inhibition. A freshly prepared DPPH solution possess a deep purple colour which has an absorption maximum at 517 nm. The purple colour fades generally, when antioxidant molecules quench DPPH free radicals and convert them into a colourless/bleached product (i.e. 2, 2-diphenyl-1- hydrazine, or a substituted analogous hydrazine), leading to decrease in absorbance at 517 nm band. Tannins are responsible for the astringency in the fermented product. They are the water soluble polyphenols which coagulate the proteins of saliva. Tannin content of raw material and final fermented product are not quite related. Fruit tannin extracted from solid fruit material goes into must during fermentation, and then undergoes chemical rearrangements to reach its final form in the beverage. This is why it is not simple to predict final fermented beverage tannin by

simply measuring fruit tannin. Flavonoids are found in the skins, seeds and stems of fruits. Anthocyanins and flavonols are majorly present in skins. Catechin and leuko-anthocyanins are present in seeds and stems. Extraction of flavonoids depends upon temperature, pH, yeast strain, etc. Sugar is the major ingredient for the production of ethanol in fermented products. Yeast utilizes sugar from glucose and fructose. It does not utilizes any other sugar for production of ethanol, (Crippen and Morrison, 1986). Major sugars present in the must are glucose, fructose and sucrose. Moderate consumption of ethanol helps to enhance meal induced insulin secretion in non- insulin dependent diabetes. Ethanol consists of a compound resveratrol which exhibits physiological effects and has ability to activate estrogen receptors. This inturn helps in protection against breast cancer.

The objective of the study is to address environmental concerns of pomace disposal, hence it will make industries more sustainable. It includes development of value added fermented beverage and decreasing production costs and to estimate physic-chemical properties including the alcohol content, acidity, ash content, tannin content and total sugars of the final product. The study also involves the estimation of shelf-life, microbiological analysis (*E.coli*, yeast and mold count) and sensory evaluation and to check the overall acceptability of the product.

II MATERIALS AND METHODS

2.1 Materials

Pomace of apple, orange, black grapes, beetroot and pineapple; sugar, yeast (*Saccharomyces Cerevisiae)*

,

2.2 Fermented Beverage Preparation

Ingredients like pomace of apple, orange, black grapes, beetroot and pineapple were collected from the local vendors whereas sugar, yeast, etc. was taken from wholesale sellers of local market. Fruit and vegetable pomaces were segregated and 3 variants were prepared A(apple: orange: beetroot; 3:3:2) , B (apple: orange : black-grapes; 3:3:2) , C (apple : pineapple : black- grapes; 3:3:2). Blanching was carried out in stainless steel vessel using muslin cloth. Pomaces were tied up in a muslin cloth and then blanched in water at a particular temperature for a particular time and followed by transferring them to a cold water vessel. Luke warm water was taken for activating 5gm yeast i.e. *Saccharomyces Cerevisiae* and 20gm sugar was added to the 500mL must. The prepared must of fermented beverage was packed in sterilized jars which were sealed and stored at temperature of 25˚C for 7 days. After 7 days, must is filtered and samples are pasteurized at 63˚C for 30 minutes. Further the fermented beverage is packed in pre- sterilized air tight labelled glass bottles.

2.3 Chemical Analysis

2.3.1 Ash Content

The empty dish was weighed (W1). 10-15mL of sample (V) was taken and evaporated on water bath. The evaporated sample was kept in muffle furnace until a greyish white was observed. The dish wash (W2) weighed with ash (AOAC, 1997).

2.3.2 pH

The meter was caliberated using standard buffer capsules. 10mL of sample was taken into 150mL beaker and to it 90mL of boiling distilled water was added with continuous stirring and the pH was determined (FSSAI, 2015).

2.3.3 Total Acidity

The normality of NaOH was standardized using standard oxalic acid in the presence of phenolphthalein indicator. 5-10mL of sample was taken in a conical flask and was titrated against NaOH in the presence of phenolphthalein and a faint pink colour was observed as end point (IS 7585:1995; 1975).

2.3.4 Alcohol Content

200mL of sample was taken and transferred to a 500mL of distillation flask containing about 25mL of distilled water and a few pieces of pumic stone. The contents were distilled for about 35 minutes and the distillate was collected in a 200mL of volumetric flask till the volume almost reaches the mark. Distillate was brought to room temperature and volume was up with distilled water and was mixed thoroughly. Specific gravity of the distillate was noted and alcohol content was observed from standard table (IS: 3752:2005; 1988).

2.3.5 Reducing Sugar

De-alcoholisation and de-colourisation , 100mL of fermented beverage sample was taken in a dish and was neutralized with NaOH, calculating the acidity and evaporated to half of it. Volume was made upto 100mL with distilled water (Ranganna, 1986).

Standard titration: Titrated of 10mL of fehling solution (5mL each of A nd B soln.) against standard glucose soln. and standardized the fehling's soln.

Total Sugars: Measure accurately 10mL of de-alcoholised fermented sample, 2mL concd. HCl was added and the flask was kept in boiling water bath for 20-25 min. After that it was cooled and acid was

neutralized with Na_2CO_3. The content of the flask were transferred quantitatively to 100mL. Volumetric flask and the volume was made upto mark with distilled water. This solution was then in the burette and was titrated against 10mL fehling' soln. The reading were calculated using standard tables.

2.3.6 Tannin Content

Preparation of Standard Curve

7 test tubes were taken and standard tannic acid (10, 20, 40, 60, 80, 100µg/mL) was taken in 6 test tubes containing 2.5mL of distilled water. 0.5mL of Folin-Ciocalteu's reagent was added and 1mL of 35% of Na_2CO_3 was added after 5minutes. Test tubes were incubated for 30minutes and absorbance was measured at 760nm. 7^{th} test tube was incubated with the above reagents except tannic acid and 1mL of fermented beverage sample. Plot absorbance against mg of tannic acid/1 mL of fermented beverage sample (Ranganna, 1986).
Preparation of standard curve.

2.3.7 Ascorbic Acid Content

Preparation of Standard Curve

To dry cuvettes, pipette standard ascorbic acid (1, 2, 2.5, 3, 4 and 5 mL and make upto 5mL using 2% HPO_3). Pipette 10mL of dye, shake and take the readings within 20seconds at 100% transmission using blank 5mL of 2% HPO_3 solution and 10mL of water. Measure the red colour at 518 nm and plot absorbence against concentration. Preparation of standard curve (Ranganna, 1986).

Take sample 10mL in a 100mL volumetric flask and make it upto the mark using 2% of HPO_3.

2.3.8 Total Phenolic Content

Standard gallic acid (10, 20, 40, 60, 80, 100µg/mL) was positioned in 6 test tubes and 5mL of distilled water was added. 0.5mL of Folin Ciocalteu's reagent was mixed and 1.5mL of 20% Na_2CO_3 was added after 5 minutes. Volume was made upto 10mL and test tubes were allowed to incubate for 2 hours at room temperature. Intense blue colour is developed and absorbance was measured at 750nm. 1mL of sample with above reagents was prepared and absorbance was measured under the same (Ghasemi et. al., 2014). Preparation of standard curve ANNEXURE 1.

2.3.9 Total Antioxidant Activity

TAA of fermented beverage sample was measured using DPPH assay. 2mL of sample was taken in test tubes and 1mL of methanolic solution of DPPH (1mM) and 2mL of methanol was added to make final

volume of 5mL (Akowuah et. al., 2005). Test tubes were incubated at room temperature for 60 minutes and absorbance was measured at 517nm against blank (2mL of DPPH solution and 1mL of methanol).

2.4 Microbial Analysis

2.4.1 Total Plate Count

Total viable count was carried out using pour plate method with plate count agar. From suitable dilutions, one mL is transferred into the plate then the medium poured and mixed in. The plates were incubated at 37ºC for 24-48hrs (IS: 5402: 2002).

2.4.2 Coliform Count

Samples were diluted using phosphate buffer and Mc Conkey broth was used. Serial dilutions were made upto 10^{-2}. Samples were incubated at 37˚C for 24 hrs (IS: 5403:1999).

2.4.3 Yeast And Mould Count

Preparation of plates, under similar conditions, preparing decimal dilutions of the test sample or of the initial suspension (IS: 5403:1999). Aerobic incubation of the plates is done atat 25°C for 3, 4 or 5 days.

2.5 Sensory Analysis

Sensory evaluation of all the variants of fermented beverage sample was conducted on the day of preparation. The panelists consisted of 20 untrained and semi trained members based on their interest and availability- who were Btech students of food technology and staff members in the Department of Food Science and Nutrition, University of Delhi, evaluated the sensory characteristics (appearance, color, odor, after taste, taste, and overall acceptability). Training sessions were conducted prior to evaluation in which the panelists were trained to be familiar with attributes and scaling procedures of fermented beverage samples. Sensory attributes were evaluated using a nine point hedonic scale with 1- the lowest or extremely dislike and 9 - the highest or extremely like. All samples were randomly coded and presented to the panelists. Lighting of the room was the same throughout the analysis, which was conducted in the Food Safety and Quality Control Lab, University of Delhi. Water was provided to the panelists to cleanse their palates between samples.

2.6 Statistical Analysis

The results presented for sensory evaluation as average values obtained for 3 variants were tabulated and statistically analysed by ANOVA. Evaluation for sensory was conducted on different basis i.e., taste, after taste, appearance, colour, aroma and overall acceptability.

III RESULTS AND DISCUSSION

3.1 Chemical Analysis

3.1.1 Phenolic Content

Phenolic compounds greatly affect the appearance, colour, taste, mouthfeel, etc of the fermented beverages. They are complexed compounds which help the fermented products to maintain their quality (Singleton, 1982). Due to the high content of phenolic compounds in citrus products, these can be exploited for pharmaceutical and food industries for functional foods production. As per below resulted table I, maximum amount of phenolic content was observed in sample A and sample B consisting of citrus fruit pomace as an ingredient. (Brunet et. al., 2016) stated in their study that beetroot is also a good source of phenolic acids which can also be a reason for high level of phenolic content in sample A as compared to sample C. Total phenolic content was found to be maximum in variant B i.e. 10.7mg(GAE)/100mL. Similar results were observerd by Johnson et. al., (2015), which had high total phenolic content from fermented was observed to be in range 65.1- 2033.6 mg (GAE)/mL.

TABLE 1 Estimation of phenolic content

Variants	mg(GAE)/mL
A	10±0.6
B	10±0.7
C	10±0.5

3.1.2 pH and Acidity

pH of the variants was observed between 3.8-4 after fermentation as resulted in table II. Maximum pH was observed in sample C after fermentation. pH also refers to the tartness and freshness of the product. pH also has an effect on alcohol production and also effectes the yeast's alcohol production activity. Optimal pH for alcohol production was observed to be 5. Similar results were stated by (Lin et. al., 2012), who conducted the study on Factors affecting ethanol fermentation using *Saccharomyces cerevisiae* BY4742. As per studies conducted by (Prado et, al., 2015), also observed decrease in pH after fermentation of coconut water for a particular period of time. pH before fermentation was observed to be 5.67 whereas after fermentation for 28 days pH was upto 3.

Acidity increases the inhibition property against microbial growth. As per resulted in table 2a, maximum acidity was found in sample C which comprised of ingredients i.e., apple, pineapple and black grapes after

fermentation. Calculated results also show a significant increase in acidity after fermentation. Results as per (Ogodo et. al., 2015) stated in their study of development of papaya, banana and watermelon wine, observed a significant increase in the acidity of the product after fermentation.

TABLE 2a Estimation of pH of given variants

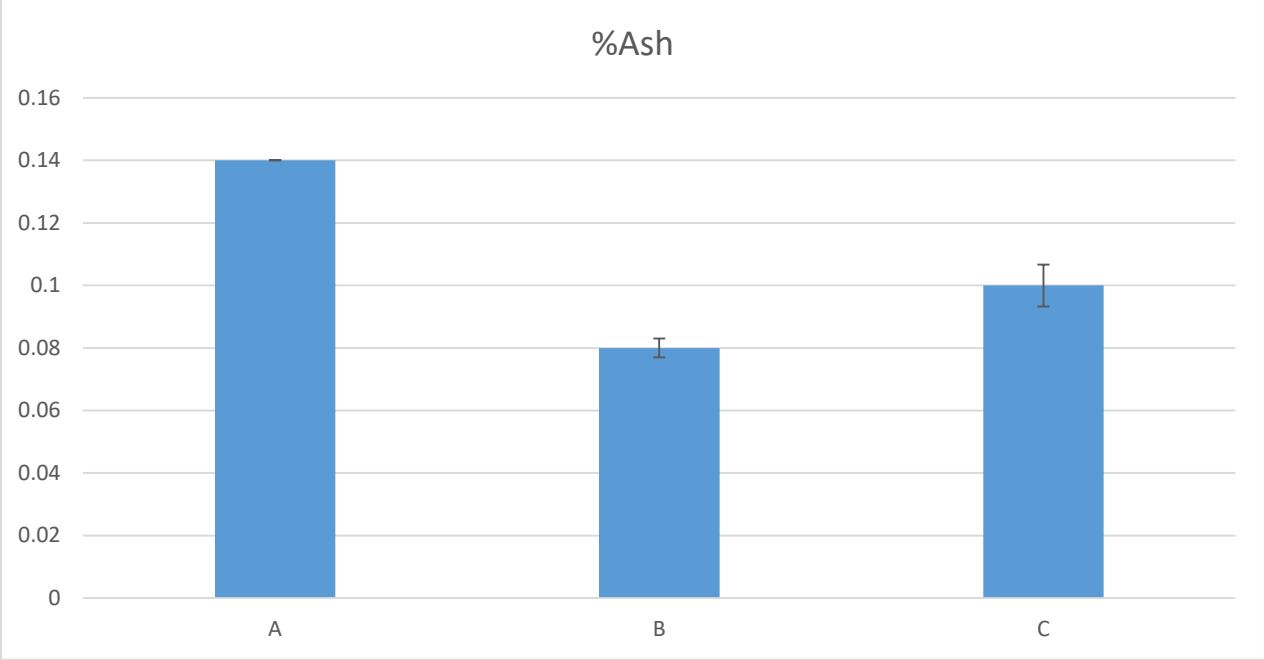
Variants	pH (before fermentation)	pH(after fermentation)
A	5±0.2	3±0.8
B	5±0.1	3±0.8
C	5±0.2	4±0.02

TABLE 2b Estimation of %acidity in the variants

Variants	% acidity (before fermentation)	%acidity(after fermentation)
A	0±0.01	0±0.02
B	0±0.01	0±0.02
C	0±0.02	0±0.03

3.1.3 Ash Content

The figure 1, below shows that highest amount of mineral content was observed in sample A that comprises of ingredients i.e., apple, beetroot and orange. Blanching is a preservative measure which is often a reason for loss of nutrients commonly minerals and vitamins. This generally occurs due to leaching of important minerals. Ash content is considered as the mineral content of the product. Amount of mineral present in the present is important due to its health impact and their role in the stability of the fermented products, (Friaz et al., and 2002).



3.1.4 Ascorbic Acid Content

Maximum amount of ascorbic acid content was found in sample A and B which had a significant amount of orange pomace as an ingredient, shown in table IV. Peels of citrus fruits has more ascorbic acid content as compared to their juice according to USDA National Nutrient Database. It is the vitamin C content of the product. Vitamin C and carotenoids of the peels and fruits by products are considered as an active ingredient in production of functional foods when designing a healthy product or a synthetic preservative substitute, (Ignat et al., 2011).

TABLE 4 Estimation of ascorbic acid content in given variants

Variants	mg/100mL
A	0±0.18
B	0±0.19
C	0±0.10

3.1.5 Total Antioxidant Content

Maximum antioxidant activity was observed in sample B i.e., 55.87% that has apple, orange and black grapes as ingredients and minimum amount of antioxidant activity was found in sample A i.e., 46.16%.

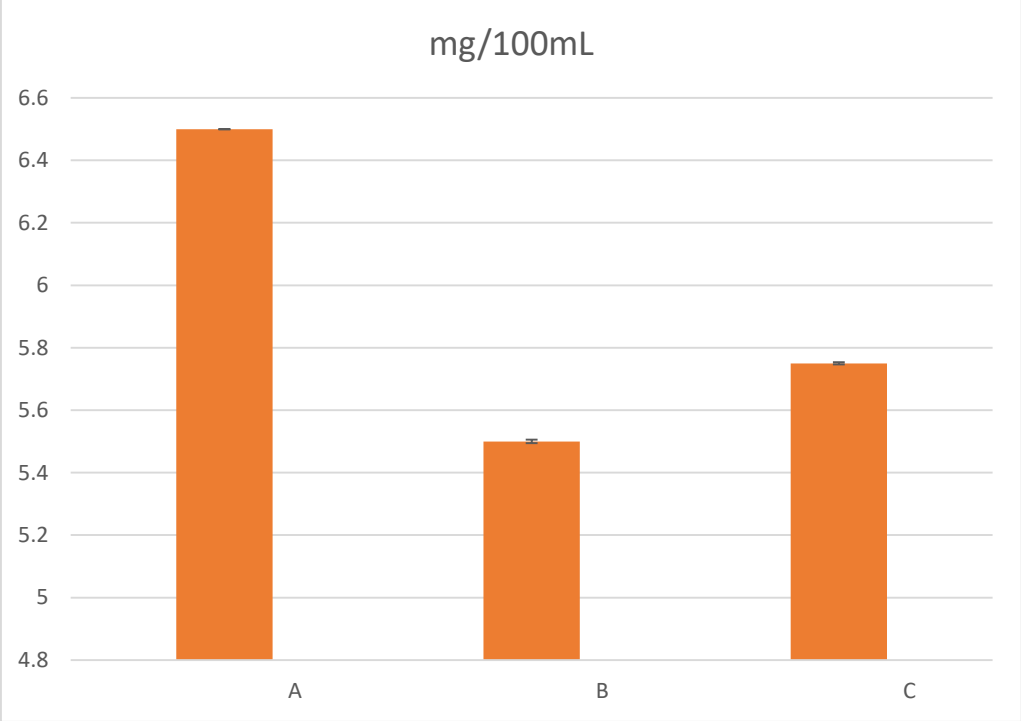
A freshly prepared DPPH solution possess a deep purple colour at an absorption maximum 517 nm. Grape fruits are considered a potential source of compounds to be applied as natural antioxidants in food as compared to other fruits, (Sharma et al., 2016). Similar studies was observed by (Corona, 2015), in which kefir like beverage was prepared and final anti-oxidant content was found to be in the range of 14-75%.

TABLE 5 Estimation of antioxidant content in the given variants

Variants	TAA%
A	46±0.16
B	55±0.87
C	52±0.36

3.1.6 Tannin Content

Maximum tannin content was observed in sample A comprising of ingredients i.e., apple, beetroot and orange. As per below results, maximum sugar level is obtained in variant A i.e., 8.6 mg/100mL whereas minimum sugar level in sample C i.e., 5.7 mg/100mL. Similar results were observed by (Nazarni, 2016), who studied the effect of fermentation on tannin content. Final tannin content was observed to be 55mg/100mL after fermentation.



3.1.7 Total Reducing Sugar

The final sugar level is due to the action of yeast in must and blanching has no effect on final sugar level of the product. As per table 6 results, maximum sugar level is obtained in variant A i.e., 8.6 mg/100mL whereas minimum sugar level in sample C i.e., 5.7 mg/100mL which decreased after the action of yeast. As per studies conducted by Prado et, al., (2015), decrease in reducing sugar was observed after fermentation for a particular period of time. It was observed in the range of 36.73-44.77 gm/L.

TABLE 6 Estimation of total reducing sugar content

Variants	mg/100mL
A	8±0.6
B	6±0.5
C	5±0.7

3.1.8 % Alcoholic Content

Alcohol content of the product was measured at both 5th day and 7th day at temperature variation of 25˚C and 30˚C as shown in table 2. There is a significant increase in the alcohol content from 5th day to 7th day

at 25˚C. Maximum alcohol content on 7th day was observed in sample A and minimum in sample B. There was no significant increase in temperature from 5th day to 7th day at 30˚C. Alcohol content was higher at 25˚C than 30˚C. Whereas, 25˚C is the optimum temperature for the fermentation.

TABLE 7 Estimation of ethanol content (%) at variant temperatures

	25˚C		30˚C	
Variants	**(5th day)**	**(7th day)**	**5th Day**	**7th Day**
A	3±0.05	5±0.12	2±0.75	3±0.01
B	3±0.04	5±0.03	2±0.8	3±0.03
C	3±0.34	5±0.07	3±0.01	3±0.04

3.2 Sensory Evaluation

As per data shown maximum score for appearance is given to variant C. According to the taste perception maximum score is given to variant C and minimum to variant B. Whereas as per score given to colour of the product variant B and variant C has the same score which is due to the presence of black grapes in both the samples. Maximum after taste was felt in variant B and least in variant A. Above calculated data suggests that variant C has the highest overall acceptablilty i.e., 7.92±0.21 whereas lowest of variant A i.e., 7.44±0.13. Variant C got maximum score for aroma whereas minimum score was given to variant B. The statistical analysis showed that all parameters assessed for 3 variant have a statistically significant effect ($P < 0.05$).

TABLE 8 Sensory evaluation of the variants

Variants	**Appearance**	**Colour**	**Aroma**	**Taste**	**After taste**	**Overall acceptability**
A	7.92± 0.81	8.24± 0.81	7.88±0.54	6.84± 0.25	7.08±0.35	7.44±0.13
B	8.28± 0.21	8.64± 0.23	7.48± 0.81	6.48± 0.31	7.52±0.25	7.48±0.31
C	8.44±0.41	8.64±0.33	7.96± 0.76	6.92± 0.54	7.28±0.62	7.92±0.21

3.3 Microbial Analysis

3.3.1 Before Pasteurisation

Pasteurisation, an important heat treatment given to products in order to increase the shelf life and also to increase its potential to inhibit microbial growth. The above samples were given no heat treatment. Though there was no coliform present but TPC resulted 9-10 cfu/mL in all the variants. Yeast and mould count was also quite objectionable.

TABLE 9 Microbial analysis before pasteurisation

Variants	TPC (cfu/mL)	Coliform (cfu/mL)	Yeast and mould (cfu/mL)
A	10	ND[a]	1.2×10^4
B	10	ND[a]	1.5×10^4
C	10	ND[a]	1.32×10^4

3.3.2 After Pasteurisation

As per given results, coliform was absent and TPC was less then the detectable limit. Yeast and mould was also less then the detectable limit which suggests that blanching and pasteurization given to the products was effective.

Table 10 Microbial analysis after pasteurisation

Variants	TPC (cfu/mL)	Coliform (cfu/mL)	Yeastandmould (cfu/mL)
Sample A	ND[a]	ND[a]	ND[b]
Sample B	ND[a]	ND[a]	ND[b]
Sample C	ND[a]	ND[a]	ND[b]

ND[a] < 1cfu/mL

ND[b] < 1 yeast and moulds/mL

3.4 Shelf Study Analysis

The above study conducted shows absence of coliform and TPC was less then the detectable limit for all the variants.Yeast and mould was also less then the detectable limit which suggests that blanching and pasteurization given to the variants was effective. The growth was less then the detectable limit for 21 days which suggests that

product is fit for consumption for 21 days. Hence, variants A,B and C has a shelf life of 21 days.

TABLE 11 TPC ,YMC and coliform count of the variants during the Shelf life Study (A, B and C)

Days	TPC (cfu/mL)	Yeast and mould (cfu/mL)	Coliform (cfu/mL)
0	ND[a]	ND[b]	ND[a]
7	ND[a]	ND[b]	ND[a]
14	ND[a]	ND[b]	ND[a]
21	ND[a]	ND[b]	ND[a]

FIGURE 3 : Final product

IV CONCLUSION AND FUTURE DIRECTIONS

From above conducted study in regards to different temperature and day's variation for fermentation, conclusions can be made that optimum temperature for yeast activity is 25˚C. On increasing temperature, yeast activity decreases hence, leads to less production of ethanol. Optimum pH for production of alcohol is 5. On making the conditions more acidic or basic, declines the conditions for ethanol. Addition of ginger (≤ 1g) to the product during fermentation masked the off flavor production and enhanced the odour. Increasing the amount of ginger, made ginger more prominent and less acceptable. Best ratio of ingredients i.e. was found to be 3:3:2 (3: apple/ pineapple/ orange; 2: black grapes/ beetroot). Blanching and pasteurization are effective method for preserving the product quality and maintaining the shelf life.

The present study was conducted to explore the utilization of fruits and vegetable pomace by fermentation. The study was conducted at different variations of days, temperature, pH, acidity, etc. In regard to this study further improvisations can also be made such as :

 Study can be conducted by variating factors like sugar, pomace ratio, etc. and incorporation of different flavours and other nutritive compounds.

 Quality of beverage can be enhanced by increasing phenolic compounds, tannin content

and antioxidant property.

 Variations can be made by using vegetable pomace in higher ratio.

 Study can also be conducted by inoculating different levels of yeast culture and assessing

physicochemical, microbiological and organoleptic quality of fermented beverage.

ACKNOWLEDGEMENT

This research project is the primacle of life long process of learning and growing, and is a consequence of patience, hard work, guidance and caring. It gives me immense pleasure to express my deepest sense of gratitude, grateful appreciation and indebtness to Dr. Manisha Sabharwal and Ms. Prabhjot Kaur Sabharwal for their overlasting affection, keen interest and judicious guidance which helped me to work throughout my research work and preparation of research report. I am highly indebted to Dr. Anupa Siddhu, Director, Lady Irwin College and Dr. Neena Bhatia HOD (Food and Nutrition Department) for providing me facilities that enabled me to carry out all my lab work and completion of my project.

REFERENCES

Akowuah, G.A., Ismail, Z., Sadikun, A. (2005). The effects of different extraction solvents of varying polarities on polyphenols of Orthosiphon stamineus and evaluation of the free radical- scavenging activity. Food Chemistry.

AOAC . (1997). Official methods of analysis of the Association of Official Analytical Chemist, 16th edn.

Brunet, J.C., Šaponjac ,V.,Djilas, S. and Vulic,´ J. (2016). Encapsulation of Beetroot Pomace Extract: RSM Optimization, Storage and Gastrointestinal Stability.

Caplice, E. (1999). Food fermentations: Role of microorganisms in food production and preservation. *International Journal of Food Microbiology*, 50(1-2), 131–149.

Corona, O., Randazzo, W., Miceli, A., Guarcello, R., Francesc, N., Erten, H., Moschetti, G. and Settanni, L. 2015. Characterization of kefir-like beverages produced from vegetable juices. *Food Science and Technology.*

Crippen, DD. and Morrison, JC. (1986). The effects of sun exposure on the phenolic content of Cabernet Sauvignon berries during development, 243- 247.

Dasa,A. and Mondal ,C. (2013). Studies On The Utilization Of Fruit And Vegetable Waste For Generation Of Biogas. *International Journal Of Engineering And Science*, Volume -3, 24-32.

Ferreira, M.S.L., Santos, M.C.P., Moro, T.M.A., Basto, G.J., Andrade, R.M.S. and Gonçalves, É.C.B.A. (2013). Formulation and characterization of functional foods based on fruit and vegetable residue flour. *Journal of Food Science and Technology*, 52(2),822–830.

Friaz. (2002). Managing Wine Quality: Viticulture and Wine Quality.

FSSAI- Manual of Methods of Analysis of Foods (Alcoholic Beverages). (2015). New Delhi, India.

Ghasemi, K., Ghasemi, Y., Ebhrahimzadeh, M. (2014). Antioxidant Activity, Phenol and Flavonoid Contents of 13 Citrus Species Peels and Tissues. *Pakistan journalof pharmaceutical sciences.*

Gulhane,P.A., Gomashe,A.V. and Kadu,K. (2015). Apple Pomace: A Potential Substrate for Ethanol Production. *International Journal of Research Studies in Biosciences* (IJRSB), Volume-3, 110-114.

Ignat, I., Volf, I. (2011). A critical review of methods for characterization of polyphenolic compounds in fruits and vegetables, Volume-126, 1821-1835.

IS 3752:2005 (1988) Alcoholic drinks - Methods of test. Indian Standards Institution. New Delhi.

IS 5402: 2002. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Enumeration of Micro-Organisms – Colony-Count Technique at 30˚C. Indian Standards Institution. New Delhi.

IS 5403 :1999. Method for Yeast and Mould Count of Foodstuffs and animal feeds. Indian Standards Institution. New Delhi.

IS 7585:1995 (1975) ‘Wines - Methods of Analysis. Indian Standards Institution, New Delhi.

Johnson, M.H., Walliga, M., Vital, D.A.L. and Mejiaa, E.G. (2015). Alcohol-free fermented blueberry–blackberry beverage phenolic extract attenuates diet-induced obesity and blood glucose in C57BL/6J mice. *Journal of Nutritional Biochemistry*.

Kumar,A. (2004). Industrial Pollution and Management.

Lin, Y., Zhang, W., Tanaka, S. and Kong, H. (2012). Factors affecting ethanol fermentation using *Saccharomyces cerevisiae* BY4742.

Many, J.N., Radhika, B. and Ganesan, T. (2014). Study on Tomato Wine Production and Optimization. *IOSR Journal Of Environmental Science. Toxicology And Food Technology*, 97-100.

Nazarni, R., Purnama, D., Umar, S. and Eni, H. (2016). The effect of fermentation on total phenolic, flavonoid and tannin content and its relation to antibacterial activity in jaruk tigarun. *International Food Research Journal*.

Ogodo, AC., Ugbogu, OC., Ugbogu, AE., Ezeonu, CS. (2015). Production of mixed fruit (pawpaw, banana and watermelon) wine using *Saccharomyces cerevesiae* isolated from palm wine.

Ozsoy, N., Can, A., Yanardag, R., Akev, N. (2008). Antioxidant Activity of Smilax excels leaf extracts. Food Chemistry,571- 583.

Prado, F.C., Lindner, J.D.D., Inaba, J., Soccol, V.T., Brar, S.K. and Soccol, C.R. (2015). Development and evaluation of a fermented coconut water beverage with potential health benefits. *Journal of Functional Foods.*

Rachana.S and Gupta D.K. (2010). Utilization of pomace from apple processing industries: a review. *Journal of Food Science Technology*.

Ranganna, S. (1986). Handbook of Analysis and Quality Control for Fruit and Vegetable Products.

Rudra,S.G, Nishad,J, Jakhar,N and Kaur,C. (2015). Food Industry Waste: Mine of Nutraceuticals. *International Journal of Science, Environment and Technology*,Volume- 4.

Sharma, M., Thakur, R. and Sing, V. (2016). In vitro Antioxidant Activities of Green and Black Grape (Vitis vinifera L.) Methanolic Extracts- A Comparative Study. *International Journal of Theoretical & Applied Science.*

Singleton, VL. (1982) Grape and Wine Phenolics: background and prospects, 219-227.

ANNEXURE

A.I1.1 Standard Curve for TANNINS

The tannin content of the fermented beverage was estimated using the standard curve of tannic acid and the results were expressed as mg tannic acid/100mL