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Production and quality evaluation of sea buckthorn (*Hippophae rhamnoides* L) vinegar using *Accetobacter acceti*

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Abstract: In the present study sea buckthorn (*Hippophae rhamnoides* L) vinegar was prepared from sea buckthorn pulp. The pulp and the prepared vinegar were analyzed chemically for its quality evaluation. In pulp moisture, ash, total acidity, crude fat, crude fiber, crude protein, total sugars, tannic acid, ascorbic acid and β-carotene of pulp (80.0±2.30), (0.83±0.03%), (2.24±0.06%), (1.73±0.12%), (0.80±0.04%), (1.14±0.10%), (4.79±0.25%) (0.52±0.02%), (96.0±3.10 mg/100g) and (10.3±0.70 mg/100g) was respectively. The sea buckthorn vinegar contains pH (2.8±0.1), total solid (7.79±0.11%), total ash (2.32±0.03%), total acidity (5.54±0.2%), volatile acids (1.45±0.12%), non-volatile acids (4.09±0.22%), oxidation value (340.2±0.3), alkaline oxidation value (10.4±0.42), acid value (22.35±0.53 KOH/kg), saponification value (25.45±0.1), iodine value (40±0.3), ethanol content (Nil) and ester value (60±0.13). In vinegar some minerals (Na, K, Ca, Mg, P and Fe) were carried out by Atomic Absorption Spectrophotometer, and on the basis of sensory evaluation se buckthorn vinegar showed good overall acceptability.

Keywords: Sea buckthorn pulp, vinegar, chemical composition, sensory evaluation, mineral compositions.
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INTRODUCTION

Vinegar is commonly used as food ingredient but also for its medicinal properties and for its physiological effects such as invigorating, regulator of blood pressure, diabetes mellitus regulator, appetite stimulator, digestion and absorption of calcium[1]. Natural vinegar is a superior food additive over synthetic vinegar as it carries essential amino acids from its fruit source and is reported to act as a medicine for aches and gastric troubles[2]. Consequently, acetic acid bacteria cause an important industrial interest as well as lactic acid bacteria and yeast.

Since, the acetic bacteria are involved in the production or of spoilage of food, their species identification is lead information for the technologist trying to control a bioprocess industry[3]. In recent years, their have been major advances in understanding their taxonomy, molecular biology and physiology and in methods for their isolation and identification[4].

The sea buckthorn (*Hippophae rhamnoides)* is found in Chitral and Northern Areas of Pakistan. Normally, it is spread throughout the Karakuram and Himalayan ranges. According to Chinese *H. rhamnoides* expert, Professor Rongsen, there are about 3000 hectares of *H. rhamnoides* forests in Pakistan, annually producing 1200-2500 tons of sea buckthorn, and various industries producing james, jellies, chocolates and capsules at small scale and exporting the berries abroad[5].

Sea buckthorn (*H. rhamnoides*) is known to be one of the vitamin rich berries in the plant kingdom and has been credentialed as highly valued for healthy living, improving well being, enhancing of life style and preventing the disease[6].

The functional food and nutraceutical markets, collectively estimated as a multi-billion dollar global industry has been gaining popularity[7-9]. Traditional products from the berries include juices, liqueurs, wine, jams, candy and ice cream. However, the berry's unique chemical and nutritional composition has offered economic potential as a health food[7,10]. However, it is generally ignored by both the consumer (due to the higher price) and the producer (due to the long fermentation time of 5-6 weeks).

In rural areas the population resorts to traditional fermentation methods without the use of proper cultures/cultural conditions. Moreover, there is also a lack of awareness of the properties of natural vinegar, besides the problem of the high cost of investment.

The present study was undertaken to find of the possibility of using sea buckthorn pulp for vinegar production through biotechnology process. The aim of the present study was to analyze the chemical composition of fresh sea *H. rhamnoides* pulp and to develop value added food product without losing its natural flavor and nutrients.

Keeping in view of the high demand of the *H. rhamnoides* and its products in the world market, an appropriate new process was developed for the preservation and transformation of the fruit into exportable product, which will contribute to our export earning from the non-conventional source.

MATERIALS AND METHODS

Culture collection

Culture of *Acetobactor aceti* for preparation of sea buckthorn vinegar, after isolation, identification and characterization was produced in Food Microbiology Laboratory, PCSIR Laboratories Complex Peshawar.

Fermentation process

The sea buckthorn berry was procured from Demonstration Cum Training Center PCSIR Skardu, washed with water to remove dust, stalks etc. Then the berries were dipped into water and passed through forcelin cloths and filtered up to three times in 5L flask. Covered the container with a clean cloth (ensuring that the cloth cannot come into contact with the liquid) and secured with string or elastic. Left covered filtrate for 4 - 5 days by stirring once or twice per day. Strain through a jelly bag or muslin cloth. The volume of the liquid was measured, sugar (450g) was added for each pint (600ml) of liquid. Heated gently to dissolve the sugar, bring to the boil and simmered for 10 minutes. The liquid was poured into clean, dry bottles, filled up to 1.5 cm (½ inch) below the stopper. The product was kept for 2 weeks to allow the flavors to mature.

Preparation of Sea buckthorn vinegar

Collection of sea buckthorn berry
↓
Removal of dust, stones and foreign matters
↓
Addition of water
↓
Grinding
↓
Filtration
↓
Addition of sugar
↓
Addition of yeast
↓
Addition of *Accetobacter acceti*
↓
Separation of liquid
↓
Ageing
↓
Filtration and Bottling
↓
Pasteurization
↓
Filling and Labeling

Chemical analysis

The fresh sea buckthorn pulp was chemically analyzed for their moisture, total ash, total acidity, fiber, fat, total sugars and tannic acid by standard method[11]. Crude protein was estimated by kjeldhal method as described earlier[12]. The acidity was determined by titration against 0.1 N sodium hydroxide and total soluble solids were measured by an Abbe refractometer[11, 13]. The ß-carotene content was estimated at 445 nm by UV-Spectrophotometer, Model UNICO 2100 Series Japan[14], whereas the concentration of vitamin C was determined by titrimetric method using 2, 6-Dichlorophenol indophenol method[15]. The pH, total solid, total ash, total acidity (Acetic acid), malic acid, volatile acids, non volatile acid, oxidation value, alkaline oxidation value, acidic value (KOH/kg), saponification value, peroxide value, iodine value, ethanol contents and ester value of sea buckthorn vinegar was determined according to the standards methods[11].

Minerals composition of sea buckthorn vinegar

Decomposition of samples

Sea buckthorn vinegar samples (1.0 g) each in triplicate were taken into a digestion tubes. To these, two volumes (20 ml) of concentrated nitric acid (65 %) was added and digested on low heat in a digestion chamber, until nitric oxide fumes ceased. After adding one volume (10 ml) of percholoric acid (70 %), firstly these were heated gently and then vigorously. There after the contents were evaporated until the volume was reduced to about 1-2 ml, but not to dryness. Added 20 ml nitric acid in empty digestion tube as blank and treated as samples. After cooling the flask, diluted with deionized water and filtered through pretreated Whatman No.1 filter paper. The filtrate and washing were collected in 100 ml volumetric flasks and made up the volumes up to mark with deionized water. The solutions were stored for the determination of minerals on atomic absorption spectrophotometer[16].

Instrumentation

The samples solutions were analyzed for minerals (Na, K, Ca, Mg, P & Fe) by Atomic Absorption Spectrophotometer Model Hitachi Z-8000 Japan equipped with hollow cathode lamps as radiation source using air acetylene flame. The instrument setting and operations were done in accordance with the manufacturer user's specification.

The sample solutions were appropriately diluted, if required, prior to direct measurements and calibration curves were obtained for minerals using standard solutions. They were linear and

correlation coefficient of each curve was above 0.9900, which indicated a best fit between concentration of the standard solutions and respective absorbance values. Accuracy, precision of the method was verified by standard addition/recovery method[17]. Precision was checked by coefficient of variance (CV) which varied from 0.00-10.0%. For background correction, blank was analyzed under instrumental condition. The concentration was recorded in ppm (parts per million), and was converted into gram per 100 gram.

Sensory evaluation of sea buckthorn vinegar

In order to check suitability of the product prepared, the sea buckthorn vinegar and the branded samples were subjected to sensory evaluation i.e. color, flavor, taste and overall acceptability by a panel of trained judges using the 9-point Hedonic scale[18].

RESULTS AND DISCUSSION

Sea buckthorn (*H. rhamnoides*) vinegar was prepared by using sea buckthorn pulp. In order to check its suitability for nutritional purpose the fresh *H. rhamnoides* pulp (11.8° brix) was analyzed chemically (Table 1). It was observed that the moisture, ash, total acidity, crude fat, crude fiber, crude protein, total sugars, tannic acid, ascorbic acid and β-carotene of pulp (80.0±2.30), (0.83±0.03%), (2.24±0.06%), (1.73±0.12%), (0.80±0.04%), (1.14±0.10%), (4.79±0.25%) (0.52±0.02%), (96.0±3.10 mg/100g) and (10.3±0.70 mg/100g) was respectively.

Table 1: Chemical analysis of the sea buckthorn pulp

S #	Parameters	Sea buckthorn Pulp
1.	Moisture (%)	80.0 ± 2.30
2	Total Ash (%)	0.83 ± 0.03
3	Total Acidity (%)	2.24 ± 0.06
4	Crude Fat (%)	1.73 ± 0.12
5	Crude Fiber (%)	0.80 ± 0.04
6	Crude Protein (%)	1.14 ± 0.10
7	Total sugars (%)	4.79 ± 0.25
8	Tannic acid (%)	0.52 ± 0.02
9	Ascorbic acid (mg/100g)	96.0 ± 3.10
10	β - carotene (mg/100g)	10.3 ± 0.70

Values are mean±SEM

On the basis of sea buckthorn pulp nutrients, the chemical analysis of the vinegar prepared are presented (Table 2). It was observed that the pH, total solids, total ash, total acidity (acetic acid), volatile acids and non-volatile acids of vinegar (2.2±0.1), (7.79 ±0.11%), (2.32±0.03%), (5.54±0.2%), (1.45±0.12%) and (4.09±0.22%) was respectively. The pH of our vinegar observed is in close agreement as recommended for vinegar which has pH range of 2.35 to 2.45 reported by Rehm and Reed[19]. The vinegar produced from pineapple peels has a pH 2.8[19].

Table 2: Chemical analysis of sea buckthorn vinegar

S #	Parameters	Results
1	pH	2.2 ± 0.1
2	Total solid (%)	7.79 ±0.11
3	Total ash (%)	2.32 ±0.03
4	Total acidity (%)	5.54 ±0.2
6	Volatile acids (%)	1.45 ±0.12
7	Non volatile acid (%)	4.09 ±0.22
8	Oxidation value	340.2 ±0.3
9	Alkaline oxidation value	10.4 ±0.1
10	Acidic value (KOH/kg)	22.35 ± 0.14
11	Saponification value	25.45 ±0.1
13	Iodine value	40 ±0.3
14	Ethanol contents	Nil
15	Ester value	60 ±0.13

Values are mean±SEM

The ash of the product was due the inorganic materials (minerals). All volatile organic acids short chain affects the acidity, flavor and quality of vinegar. These volatile acids, mainly acetic acids and smaller propionic as well as butyric acid come from raw materials or are generated by fermentation [20]. According to Walter [21] acetic acid and other organic acids (citric acid, tartaric acid, malic acid, succinic acid and lactic acid) determines the acidity of sea buckthorn vinegar.

The oxidation value, alkaline oxidation value, acidic value, saponification value, iodine value and ester value of Sea buckthorn vinegar were (340.2 ±0.3), (10.4 ±0.1), (22.35±0.14 KOH/kg), (25.45±0.1), (40±0.3) and (60±0.13) respectively. The ethanol content in Sea buckthorn vinegar was Nil. Most of the total sugar was converted to acetic acid via ethanol, onion vinegar have ethanol content 2 g/l [21]. The minerals of sea buckthorn vinegar are presented (Table 3). Among the minerals the concentration of sodium (1.436 ±0.04 g/100g) was higher than potassium (0.189 ±0.01 g/100g), calcium (0.110 ±0.01 g/100g), magnesium (0.077 ±0.02 g/100g) and iron (0.065 ±0.03 g/100g), while phosphorus (0.059 ±0.01 g/100g) was the lowest.

Table 3: Minerals composition of sea buckthorn vinegar

S #	Minerals	Sea buckthorn vinegar
1	Sodium (g/100g)	1.436 ±0.04
2	Potassium (g/100g)	0.189 ±0.01
3	Calcium (g/100g)	0.110 ±0.01
4	Magnesium (g/100)	0.077 ±0.02
5	Phosphorus (g/100g)	0.059 ±0.01
6	Iron (g/100g)	0.065 ±0.03

Values are mean±SEM

Sodium and potassium are the major ions in the body fluids. The regulation of proper concentration of these ions in the extra cellular and intra cellular fluid is critical for homeostasis[22]. Magnesium is important to carbohydrate metabolism. It may influence the release and activity of insulin, the hormone that helps control blood glucose levels[23]. Diets that provide plenty of fruits and vegetables, which are good sources of potassium and magnesium, are consistently associated with lower blood pressure. Calcium is essential for the clotting of blood, the action of certain enzymes and the control of the passage of fluids through the cell walls[23].

Table 4: Sensory evaluation of sea buckthorn vinegar in comparison to branded vinegar

Name of vinegar	Color	Flavor	Taste	Overall acceptability
Sea buckthorn vinegar	8.3	8.6	8.7	8.5
Branded vinegar 1	7.8	7.6	8.2	7.8
Branded vinegar 2	8.2	9.1	8.7	8.6
Branded vinegar 3	6.8	7.2	8.0	7.4
Branded vinegar 4	7.8	8.6	8.1	8.2

The sensory evaluation of the sea buckthorn vinegar and branded vinegars were carried out comparatively to check its color, taste, flavor and overall acceptability (Table 4). This evaluation plays an important role in the quality of food. The overall acceptability of sea buckthorn vinegar was 8.5 score.

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