DEVELOPMENT OF CITRUS FRUIT-BASED BEVERAGE UTILIZING EXTRACT OF A NATURAL HERB *Asteracantha longifolia (*KULIKHARA)

Hazra Nabanita[1], Dutta Ranita, Dey Anamika[1], Roy Nabamita (Chattopadhyay) Pal Jayati[1*]
[1]Department of Food Technology, Techno India, Maulana Abul Kalam Azad University
Salt Lake Kolkata, India
*E-mail: jayatipc@gmail.com

Abstract
A. longifolia (common name Kulikhara) is widely known medicinal herb with its established therapeutic properties. Citrus beverage is identified as a potential platform for enrichment with herb extract for enhancement of its nutritional and functional value. Individual extract from citrus fruits, spice blend (cardamom and cinnamon) and kulikhara leaves were prepared. Lemon and sweet-lime juice along with spice extract and sugar syrup were blended in a definite ratio with incorporation of aqueous extract of A. longifolia. Both unprocessed and heat-processed beverages were compared for iron and polyphenol content. 10% herb extract with corresponding 0.8 g herb solids in the final unprocessed beverage was found to be acceptable. Final formulation was selected by sensory evaluation with overall acceptability of 8.0. Titratable acidity content, ash content and vitamin C content of the unprocessed and processed final beverage was found to be 0.45-0.46 g/100 ml, 0.57-0.51% (w/w) and 0.056 IU. There was 51.43% (w/w) increase in iron content after heat-processing, whereas polyphenol content was found to get decreased by 68.5% (w/w).

Keywords: Herb, iron, polyphenol, pasteurization, sensory

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1. INTRODUCTION

Herbs were nurtured for their usage in multiple platforms e.g. in personal care products like ointments, perfumes, in therapeutic formulations e.g. in ayurveda or antidotes for poisons, as basic ingredients for incense etc. since ancient times. Subsequently, usage of herbs has been explored in culinary preparations as flavouring agent in different food products and beverages. They are also known to exhibit their preservative effect in food preparations. Depending on applications, they were classified as culinary, cosmetic and pharmaceutical herbs (Peter et al., 2012).It is their specific phytochemicals existing in the form of essential oil, oleoresin, oleogum and resin, which make herbs a useful ingredient in various sectors. Different parts of the plant i.e. seeds, berries, roots, leaves, fruits, bark, flowers or even the whole plants are used to impart therapeutical benefits exploited from herbs (Pan et al., 2012).

A. longifolia Nees (Fam. *Acanthaceae*) is a wild herb commonly found in most part of India, Sri Lanka. Locally, this herb is known as Kulikhara. It is a source of the Ayurvedic drug *Kokilaaksha* and the Unani drug *Talimakhana.* According to Ayurveda, the herb is sweet and bitter in taste. Studies have shown that the whole plant contains lupeol, stigmasterol, isoflavone glycoside, alkaloid and small quantities of uncharacterized bases. The seed contains asterol, asteracanthine, asteracanthicine, amino acids - histidine, lysine and phenylalanine. The fresh flowers contain apigenin-7-O-glucoside. Leaves of this plant possess liver-stimulating effect and also facilitate bile secretions, hence beneficial in hepatitis and liver diseases (Dattatrya et al., 2012). Antitumor activity, anti-inflammatory activity, antipyretic activity, analgesic activity were other therapeutic activities, exhibited by Kulikhara herb extracts. Dattatrya et al. (2012) also established antibacterial and antioxidant activities of solvent and aqueous extracts of this herb.

Traditional belief is that consumption of kulikhara leaf extract is beneficial for treatment of iron-deficiency anaemia , a common

nutritional disorder prevalent in both industrialized and developing countries among infants, children, women of childbearing age, pregnant women because of poor iron absorption. An ethanolic extract of this herb, obtained from crude drugs, was found to be effective in enhancing the hematological parameters, serum iron and serum protein (Pawar et al., 2010). Petroleum ether and chloroform extract of leaves reportedly showed hematopoietic activity by increasing the erythrocyte count, leucocyte count, and hemoglobin level significantly (Pawar et al.,2006a, 2006b).These observations very well justify inclusion of leaves of this herb in Indian system of medicine since ancient time.

Beverages are considered to be a compatible platform for enriching with functional phytochemicals such as soluble fiber or herbal extract (Gordon et al., 2003). Blending with suitable juices or extracts may result in the preparation of beverages with improved organoleptic quality and high nutritive value (Kausar et al., 2016). Citrus fruit especially lemon beverage enjoys a special platform as refreshing beverage in tropical country. According to Chatham (2012) lemon and limes have similar composition and nearly the same health benefits. Presence of vitamin C and total polyphenols in these citrus fruits are majorly responsible for those benefits. Spices also are important flavouring agents used in foods with their potential antioxidant, antimicrobial, therapeutic and nutritional properties (Peter et al., 2012). Keeping this in view, the present research study explores the development of a citrus fruit based beverage formulated with spice extract and utilizing kulikhara extracts for enrichment with natural phytochemicals and for enhancing its functional value.

2. MATERTIALS AND METHODS

This present study was conducted at Department of Food Technology laboratory, Techno India. Kulikhara leaves, lemon and sweet-lime were procured from local market. Common salt of Tata brand was used as ingredient. Whole spices (cardamom, cinnamon and black pepper) were also sourced from local market. All the chemicals were purchased of analytical grade (AR).

Preparation of spice extract

Spices (cardamom and cinnamon) were ground separately. Cardamom and cinnamon powder were mixed in the ratio 6:4 by weight. 1 g of the spice mixture was suspended in 100 ml of distilled water in a conical flask and the suspension was kept for extraction of soluble solids at 75^0C for 10 minutes. The solution was filtered in hot condition and cooled immediately. For black pepper extract, 1 g of black pepper powder was taken in 100 ml of distilled water and hot water extraction was done in the same method, time-temperature combination remaining the same. The spice extracts were kept in a refrigerated condition (7 ± 1^0C) until used.

Preparation of sugar syrup

Required amount of sugar was taken and mixed with distilled water at 45^0-50^0C with constant stirring. Brix was adjusted to 45^0 by adding required amount of distilled water. The syrup was then cooled and filtered through muslin cloth to get a clear sugar syrup. The syrup was kept in a refrigerated condition (7 ± 1^0C) until used.

Preparation of fruit juice (for both lime and lemon)

Matured lemon and lime fruits were taken, washed, peeled and cut into two halves. Juice was extracted by squeezing them and was strained through muslin cloth to remove the pulp. The juice was kept in a refrigerated condition (7 ± 1^0C) until used.

Preparation of herb extract:

Measured quantity of kulikhara leaves were taken and washed thoroughly. Leaves and distilled water in the ratio of 1:10 were cooked in pressure cooker for 10 minutes. The mixture was then screened through muslin cloth. Clear herb extract was collected. The herb extract was kept in a refrigerated condition (7 ± 1^0C) until used.

Final blending for preparation of the beverage:

Measured amount of sugar syrup was mixed with definite amount of spice extract and was

stirred well. Then herb extract was added slowly with continuous stirring followed by the addition of measured quantity of lemon and lime juice. It was then followed by the addition of distilled water to adjust the final soluble solids (Brix) to prepare the final beverage. Three different formulation trials (Formulation A, B and C) have been undertaken to fix the final formulation of the beverage. Lemon and sweet-lime juices were fixed at 5% level (wt./wt.) in all three developmental formulations with 1:1 (wt./wt.) ratio. Spice mix extract and black pepper extract were tried out in the range of 5-10%(w/w) in three different formulations. 10%, 25% and 30% (wt./wt.) herb extract were incorporated in three final formulations. Details of the trial formulations (basis 100g) have been shown in Table 1.

Processing of final beverage: Final beverage, after selection from developmental samples, was pasteurized at 90-95^{0}C for 10 min. The beverage was then filled into previously washed glass-bottles at 90^{0}C in hot condition and crown-corking was done immediately. Final beverage was gradually cooled down and was stored at ambient temperature.

Physico-chemical analysis: The kulikhara based juice samples were analyzed for their different physico-chemical properties. The total soluble solids were determined with refractometer (0-30) and the values were expressed as ^{0}Brix. Total acidity was calculated in terms of citric acid for juice samples and herbal beverage sample by titrating against 0.1(N) NaOH according to AOAC (1995) method.

Determination of total iron content: Beverage sample was taken for ashing in the muffle furnace. Total ash was collected. It was extracted in 5 ml. of concentrated hydrochloric acid, filtered quantitatively in volumetric flask and volume was made up with water. An aliquot of extract was taken and hydroxylamine hydrochloride solution was added. After 5 minutes, 10 ml acetate buffer solution and 10 ml orthophenanthroline were added. Volume was made up with water and mixed thoroughly. After standing for 30 mins., absorbance was measured in spectrophotometer at 510 nm. A standard calibration curve was plotted using standard Mohr salt solution (Rahul et al.,2013).

Determination of total polyphenol content: A definite aliquot of beverage sample was mixed with 1 ml Folin-Ciocalteau reagent and after 5 minutes, 3 ml of 20% sodium carbonate was added. It was kept undisturbed at 40^{0}C for 30 min. Absorbance was measured at 760 nm. Standard calibration curve was plotted using gallic acid (Garg et al., 2015).

Determination of vitamin C content: A definite aliquot of beverage sample was dissolved in 5% metaphosphoric acid and volume was made up to 100 ml. 5 ml of dye solution was titrated against the sample. Result was expressed in terms of I.U. of vitamin C per 100 ml of the sample (AOAC, 2012).

Sensory analysis of beverage: Sensory analysis was done on a 9 point Hedonic scale. The developmental beverage samples were evaluated for the attributes of appearance, colour, flavour, taste and mouth feel.

Statistics:

Samples were analyzed in triplicate and the results are given as averages ± standard deviation.

3. RESULTS AND DISCUSSION

Physico-chemical characteristics of herb powder and herb extract are mentioned in Table 2 and Table 3 respectively.

Table 1. Trial formulations (basis 100g) of the herbal beverage

Ingredients	Drink A	Drink B	Drink C
Lemon juice (g)	5	5	5
Sweet lime juice (g)	5	5	5
Sugar syrup (g)	50	50	50
Spice mix extract (g)	10	8	5
Black pepper extract (g)	10	7	5
Salt (g)	0.25 g.	0.25g.	0.25g.
Herb extract (g)	10	25	30
Water (g)	10	nil	Nil

Analysis of kulikhara powder in Table 2 revealed that ash content and iron content were 16.69% (w/w) and 10.67 mg/100g on dry matter basis. Result of ash content of leaves matches with that of the findings of 14.2% (Dash et al.,2012). Mukherjee et al. (2017) reported 7.03 mg/100g of iron content in fresh tap-dry leaf (14.06-17.57 mg/100g on dry basis), which closely matches with the observed iron content of herb powder. Iron content in hot-water extract of herb (100g) was found to be higher than that of herb powder (100g), as indicated by Table 3 (12.3 mg/100 g). This finding is also supported by the observations made by Dash et al (2012).

It was observed from Table 4 that drink B and C having 25% and 30% (wt./wt.) herb extract were found to have much less sensory score with respect to appearance, color, flavor and taste from Drink A, made with 10% (wt./wt.) herb extract. Mouth feel attribute was also less for both Drink B and C, compared to drink A. This resulted in less overall acceptability of the two drinks, made with 25% and 30% herb extract. Drink A with 10% herb extract came out as the superior one among three formulations of the herbal beverage. This resulted in 0.8 g. of herb solids in the final formulation.Table 5 showed that sensory analysis revealed that there was not much change with respect to appearance, color and flavour of the beverage after heat-processing. Taste score decreased, whereas mouthfeel score increased. Final heat-processed beverage was found to be comparable to the unprocessed drink with respect to overall acceptability score.

Table 2. Analysis of herb powder

Parameter	Percentage values (% w/w)
Moisture	8.96 ± 0.02
Ash	15.19 ± 0.51
Iron	9.71 ± 0.38 mg/100 g.

Table 3. Analysis of herb extract

Parameter	Percentage values (% w/w)
Total solid	8.81 ± 0.09
Ash	2.94 ± 0.06
Iron	12.3 ± 0.67 mg/100g.

Table 4. Sensory analysis of three trial formulations (Formulation A, B and C)

Attributes	Drink A	Drink B	Drink C
Appearance	8 ± 0.5	6.57 ± 0.94	5.53 ± 0.41
Color	8.17 ± 0.29	6.25 ± 0.20	5.33± 0.24
Flavor	8 ± 0.5	5.9 ± 0.08	5.83 ± 0.24
Taste	8.33 ± 0.29	6.33±0.24	5.00 ±0.08
Mouthfeel	8.16 ± 0.29	7.20 ±0.22	6.23 ±0.05
Overall acceptability	8.03 ± 0.06	7.53±0.05	6.34±0.34

Table 5. Sensory analysis of final beverage before and after heat-processing

Attributes	Beverage before processing	Beverage after processing
Appearance	7.58± 0.31	7.66 ± 0.24
Color	7.83± 0.24	7.50± 0.41
Flavor	7.25± 0.20	7.33 ± 0.24
Taste	8.00± 0.08	7.03 ± 0.05
Mouthfeel	7.66 ± 0.24	8.20± 0.22
Overall acceptability	8.25 ± 0.20	8.42 ± 0.12

Table 6. Analysis of herb-beverage

Parameter	Without heat-processing	With heat-processing
Brix	24^0	24^0
Acidity(%)	0.46 ± 0.01 %	0.45 ± 0.03%
Ash (%)	0.57 ± 0.11	0.51 ± 0.09
Vitamin C	0.056 IU	0.045 IU

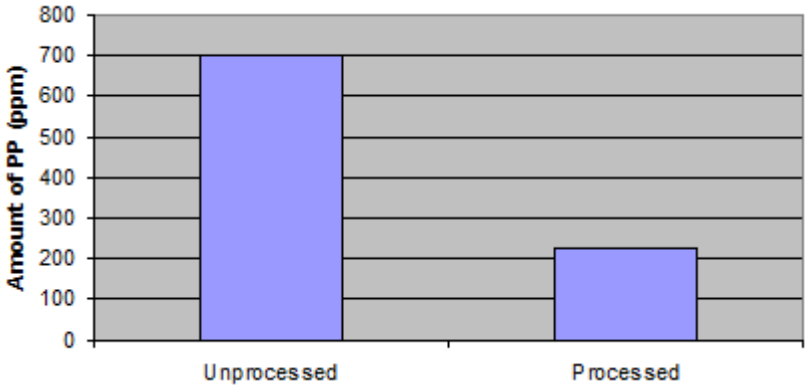

Fig.1. Effect of heat-processing on total polyphenols

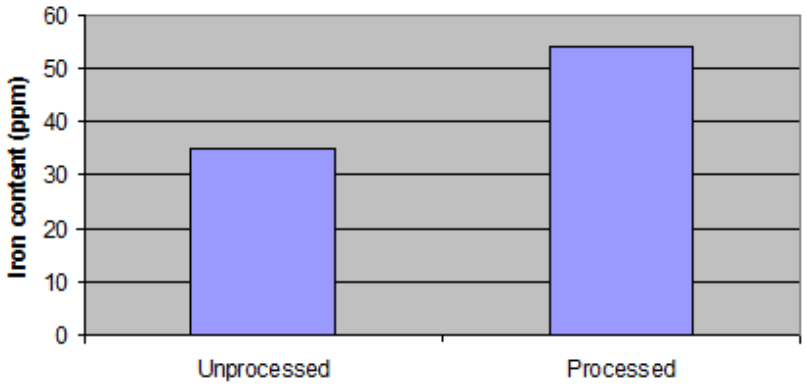

Fig. 2. Effect of heat-processing on iron content

Figure 1 and Figure 2 clearly showed the effect of heat-processing on phytochemicals of final beverage. It was observed that heat-processed beverage was found to have less polyphenol content compared to the unprocessed beverage and the decrease in content was by 2.86 times. Maskat et al. (2011) reported percentage reduction of total polyphenol content in herb (Mengkudu) extract, when gradually heated from 30 to 90^0C. High-temperature processing of beverage might lead to degradation of flavonoid glycoside to other forms resulting in reduced antioxidant activity (Weil et al., 2001). It was observed that there was 51.43% increase in iron content of the final beverage compared to its content in unprocessed one. Increase in ash content with respect to cooking time was also observed (Orhevba , 2011). Hazell and Jhonson (2007) also found that processing of foods might increase diffusible iron in the food matrix as compared to the unprocessed food. This increase might be due to release of ferrous ion in the medium, which might also increase iron bio-availability of final beverage. Heat-processing might improve mineral bioavailablility by increasing solubility due to cell-wall disruption, protein denaturation and release of organic acids. Increase in soluble iron in the final heat-processed beverage might also be due to alteration of concentration of food tannins by thermal processing (Reddyet al., 2009).

4. CONCLUSION

A citrus-based beverage has been developed with incorporation of a natural herb Kulikhara (A. longifolia). Both herb powder (0.97 g/100g) and herb extract (123 ppm) were found to be much high in iron content. In final beverage formulation, 10% herb extract corresponding to 0.8 g herb solids was found to be acceptable after sensory analysis. After heat-processing also, overall acceptability was found to be comparable with the unprocessed beverage. There was not much change in total soluble solids (%), acidity (%), ash(%) and vitamin C content between heat-processed and unprocessed beverage. Phytocomponents i.e. iron content and polyphenol content were found to get affected by heat-processing. Heat-processed beverage was found to have increased iron content, but decreased polyphenol content compared to unprocessed beverage.

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