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Evaluation of *karonda* (*Carissa carandus* L.) derived natural colourant cum nutraceuticals-supplement

Hare Krishna[1], Nitesh Chauhan and B.D. Sharma

ICAR-Central Institute for Arid Horticulture, Beechwal, Bikaner -334 006, Rajasthan, India

[1]Email: kishun@rediffmail.com

ABSTRACT

A natural 'food colourant cum nutraceuticals-supplement' was prepared from the ripe karonda fruits. The formulation had been christened as 'Lalima'. An ml of this pigment suspension formulation is sufficient to give pleasing red colour to one serving (100 ml) of any colourless beverage such as lemon based beverages. One serve of such supplemented beverage may additionally contain 469.2 µg anthocyanin (cyaniding-3-glucoside equivalent), 14.1 mg phenol (gallic acid equivalent), 12.7 mg flavonoids (rutin trihydrate equivalent), with total antioxidant activities (CUPRAC) to be 390 µM Trolox Equivalent. Lemon sherbet supplemented with 'Lalima' was found to be more acceptable in terms of flavour and appearance among the testers than the plain lemon sherbet. The development of technology for value addition of the food items through alternative uses of karonda would help regulate the availability of such antioxidant rich sources for nutritional security.

Keywords: Karonda, natural colourant, flavonoids, phenolics, antioxidant, nutraceuticals-supplement

INTRODUCTION :

The old adage 'first to feast is the eyes' underlines the importance of colours in our life. People's perception towards food items is generally influenced by their appearance. Colour is an important component of appearance, which govern the quality of foods. Colour of a food or beverage play a profound role in flavour perception (Krishna *et al*., 2014). One way of colouring food through natural means is addition of a strongly coloured food to the intended food item, which is to be coloured. This is a common approach practiced in home-cooking. However, for industrial food production such approach may not be desirable due to issues like low concentration of pigments in most foods (which leads to addition of a large amount of pigmented food items to get the desired shade), unwanted flavour and insoluble matter (e.g., peel and seeds). Therefore, pigments are extracted to overcome such potential glitches (Naderi *et al*., 2010). The naturally extracted pigments/colours are perceived by the consumer as safe to consume than the synthetic colours. Further, an added advantage of using such colours derived from natural sources is that they are bioactive.

Karonda or Christ's thorn (*Carissa carandas* L.) of the family Apocynaceae, is a hardy evergreen spiny shrub of Indian origin. It is a multi-branched bushy tree and is generally planted as a hedge. It can tolerate the hot and dry conditions and intense solar radiation in the arid and plateau region (Ghosh, 2014). In India, *karonda* plants are found in wild form in several regions including the Siwalik Hills, Bihar, West Bengal, the Western Ghats, Karnataka and the Nilgiri hills. Most *karonda* shrubs are ornamental, though the tribal areas of Madhya Pradesh, Chhattisgarh, Rajasthan, Gujarat and Jharkhand are known to grow the fruit on a limited scale. In Varanasi district of Uttar Pradesh and South 24-Parganas district of West Bengal, it is grown on a commercial scale (Panda *et al*., 2014). *Karonda* is commonly used as a condiment or additive to Indian pickles and spices. Traditionally the plant has been used in the treatment of scabies, intestinal worms, pruritus, biliousness and also used as antiscorbutic, anthelmintic. The notable biological activities reported are analgesic, anti inflammatory, anti pyretic, cardiotonic and histamine releasing (Maheshwari *et al*., 2012). The health benefits of *karonda* (*Carissa carandus* L.) are attributed mainly to the presence of some phytochemicals, which are referred as antioxidants (Panda *et al*., 2014). Despite, its multiple usefulness, it remained an underexploited fruit, probably, due to its small berry size and sour taste. However keeping in view the rising awareness among the consumers for health foods, alternative form of utilization may be devised to encourage its increased consumption by the general public. One such use could be extraction of natural pigments from ripe fruits as some genotypes of *karonda* develop dark red pulp upon ripening. *Karonda* genotype, CIAH Selection-1, which turns dark red-purple upon maturity; could be a likely candidate as a source of natural food colourant and antioxidants for its potential domestic and industrial application. Therefore in the present study, an attempt was made to extract natural pigments from the ripe *karonda* fruits for its possible use as 'food colourant cum nutraceuticals-supplement'.

MATERIALS AND METHODS

Preparation of food colourant cum nutraceuticals-supplement

Fruits of *karonda* genotype CIAH Selection-1 which has green fruits with reddish tinge at maturity and turns dark red-purple upon ripening (Fig. 1) were harvested from Experimental Block of ICAR-Central Institute for Arid Horticulture, Bikaner, Rajasthan, India. Fruits were harvested between 2nd to 3rd weeks of October and were selected according to the uniformity of shape and colour. For colour extraction, after washing and cleaning ripe fruits were cut into halves. Seeds were removed before subjecting fruits to dehydration. The dehydrated fruits were later grounded into powder with the help of an electric mill with 0.5mm sieve. Powdered fruit pulp was cold extracted thrice with ethanol and supernatant were pooled together, filtered through 2.5μ filter and later air dried at 45 ^{0}C in hot air oven. The dried concentrated 'colour pigments' which predominantly contained anthocyanin and phenolics were then dissolved in water to get ready-to-use 'food colourant cum nutraceuticals-supplement'. Benzoic acid @ 600ppm was added to enhance the shelf life of the product. To make it user-friendly, the formulation was packed in 10 ml plastic dropping bottles. This packed bottle had pigments extracted from the 10g dried fruits. Subsequently, a study was undertaken to compare the plain lemon *sherbet* and lemon *sherbet* supplemented with *karonda* derived pigment suspension formulation in terms of their photochemical contents, antioxidant activities and overall sensory acceptability. Lemon *sherbet* was prepared by adding 10% lemon juice to water and adjusting final soluble solid contents of *sherbet* to 10%. Plain lemon *sherbet* served as control, in this study.

Estimation of phytochemicals and antioxidant activities

The ascorbic acid contents of the plain and supplemented lemon *sherbet* were estimated according to Ranganna (1986). Total phenolics in samples was estimated spectrophotometrically using Folin–Ciocalteu reagent and results expressed as gallic acid equivalents (mg GAE 100g^{-1} FW) (Krishna and Parashar, 2013). The quantification of total flavonoids was carried out by means of the aluminium chloride colorimetric method (Krishna and Parashar, 2013). Results were expressed as mg of rutin equivalents 100g^{-1} FW. The estimation of flavanols was performed according to the method described by Thimmaiah (1999) using the Vanillin reagent. The results were expressed as mg phloroglucinol equivalents 100g^{-1} FW. For *0*-dihydric phenol, the supernatant was mixed with 0.05 N HCl, Arnow's reagent, and 1 N NaOH. The absorbance was read at 515 nm. Standard curve was prepared using pyro-catechol (Thimmaiah, 1999). The total monomeric anthocyanin content was determined on a UV-visible spectrophotometer by the pH-differential method (Giusti and Wrolstad, 2001). The pigment content was calculated and expressed as mg cyaniding 3-glucoside (Cyd 3-glu) per L, using an extinction coefficient (^{a}) of 26,900 L/cm/ mol and a molecular weight of 449.2 gmol/L. Antioxidant activity was measured by cupric reducing antioxidant capacity (CUPRAC) and ferric reducing antioxidant power (FRAP) methods as suggested by Krishna and Parashar (2013).

Organoleptic evaluation for acceptability

Organoleptic evaluation was performed on beverage preparations by a ten-member trained panel. For each sensory parameter, such as colour & appearance, body or texture, flavour, taste and overall acceptability, 100 marks were allotted and the products were given to the panelist in coded form (Krishna and Attri, 2016). The panelists washed their mouths with water intermittently to evaluate samples.

Statistical analysis

Experiments were laid in complete randomized design with three replications. Duncan's Multiple Range Test was used to determine significant differences. Significance was determined at $P \leq 0.05$.

RESULTS AND DISCUSSION

Color is an important characteristic of food. Based on the color of the food, first impressions are made (Mortensen, 2006; Rodriguez-Amaya, 2016). The use of colorants as additives for food and drinks is a significant factor to food manufacturer and consumer in determining the acceptability of foods (Muntean, 2005). The pigment suspension formulation derived from *karonda* 'CIAH Selection-1' had been christened as 'Lalima'. One ml of this pigment suspension formulation is sufficient to give pleasing red colour to one serving of any colourless beverage such as lemon based beverages (Fig. 2). Colorants derived from vegetables and fruits may impart nutritional value to the colored food item (Naderi *et al*., 2010) as they contain several pheolics. The phenolics contribute to the protection against degenerative diseases, and their effects on health have been mostly attributed to their antioxidant properties (Krishna *et al*., 2016). One serve of *karonda* derived pigment supplemented beverage may additionally contain H"2.0 mg *o*-dihydric phenol (pyro-catechol equivalent) and H"14.0 mg total phenols (gallic acid equivalent) over plain lemon *sherbet* or control. Several studies have emphasized that flavonoids can act as powerful antioxidants, even more so than can the traditional vitamins (Krishna *et al*., 2014; Sánchez-Salcedo *et al*., 2015). In the present study, the total flavonoids (15.78 mg) and flavanols (3.92 mg) contents were found to be significantly higher in 'Lalima' supplemented lemon *sherbet* than plain lemon *sherbet* (Table 1). Since, pigment suspension formulation did not have ascorbic acid, addition of 'Lalima' did not result in any increase in ascorbic acid content of supplemented *sherbet* (Table 1). Anthocyanins hold special importance as they have been reported to have high free radical scavenging activity and; hence, considered vital from the nutrition point of view (Sánchez-Salcedo *et al*., 2015; Chung *et al*., 2016). In the present study, 'Lalima' supplemented *sherbet* had 469.2 µg anthocyanin (cyaniding-3-glucoside equivalent), which imparted pleasing red colour to otherwise colourless lemon *sherbet*. Anthocyanins are responsible for pigments in nature, namely red, purple, violet and blue and this can be transposed to food when they are used as colorants. Their main applications are in soft drinks, confectionary products and fruit preparations (Baines and Seal, 2012).

The antioxidant capacity is governed by the different mechanisms of action of their antioxidant components (Donno *et al*., 2015); therefore, it is advisable to evaluate it by a variety of methods pertaining to the respective mechanisms (Krishna and Parashar, 2013). Consequently, FRAP and CUPRAC assays were used to assess the antioxidant activity of lemon *sherbets* (Table 1). A remarkably higher antioxidant activity was registered in the 'Lalima' supplemented *sherbet* with total antioxidant activities (FRAP; CUPRAC) to be H"317 and H"390 µM Trolox Equivalent, respectively. Edible grade dye and pigments from natural sources had previously been attempted by several workers in past e.g. from *Cucurbita pepo* (Muntean, 2005), marigold flower (Jothi, 2008), annatto (Chowdhury *et al*., 2010), paprika (Carocho *et al*., 2015) and *Hylocereus polyrhizus* (Naderi *et al*., 2010); however, ours could be the first such study on *karonda*.

'Lalima' supplemented lemon *sherbet* was also subjected to sensory evaluation. Results of the trained sensory panel are presented in Table 2. Feedback collected from the trained panel revealed significant (P <0.05) difference in appearance and colour upon sensory evaluation. Based on ranking, 'Lalima' supplemented lemon *sherbet* was found to be more acceptable (P <0.05) in overall appearance and colour (Fig. 2) than plain lemon *sherbet*. Addition of 'Lalima' does not affect flavour and taste of the *sherbet*. However, it improved the appearance of lemon *sherbet*. The higher acceptance of 'Lalima' supplemented lemon *sherbet* among testers, in comparison to plain lemon *sherbet*, could be owed remarkably to its attractive red colour and appearance. Krishna *et al*. (2014) also observed that plain *Rhododendron* squash scored better in terms of colour and appearance over ginger-blended squash owing to its appealing red colour.

Figure 1. Transition of *karonda* 'CIAH Selection-1' fruit colour at different fruit maturity stages

Figure 2. Lemon *sherbets* (from left to right: plain *sherbet* and 'Lalima' supplemented *sherbet*; Inset: 'Lalima').

Table 1: Antioxidant properties of lemon *sherbet* per serve (100 ml)

S. No.	Antioxidant attributes	Beverage		SEm±
		Lemon *Sherbet*	Supplemented *Sherbet*	
1.	Ascorbic acid (mg100 ml^{-1})	3.93a	3.88a	0.001
2.	Total phenolics (mg 100 ml^{-1})	4.61a	18.79b	0.004
3.	*o*-dihydric phenol (mg100 ml^{-1})	0.26a	2.29b	0.002
4.	Total flavonoids (mg100 ml^{-1})	2.94a	15.70b	0.046
5.	Flavanol (mg100 ml^{-1})	0.39a	4.12b	0.003
6.	Total anthocyanin (mg l^{-1})	-	463.13b	5.121
7.	Total antioxidant activities (FRAP; µM TE 100 ml^{-1})	103.47a	482.65b	70.833
8.	Total antioxidant activities (CUPRAC; µM TE 100 ml^{-1})	126.37a	516.76b	50.667

*Row values followed by the same letter are not significantly different ($P < 0.05$).

Table 2: Sensory evaluation of plain and 'Lalima' supplemented lemon *sherbets*

Attributes	Score Plain	Supplemented	SEm±
Colour & appearence	12.1a	18.3b	0.040
Body/texture	16.4a	16.6a	0.025
Flavour	16.3a	16.2a	0.026
Taste	16.5a	16.1a	0.021
Overall quality	12.7a	18.5b	0.037
Total	74a	85.7b	4.000

*Values followed by the same letter are not significantly different ($P < 0.05$).

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