Research Article

Effect of Addition of Honey, Herbs and Spices on Nutritional, Antioxidant, Antimicrobial and Sensory Quality of Seasoned Apple Cider Vinegar

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Abstract

Apple cider vinegar, a traditional condiment, is produced from apple fruits/ juice by a two-step process comprising of anaerobic conversion of sugar to ethanol and the aerobic oxidation of ethanol to acetic acid. In the present study, efforts were made to standardize and formulate seasoned (flavored) apple cider vinegar by using different concentrations of honey, medicinal herbs and spices to make it more palatable, nutritious and sweat in taste. Out of different formulations, the formulation consisting of 70 % vinegar (4.5% acidity), 10 % honey, 1 % black salt, 3 % black pepper, 4 % cumin seed, 1 % large cardamom, 2 % *pudina*, 4 % ginger powder, 5 % lemon juice was adjudged to be the best by the panelists based on better sensory score for aroma, taste and overall acceptability. Besides improving the sensory quality, addition of herbs and spice extract has also improved the nutritional, antioxidant as well as antimicrobial potential of the cider vinegar. The seasoned vinegar developed contained higher amounts of ascorbic acid (25.86 mg/100g) and total phenols (123.4 mg/ 100g) and thus has good health promoting properties. Hence, its availability in the market will definitely benefit the health conscious people at reasonable prices.

Keywords: Apple, apple cider vinegar, flavored vinegar, fermentation, seasoning

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Introduction

Vinegar is defined as “a liquid fit for human consumption and produced from a suitable material of agricultural origin containing starch or sugar or both by the process of double fermentation and contains a specific amount of acetic acid” [1]. It is a traditional acidic condiment and widely produced from rice, malt, apples, wine and various other agricultural materials [2,3]. Vinegar fermentation is essentially a two step process, first being the anaerobic conversion of fermentable sugars to ethanol by yeasts, usually *Saccharomyces* species and the second being aerobic oxidation of ethanol to acetic acid by bacteria, usually *Acetobacter* species [4-5]. Vinegar has been a part of the human diet as a condiment and food preservative, as well as the basis for simple remedies for people and animals, since time immemorial [2]. It is commonly used for pickling of fruits and vegetables and in the preparation of mayonnaise, salad dressings, mustard, and other food condiments and as a food ingredient for flavoring. Furthermore, natural apple cider vinegar or cider vinegar is brewed vinegar, which is made from fresh, crushed, organically grown apples and is allowed to mature in wooden barrels [6-8]. But, nowadays, cider vinegar is made mainly by submerged culture from apple fruits, juice and apple juice concentrate [9]. Cider vinegar is a popular folk medicine and is suggested as a remedy to various diseases such as obesity, overweight, arthritis, asthma, coughs, diarrhoea, colitis, eczema, and many other conditions. Whereas, more conventional uses of cider vinegar are as a flavoring agent and as a food preservative like other brewed vinegars [10]. Despite, tremendous medicinal as well as heath benefits of cider vinegar, it is still used as a condiment and not much accepted as a medicine by the general consumer due to its high acidity and a harsh taste. So, seasoning of cider vinegar with herbs and spices extract can be attempted to make it more palatable and sweat in taste. Seasoned vinegar or also known as flavored vinegar is a type of brewed vinegar in which extract of spices and herbs is used primarily for their strong flavor, aroma and natural antioxidants. Flavored vinegar has been produced and sold as a commercial product for approximately 5000 years. The Babylonians produced and sold vinegars flavored with fruit and honey until the 6th century [11-12].But, not much work has so far been conducted in this area in India. Therefore, keeping all these points in view, the present study was undertaken to optimize different proportions of honey, herbs and spices for the preparation of seasoned apple cider vinegar having high nutritive value, antioxidant potential and strong antimicrobial activity for the benefit of masses in general and health conscious people in particular.

Material and Methods

Raw material

Apple fruits (Golden Delicious) were procured from the local market and the juice was extracted as per the method described earlier [8]. The fruits were grated and the juice was extracted by using hydraulic press. It was then used for alcoholic fermentation to prepare the base wine (cider).

Apple cider vinegar (ACV)

Vinegar production is a two stage process. In first stage, alcoholic fermentation of apple juice was carried out to prepare base wine (cider), whereas, in the second stage, the base wine so produced is converted in to acetic acid (vinegar) by acetic acid fermentation. First of all, apple base wine (cider) was prepared in lab scale fermenter (Bio-Flow 2000). The fermentable apple juice (*must*) was prepared using already standardized parameters viz. 14.00 ^{o}B initial TSS, 0.1% DAHP and 50 ppm sulphur dioxide [13]. This apple juice was then filled in the glass container (4 L) through sampling port of the fermenter and the temperature and DO probes were connected. After two hours, culture of wine yeast (*Saccharomyces cerevisiae* var. *ellipsoideus*) culture @0.5% was added through inlet port. The fermentation of apple juice was carried out under controlled conditions viz. temperature 25°C, DO 50 and rpm 50. After completion of the fermentation, the fermented juice (base wine)was siphoned and pasteurized at 65ºC for 20 min. The base wine so produced was used for preparation of cider vinegar. Acetic acid fermentation of fermented apple juice (apple base wine) was carried out with natural consortia (mother vinegar) and using different growth factors viz. 5% initial alcohol, 0.5% K_2HPO_4 and 0.25% ammonium sulphate [10]. After achieving the desired acidity of 5.0%, the acetification process was terminated and the apple cider vinegar (ACV) so produced was siphoned, filled in glass bottles, pasteurized and kept for further use (**Plate 1** and **Figure 1**).

Plate 1 Acetic acid fermentation of apple cider (base wine)

Seasoned apple cider vinegar

Vinegar is a condiment having high acidity and a harsh taste. According to Indian Food Law, vinegar contains not less than 4gm of acetic acid per cubic centimeter at 20^{o}C. In the present study, apple cider vinegar (ACV) having 4.5% acidity was used for the preparation of seasoned/ flavored vinegar. Seasoning of cider vinegar with honey, lemon juice and different herbs and spices was attempted to make it more palatable and sweat in taste as per the detail of treatments given in **Table 1**. Calculated amount of herbs and spices were dried in an oven, ground into powder and tied in a muslin cloth, which is then dipped in the vinegar containing container for about 24 hrs so that the extract of herbs and spices get extracted and infused in the vinegar whereas, lemon juice and honey were directly added in the cider vinegar. The unit operations for preparation of seasoned vinegar are illustrated in Figure 1.

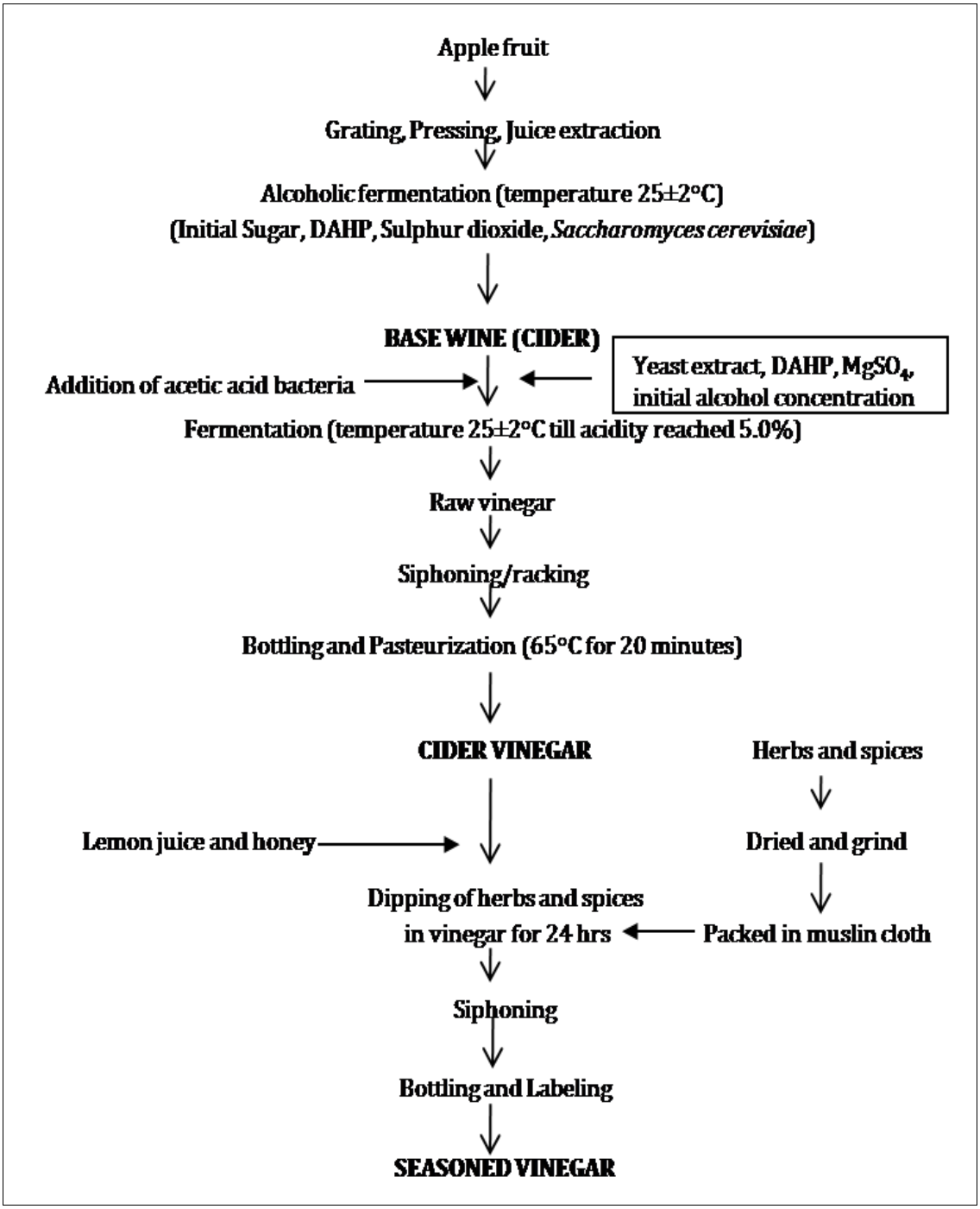

Figure 1 Flow sheet for the production of apple cider vinegar and seasoned vinegar

Table 1 Detail of treatments for the preparation of seasoned apple cider vinegar using honey and herbs and spices

Treatments	Vinegar	Honey	Black Salt	Black Pepper	Cumin Seed	Large Cardamom	Mint leaves	Ginger Powder	Lemon Juice
T_1	65	10	3	3	4	3	2	4	6
T_2	65	10	3	4	2	4	3	5	4
T_3	65	20	1	4	3	4	2	4	5
T_4	70	8	2	4	3	3	2	4	4
T_5	70	8	3	3	2	2	3	5	4
T_6	70	10	1	3	4	1	2	4	5
T_7	75	6	2	3	2	1	2	5	4
T_8	75	8	2	3	1	2	1	5	3
T_9	75	9	1	3	2	1	1	4	4
T_{10}	Control	-	-	-	-	-	-	-	-

Nutritional and sensory quality analysis

All the samples were analyzed for their nutritional characteristics viz. TSS, pH, titratable acidity, total sugars, total phenols and ascorbic acid as per standard analytical methods as described by Ranganna [14]. The residual ethanol in various samples was measured spectrophotometrically by potassium dichromate method [15]. The sensory evaluation of the products was conducted by a panel of 15 semi-trained judges using 9- point hedonic scale system for different parameters like appearance, aroma, taste and overall acceptability. Seasoned vinegar was used as a salad dressing (cucumber) during sensory evaluation of seasoned vinegar and the judges were asked to score the samples on 9-point hedonic scale with a maximum score of 9 for "*like extremely*" and minimum of 1 for "*dislike extremely*" as per the procedure described by Joshi [16].

Antimicrobial and antioxidant activity

The antimicrobial activity of the developed beverages against *E. coli* was measured by well diffusion method [17]. The in153inoculum was spread with the help of swab uniformly on the plate and a standard cork borer of 7 mm diameter was used to cut uniform wells on the surface of solid medium. The antimicrobial activity was expressed in terms of mean diameter of the zones of inhibition measured. Antioxidant activity (Free radical scavenging activity) was measured as per the method of Brand –Williams *et al*. [18], where DPPH (2, 2 diphenyl-1-picrylhydrazyl) was used as a source of free radical. The per cent antioxidant activity was calculated using the following equation:

$$\text{Antioxidant Activity (\%)} = \frac{Ab_{(B)} - Ab_{(S)}}{Ab_{(B)}} \times 100$$

Where Ab (B) = Absorbance of Blank; Ab (S) = Absorbance of sample

Statistical analysis

All the analytical parameters were recorded in triplicates and the mean values of each parameter were described. The data of quantitative estimation of biochemical characteristics were assessed by CRD whereas the data pertaining to sensory evaluation were analyzed by RBD as per standard statistical methods [19].

Results and Discussion

Physico-chemical and Nutritional quality

Data pertaining to physico-chemical characteristics of seasoned vinegar is presented in **Table 2**. It was observed that the total soluble solid (TSS) contents among different samples ranged between 6.9^0B to 18.2^0B. The highest TSS (18.2^oB) was observed in treatment T_3 having 65 % vinegar, 20 % honey, 5 % lemon juice and other spices whereas, lowest TSS of 6.9 ^{0}B was observed in T_{10} control sample i.e. vinegar without honey and herbs extract. Relatively higher amounts of honey and lemon juice added in treatment T_3 might have resulted in higher TSS compared to rest of the treatments. Total titratable acidity (TA) of the seasoned vinegars was found to vary significantly with the addition of vinegar and lemon juice at different concentrations (Table 2). The maximum TA (4.5 %) was recorded in control sample (100% vinegar)whereas lowest value (4.0 %) was recorded in treatment T_2 having 65% vinegar, 10 % honey, 3 % black salt, 4 % black pepper, 2 % cumin seed, 4 % large cardamom, 3 % pudina, 5 % ginger powder, 4 % lemon juice. Further, the treatment T_2 was found statistically at par with treatment T_1, T_3, T_4, T_5, and T_6. The pH value of different seasoned vinegars varied non-significantly between 3.0 and 3.5. The residual ethanol content of seasoned vinegars under the study varied between 1.22-1.28 %. The total sugar contents were found to vary significantly ($p<0.05$) according to the amount of honey used in different recipes (Table 2). The highest total sugar contents (11.33%) were recorded in treatment T_3 having 20 % honey whereas lowest total sugar contents (2.23 %) were found in the control sample (vinegar without honey).

Perusal of data presented in Table 2 revealed that highest amounts of ascorbic acid content (25.86 mg/100g) and total phenols (123.4 mg/ 100g) were recorded in treatment T_1 whereas lowest (4.82 mg/100g, 40 mg/ 100 g) were observed in T_{10} i.e. cider vinegar without herbs and spice extract. However, the treatment T_1 was found statistically ($p<0.05$) at par with treatment T_3 and T_6. It might be mainly due to almost equal proportions of lemon juice used in these recipes. According to Sigh and Gaikwad [20], lemon juice being rich in ascorbic acid and phenolics can be used to improve the ascorbic acid content as well as total phenolic contents of the beverages.

Table 2 Physico-chemical characteristics of seasoned vinegar

Treatments	TSS (^oB)	Total Acidity (%)	pH	Residual ethanol (%)	Total Sugars (%)	Ascorbic acid (mg/100g)	Total Phenols (mg/100g)
T_1	15.0	4.2	3.3	1.22	10.20	25.86	123.4
T_2	15.0	4.0	3.2	1.23	9.71	22.38	115.0
T_3	18.2	4.2	3.1	1.22	11.33	25.00	123.3
T_4	15.2	4.1	3.4	1.24	9.88	20.12	122.0
T_5	15.4	4.1	3.5	1.23	9.56	20.76	118.5
T_6	16.0	4.2	3.0	1.24	10.28	25.00	123.0
T_7	15.3	4.4	3.4	1.26	9.82	19.40	114.6
T_8	15.0	4.3	3.2	1.25	9.85	15.86	108.0
T_9	15.5	4.4	3.0	1.26	10.26	15.36	115.4
T_{10}	6.9	4.5	3.0	1.28	2.23	4.82	40.0
$CD_{0.05}$	0.85	0.20	NS	0.03	0.97	0.98	0.46

The developed products were also assessed for their antimicrobial activity as well as antioxidant potential. Data presented in **Figure 2** revealed that besides improving the nutritive value of cider vinegar, the addition of herbs and spice has also improved its antioxidant as well as antimicrobial potential. The highest antioxidant activity (78.03 %) was recorded in treatment T_6 containing 70% vinegar, 10% honey, 1 % black salt, 3 % black pepper, 4 % cumin seed, 1 % large cardamom, 2 % *pudina*, 4 % ginger powder and 5 % lemon juice which remained statistically at par with treatment T_1. Whereas the lowest antioxidant activity (30.6 %) was recorded in control sample (vinegar without herbs & spice extract).Similarly, the seasoned apple cider vinegar has shown highest antimicrobial activity against human pathogen *E Coli*. (52 mm) compared to that of apple cider vinegar (38 mm). Whereas, apple cider has shown negligible antimicrobial activity against *E. Coli* in the present study (**Figure 3**). The experimental photographs of apple cider and seasoned apple cider vinegar developed under the present study are shown in **Plate 2**. Acetic acid, species and herbs, for instances are well known to inhibit bacteria, yeasts and molds and have traditionally found wide applications in food preservation [21-22].

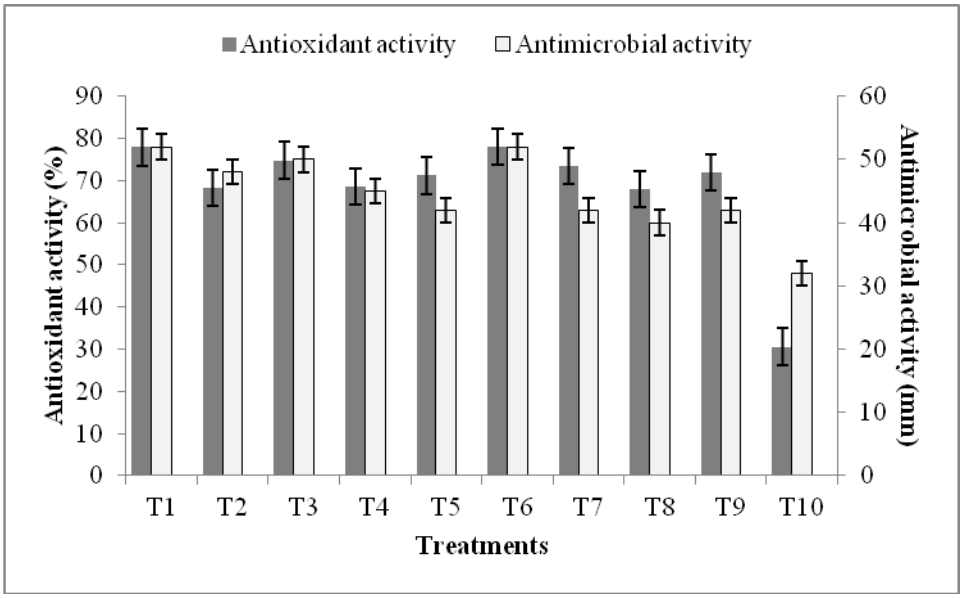

Figure 2 Antioxidant and antimicrobial activity of different seasoned vinegars

Sensory analysis of seasoned vinegar

In order to perform sensory evaluation of seasoned apple cider vinegar, it was used as a salad dressing (cucumber). The samples were presented in random order and the judges were asked to score the samples on 9-point hedonic scale. Data presented in **Figure 4** showed that seasoning of cider vinegar with different herbs and spices has significantly improved its sensory quality. Addition of spices, herbs and salt has also been attempted earlier by many authors to improve the sensory qualities of fruit based beverages [23-24]. Out of various recipes, 6^{th} recipe (T_6) was the best conducted by hedonic scale sensory evaluation for appearance, aroma, taste and overall acceptability. Treatment

$T_6$was found statistically non-significant ($p<0.05$) with treatment T_1. However, apple cider seasoned vinegars of almost all the treatments execpt control fell above '*like moderately*' category of 9-point Hedonic scale. The highest score for overall acceptability observed in 6th recipe having 70% vinegar, 10% honey, 1 % black salt, 3 % black pepper, 4 % cumin seed, 1 % large cardamom, 2 % pudina, 4 % ginger powder and 5 % lemon juice might be due to the balanced taste and flavor contributed by honey, lemon juice and other seasonings like, black salt, black pepper, ginger powder etc. and hence was optimized.

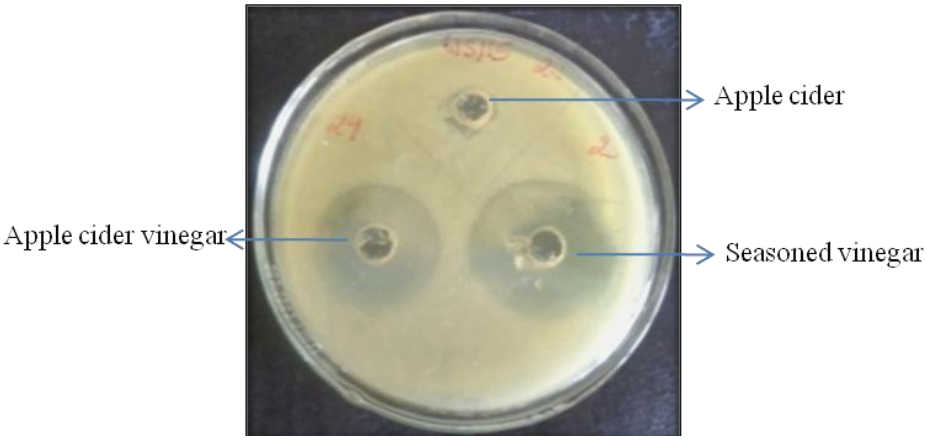

Figure 3- Antimicrobial activity of seasoned vinegar and cider vinegar against *E coli.*

Plate2 Apple cider and seasoned apple cider vinegar

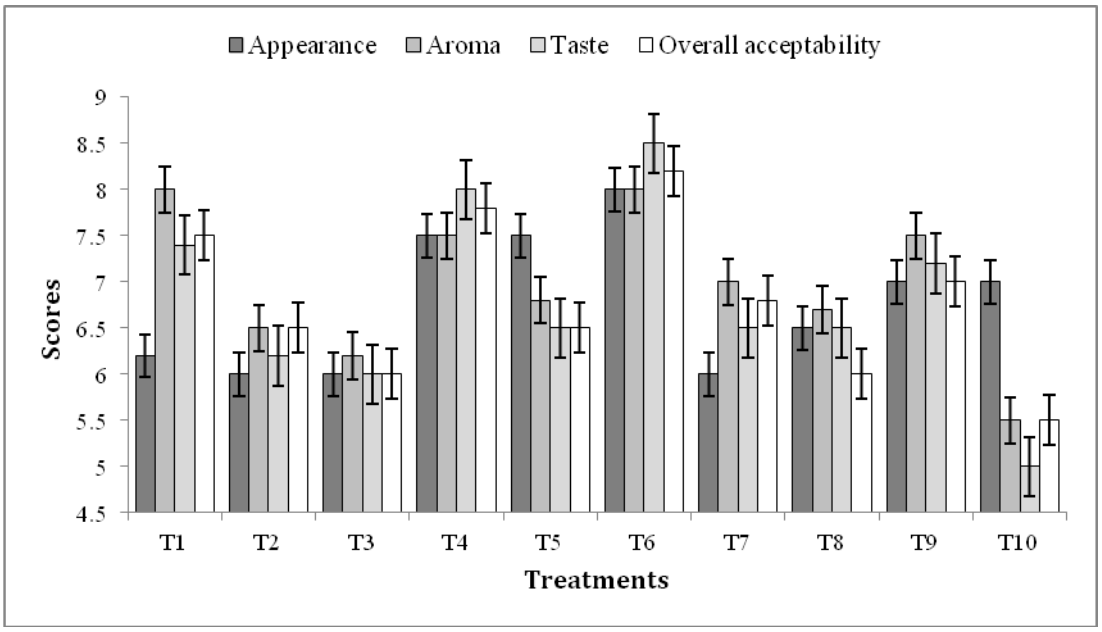

Figure 4 Effect of different treatments on sensory quality attributes of seasoned vinegar

Conclusion

From the present investigation, it emerged that seasoning of apple cider vinegar with different herbs and spices has improved its nutritional, sensory and medicinal properties. Out of different recipes, the recipe consisting of 70 % vinegar (4.5% acidity), 10 % Honey, 1 % Black Salt, 3 % Black Pepper, 4 % Cumin Seed, 1 % Large Cardamom, 2 % Pudina, 4 % Ginger Powder, 5 % Lemon Juice was adjudged to be the best by the panelists based on better sensory score for aroma, taste and overall acceptability. So, it can be concluded that seasoned apple cider vinegar has good health promoting potential and its availability in the market will definitely benefit the consumers. Furthermore, seasoning of apple cider has not affected the cost of production to a greater extent. Hence, commercial production of the developed product seems to be a profitable proposition.

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