

Optimization and storage study of value-added vinegar from guava (*Psidium guajava*) cultivars

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ABSTRACT

Two strains of *Acetobacter aceti* (MTCC 2945 and MTCC 3245) were compared on the basis of variation in inoculation size (5, 10 and 15%) and incubation time (22, 48 and 72 hours) to produce vinegar from guava (*Psidium guajava* L.) cv. Hisar Surkha and Hisar Safeda at CCS Haryana Agricultural University, Hisar, Haryana. Vinegar was further value-added by ginger (*Zingiber officinale* L.), garlic (*Allium sativum* L.), and beetroot (*Beta vulgaris* subsp. *Vulgaris*). Value-added garlic vinegar had maximum overall acceptability, hence was further stored for 45 days and examined for its physicochemical analysis. During storage value-added garlic vinegar had low acetic acid, ascorbic acid, antioxidant activity and alcohol content. The value-added garlic vinegar can be served as a functional food, and can be used for preservation of food products like pickles, gherkins, sauerkraut etc.

Key words: Acetic acid bacteria, Value-added vinegar, Storage, Incubation time, Probiotic

Guava (*Psidium guajava* L.) is a tropical fruit (Chopra *et al.*, 2024), highly consumed in Indian diet. It contains high amount of vitamin C, flavonoid, antioxidant and phenols (Harendra, 2023). Just like strawberry, mango, pineapple *etc.*, guava can also be used for an integrated development protocol for production of diversified products, *viz.* probiotic drink, squash, jam, jelly, candy and wine (Garg *et al.*, 2024). If vinegar is prepared from guava, it will be of better quality. On the other hand, to enrich the nutritional properties of vinegar or to produce value-added vinegar, it can be fortified with some extracts of functional food like ginger, garlic and beetroot. Ginger is a commercial spice crop (Chhetri *et al.*, 2023). Ginger and garlic extracts are used as medicine, whereas beetroot extract possess natural pigments and they all contain high amount of flavonoids, anti-oxidants and said to have anti-inflammatory properties (Timba *et al.*, 2019). In the present study, screening of different *Acetobacter* strain was performed to produce guava vinegar. Further, fruit vinegar was value-added with extracts of ginger, garlic and beetroot.

MATERIALS AND METHODS

Guava cultivars Hisar Surkha and Hisar were procured from local market of Hisar, Haryana. Lyophilised cultures of *Acetobacter aceti* (MTCC 2945 and MTCC 3245) were obtained from Institute of Microbial Technology, Chandigarh. Culture was maintained on Yeast extract Peptone Mannitol (YPM)

medium. *Acetobacter aceti* cultures were maintained by sub-culturing them on YPM agar slants and thereafter stored in refrigerator at 4°C till further use. Fruits were processed to obtain juice, total soluble solids (TSS) adjusted to 20°B by addition of sugar and pH was adjusted to 5 for alcoholic fermentation. Potassium metabisulfite was added @ 100 ppm, and kept for 48 hours to inactivate unwanted microflora.

Fruit juice was inoculated with 5% of 10⁶ cells/ml of *Saccharomyces cerevisiae* (MTCC 170) strain. It was then kept in incubation for 14 days at 28°C, for alcohol production under anaerobic condition. Alcohol content was determined as per method of Zoeckleim *et al.* (1990) with slight modification. After alcoholic fermentation, fermented worts were allowed to settle at 4–5°C and before proceeding for acetous fermentation, wine was diluted with water in a 1:1 ratio so the final ethanol content in wine is reduced to half, further divided equally and kept in different trays, in order to increase surface area and oxygen availability for acetous fermentation.

On the other hand, loopful culture of each *Acetobacter aceti* strain (MTCC 2945 and MTCC 3245) from slants were inoculated in sterilized YPM broth and kept for 24 h at 28°C on shaker at 150 rpm. Then were added separately to partially clarified wine by varying the inoculum size (5, 10 and 15% of 10⁴ cells/ml) and incubation time (22, 48 and 72 hours). Sterile wooden shavings were added at 1% level which served as platform for *Acetobacter* cells to grow and helped in reducing time for acetous fermentation. Acidity was periodically measured till constant value was obtained. After acetous fermentation, vinegar was stored at 4°C for 3–4 days. Then it was clarified by passing through muslin cloth.

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For value addition, chopped garlic (100 g), grated beet root (100 g) and grated ginger (100g) were packed separately in clean muslin cloth and allowed to remain suspended in each 2 litre of vinegar for 48 hr to produce value-added vinegar. After it, spice containing bags were removed and vinegar was filled in glass bottles. Samples were pasteurized at 65°C for 15 minutes and stored at room temperature. Value-added garlic vinegar had maximum overall acceptability, hence it was further stored for 45 days and examined for physico-chemical and sensory parameters at fortnightly intervals.

Titrateable acidity, pH and acetic acid in vinegar were estimated by AOAC, 2019. Ascorbic acid and antioxidant activity was estimated as described by Ranganna, (2014). Yield of vinegar was calculated in terms of acetic acid by determining the amount of ethanol converted to acetic acid. Turbidity (NTU) was recorded by using Nephelometer. Total plate count was estimated on plate count agar by serial dilutions technique. Sensory evaluation was performed in terms of overall acceptability of value-added vinegars by a panel of semi-trained judges using 9-point hedonic scale. Treatments were performed in triplets and the statistical analysis done by ANOVA using OPSTAT software.

RESULTS AND DISCUSSION

Maximum amount of alcohol content was estimated in guava cv. Hisar Surkha (9.62%) followed by Hisar Safeda (7.36%). Highest amount of acetic acid was recorded in Hisar Surkha after 72 hours of incubation with 15% inoculum of MTCC 3245 strain *i.e.* 4.56% (Fig. 1).

Both the strains depicted to produce high amount of titrateable acidity in Hisar Surkha after 72 hour of incubation (Table 1). A significant increase in acidic acid was observed as the inoculum size and incubation temperature increased (Table 1). MTCC 2945 when kept for 113 hours at 10% concentration produced maximum amount of mango vinegar (Harika *et al.*, 2017). CV 01 *Acetobacter* strain produced high alcohol at high temperature (Mounir *et al.*, 2016). When 40% concentration of mother vinegar added in papaya pulp produced highest acetic acid after 70 hours (Kong *et al.*, 2018). Maximum soursop vinegar was produced after incubating for 120 hours at pH 5.5 (Ho *et al.*, 2017).

Table 1. Effect of inoculums size and incubation time on titrable acidity of vinegar (%) with *Acetobacter aceti* (MTCC 3245)

Cultivar	Inoculum size (%)	Incubation period (h)		
		24	48	72
Hisar Surkha	5	2.54	3.05	3.62
	10	2.69	3.26	4.06
	15	2.84	3.78	4.56
Hisar Safeda	5	2.46	2.82	3.14
	10	2.54	3.26	3.57
	15	2.67	3.48	4.06

The vinegar yield was observed maximum in guava cv. Hisar Surkha. However, acetic acid content and antioxidant activity in vinegar was higher for guava cv. Hisar Safeda than others (Table 2).

Table 2. Yield and quality of wine and vinegar obtained from guava

Parameter	Hisar Surkha	Hisar Safeda
Yield of vinegar (% v/w)	34.8	31.6
Turbidity of vinegar (NTU)	972	852
Acetic acid content (% v/v)	4.02	4.36
pH of vinegar	2.0	2.1
Colour of vinegar	Pinkish	Creamish
Antioxidant activity of vinegar (% scavenging of DPPH)	35.4	38.6

After value-addition, guava cv. Hisar Surkha value-added with garlic possessed maximum overall acceptability than others; therefore, it was further kept for storage. During storage, a significant and progressive increase in turbidity and TSS of vinegar was observed. Increased turbidity during may be due to presence of large particles like bacterial cells, polyphenols, carbohydrates and improper filtration. The acidity, ascorbic acid, acetic acid and alcohol contents of vinegar decreased during storage. The decrease in ascorbic acid may be due to decline in vitamin C as during storage in open with aerobic environment vitamin C degrade easily (Davies *et al.*, 2017). The antioxidant activity decreased during storage and the results were in similarity with Davies *et al.*, 2017. The total plate count increased with increasing storage period.

Value addition by garlic resulted in significant increase in turbidity and TSS with the increase in storage period. However, the acidity, ascorbic acid, acetic acid and alcohol contents of vinegar were lower in value-added vinegar over control. The antioxidant activity and total plate count were also found lower in value-added vinegar (Table 3). There was slight but significant decrease in overall acceptability with the increase in storage duration. The vinegar remained acceptable upto 45 days period of storage.

CONCLUSION

In acetous fermentation, highest amount of acetic acid (4.56%) was recorded in Hisar Surkha after 72 hours of incubation with 15% inoculum of MTCC 3245 strain. Value-added vinegar with spices and aromatic herbs complements its taste and medicinal value. The value-added vinegar obtained can be further be used in preparation of functional drink which can be useful for diseased patients.

Table 3. Effect of storage period on various parameters of guava vinegar

Treatment	Storage period (days)								
	0	45	Mean	0	45	Mean	0	45	Mean
	Turbidity (NTU)			TSS (%)			Acidity (%)		
Control	769	942	855	3.33	3.57	3.45	4.80	4.46	4.63
Garlic +	781	1062	921	4.39	4.57	4.48	4.67	4.36	4.51
Mean	775	1002		3.86	4.07		4.73	4.41	
CD(5%)	T= 7.4; S = 7.4; T × S = 14.9			T= 0.03; S = 0.03; T × S = 0.06			T= 0.02; S = 0.02; T × S = 0.04		
	Acetic acid (%)			Alcohol content (%)			Ascorbic acid (mg/100 ml)		
Control	3.75	3.29	3.52	0.73	0.47	0.60	18.4	11.6	15.0
Garlic +	3.76	3.21	3.48	0.72	0.43	0.57	16.5	10.6	13.5
Mean	3.75	3.25		0.72	0.45		17.4	11.1	
CD(5%)	T= 0.02; S = 0.02; T × S = 0.05			T= 0.04; S = 0.04; T × S = 0.08			T= 0.08; S = 0.08; T × S = 0.17		
	Antioxidant (%)			Overall acceptability (9-point hedonic)			Total plate count (log ₁₀ cfu/ml)		
Control	38.1	19.3	28.7	7.9	7.5	7.7	4.31	4.61	4.5
Garlic +	34.2	18.3	26.3	8.2	8.1	8.2	4.10	4.40	4.3
Mean	36.1	18.8		8.1	7.8		4.2	4.5	
CD(5%)	T= 0.44; S = 0.44; T × S = NS			T= 0.14; S = 0.14; T × S = NS			T= 0.007; S = 0.007; T × S = 0.015		

NS, Non-significant

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