DEVELOPMENT OF A SUGAR FREE, NUTRA RICH CONFECTIONERY JELLY

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ABSTRACT
A nutra rich confectionery jelly was prepared using extracts of coleus aromaticus which is a source of natural colour, antioxidants for added nutra property. Fructooligosaccharides (FOS) a low-energy bulk sweetener was used to replace sugar. Freeze dried water extract of the herb Coleus aromaticus was added at 0.5, 1.0, and 1.5%. The effect of FOS along with the added herb on colour, texture and sensory quality of the product were studied and compared to that of sugar jelly. Storage studies indicated that the characteristic chewy texture of confectionery jelly was intact in the product prepared with FOS while that prepared with sugar gradually crystallized. Addition of the herbal powder showed DNA protecting activity at 1.5% level of incorporation. This study indicates the potential of the herb as a source of natural colour, antioxidant or nutraceutical that could be of use in Indian sweets with a potential application to reduce oxidative stress in living system.

Keywords: Confectionery jelly, DNA protecting activity, Antioxidant property.

INTRODUCTION
Confectionery jellies are prepared using hydrocolloids such as starches, pectins, gums, agar agar, gelatin etc, either alone or in combinations. Jellies have a unique texture ranging from firm and elastic to smooth and viscous. Bombay halwa/Karachi halwa is a popular Indian confection similar to confectionery jelly, made with wheat flour, sugar, ghee, flavoured with cardamom and garnished with nuts. The most desirable characteristics of a confectionery jelly are its translucency and elasticity (chewiness). However this confection contains a small amount of fat, added nuts, which adds to the taste and mouth feel. A simplified process for preparation of Bombay halwa has been reported by Jeyarani et al. (1996) using wheat, pregelatinized maize and waxy maize starches in presence of acid. They studied the changes in the physicochemical properties and concluded that certain extent of degradation of starch was necessary to get the desired texture and stability. This traditional confection was prepared using an alternative sweeteners FOS and an herb Coleus aromaticus.

Fructooligosaccharides (FOS) also sometimes called oligofructose or oligofructan and is used as an alternative sweetener. It occurs naturally, and its commercial use emerged in the 1980s in response to consumer demand for healthier and calorie-reduced foods. FOS resists hydrolysis by salivary and intestinal digestive enzymes. In the colon, they are fermented by anaerobic bacteria. In other words, they have a lower caloric value, while contributing to the dietary fiber fraction of the diet. FOS is more soluble than inulins and is, therefore, sometimes used as an additive to yoghurt and other products (Roberfroid MB, 1997, Gibson GR, Roberfroid MB, 1995). FOS has been used especially in combination with high-intensity artificial sweeteners, to improve sweetness profile and aftertaste. FOS also acts as a prebiotic by stimulating the growth of 'healthy' bacteria in the colon. FOS can be used for its nutritional advantages or technological properties, but it is often applied to offer a dual benefit: an improved organoleptic quality and a better-balanced nutritional composition. FOS has technical properties that are comparable to those of sugar and glucose syrups. It provides 30–50% sweetness compared with table sugar (Gibson GR, Roberfroid MB, 1995). These have been utilized in many food products.

Medicinal plants play a vital role to preserve human health. Herbs have been valued for centuries as a traditional medicine and have been used to treat various human ailments. The leaves of Coleus aromaticus are useful in anorexia, dyspepsia, flatulence, colic, diarrhoea, cholera, halitosis, convulsions, epilepsy, cough, asthma, hiccup, bronchitis, hepatopathy and malarial fever. Plants are good source of a wide variety of nutraceuticals, such as phenolic compounds, terpenoids, nitrogen containing compounds, vitamins, and secondary metabolities, which have antioxidant, antimicrobial, anti-inflammatory, antitumor, anti-mutagenic, anti-carcinogenic and diuretic activities (Coussement P, 1999).

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The use of medicinal plants to alleviate and cure illnesses has been prevalent since the early times.

_Coleus aromaticus_ is an edible, nutritive plant, which contains proteins, vitamins, minerals, soluble and insoluble dietary fibers etc, it is a good source of nutritious compounds and can be used as a food supplement. This plant also has chlorophyll, total xanthophylls, neoxanthin, violaxanthin, leutin and carotenoids (Coussenheim P, 1999, Khare RS, et al, 2011, Rout OP, et al, 2012). It is recorded in the Indian system of medicine as one of the sources of _Pashanabhedha_ (Rout OP, et al, 2012). The leaves of the green type of country borage are often eaten raw with bread and butter. The chopped leaves are also used as substitute for sage (_Salvia officinalis_ L) in stuffing. _Coleus aromaticus_ is used for seasoning meat dishes and in food products, while a decoction of its leaves is administered in cases of chronic cough and asthma. It is considered to be an antispasmodic, stimulant and is used for the treatment of headache, fever, epilepsy and dyspepsia. It is used to treat conditions such as indigestion, diarrhea, nervous tension, insect bites, toothache, earache, rheumatism, whooping cough, and bronchitis (Hebbani AV, et al, 2012). The plant also finds prominent importance in modern medicine (Chopra RN, et al, 1956, Warrier PK, et al, 1995).

Oxidation is essential to many living organisms for the production of energy necessary for biological processes. Oxygen-centered free radicals, also known as reactive oxygen species (ROS), including superoxide, hydrogen peroxide, hydroxyl (HO), peroxyl (ROO), and alkoxyl (RO), are produced in vivo during oxidation (Faleiro L, et al, 2005). ROS are not only strongly associated with lipid peroxidation, leading to food deterioration, but are also involved in development of a variety of diseases, including cellular aging, mutagenesis, carcinogenesis, coronary heart disease, diabetes, and neurodegeneration (Dragland S, et al, 2003, Blokhina O, et al, 2003). Biochemical reactions in the body generate reactive oxygen species which can damage important biomolecules, leading to several disease conditions. The harmful action of the free radicals can be blocked by antioxidants which scavenge the free radicals and nullify their damaging effect on cellular constituents. Although almost all organisms possess antioxidant defense and repair systems to protect against oxidative damage, these systems are insufficient to prevent the damage entirely (Halliwell B, Gutteridge JMC, 1999, Moskovitz J, et al, 2002). In certain biological conditions, free radical is the threatening factor and they are involved in cell apoptosis, by scavenging these radicals apoptosis can be minimized and DNA protection assay helps in evaluating the extent of damage.

Antioxidants are widely used as ingredients in dietary supplements to maintain health and preventing diseases, in addition to protect food against deterioration. But use of synthetic antioxidants is not safe due to their health risks and toxicity (Simic MG, 1988). The search for antioxidants from natural sources has received much attention to replace the synthetic ones have shown antioxidant activities of the ethanol extract of _Coleus aromaticus_ (Gülçin D, 2006). Phenolic and polyphenol constituents namely carvacrol, flavonoids, osmariacin acid, caffeic acid and chlorogenic acid are reported to be responsible for antioxidant activity of _Coleus aromaticus_. As this plant is a rich source of biologically active compounds with potential therapeutic values, it was incorporated into the confectionery jelly prepared with alternative prebiotic sweetener FOS for improved health benefits. In the present study this herb along with FOS was incorporated into confectionery jelly for improved health benefits.

**MATERIALS AND METHODS**

**PLANT MATERIAL, PREPARATION AND EXTRACTION PROCEDURES**

The leaves of _Coleus aromaticus_ were procured locally. Fresh leaves were washed, ground and the water extract was freeze dried in the institute using a lyophilizer (Lyophilization Inc USA, Model LT 5S) Fructooligosaccharide (FOS) was obtained from Sweetos, Pune, India. Confectionery jelly was prepared using sugar, corn starch and ghee. Indian Borage (_Coleus aromaticus_) powder, a natural colourant was incorporated at various concentrations (0, 0.5, 1.0, p1.5) on total product weight basis.

**PREPARATION OF CONFECTIONERY JELLY**

Confectionery jelly was prepared by heating sugar (100g) or FOS (125g) with water, a slurry of corn starch (25g) dispersed in water (100ml) along with tartaric acid (0.3g) was added to the sugar or FOS syrup. FOS had a TSS content of 80-82 °B and the commercial product had added sucralose for equi-sweetness level. This mixture was heated with continuous stirring to avoid lumps; ghee was added intermittently to prevent charring in the pan. The mixture was heated to about 120 °C or 80 °B the freeze dried herb was dispersed in 10 ml of alcohol and thoroughly mixed before pouring the mass into steel moulds (rings of 50mm dia and 15mm in height) to set. This addition was carried out at the end of cooking in order to prevent loss of any nutraceuticals. After cooling, the jelly was demoulded and packed in polypropylene pouches 150 microns at room temperature (27±2 °C) and used for further studies up to 15 days.

**EXTRACTION OF PHENOLICS FROM THE SAMPLES**

The samples were homogenized and phenolics extracted according to the method followed by (Kahl R, Kappus H, 1993). The herbal and jelly extracts and (in triplicate) were extracted (1:50, w/v) in 70% ethanol (3 X 50 ml, 2 h each), and the supernatants were obtained by centrifugation (Sigma 3-16K, USA) at 3000g for 15 min and concentrated by flash evaporation (Bucli 011, Switzerland); the pH was adjusted to 1.5 with 4 N
The color of confectionery jelly was measured with 10° view angle using CIELAB measuring system (Model, Lab scan, USA). The three parameters such as L*, a*, b*, ΔE and opacity were measured. L* represents lightness of the product, a*+ redness, -a* represents green, +b* yellow, -b* represents blue and ΔE represents the total color difference of the samples. Opacity of the confectionery jelly was measured to check translucency which is a desirable characteristic of a confectionery jelly and this is inversely proportional to opacity.

TEXTURE PROFILE ANALYSIS OF CONFECTIONERY JELLY

A texture analyzer (Model TA XT2i, Stable Micro Systems, and Surrey, England) was used to measure the texture of confectionery jelly. A plate (diameter 7.5 cm) was used to compress 50% of the confectionery jelly cube (10 mm × 10 mm × 10 mm) placed on a mounted fixed table. The load cell was calibrated with a 5 kg weight. The equipment was set to zero automatically lowering the plate until the bottom surface of plate just contacted the table.

SENSORY ANALYSIS OF CONFECTIONERY JELLY

Sensory evaluation was carried out with the help of a panel of 12 trained judges, following the method of quantitative descriptive analysis (Stone et al., 1974). Coded confectionery jelly samples were served, one at a time. The judges were asked to mark the perceived attributes such as colour, translucency, hardness, chewiness, sweetness, off taste and overall quality by drawing a vertical line on the scale.

STATISTICAL ANALYSIS

All analyses were carried out in triplicate. Data are expressed as means ± standard deviation. Duncan’s Multiple Range Test was applied to differentiate among the means of different groups. (p ≤ 0.05) (Duncan 1955) (Kumar SG, et al, 2006).

RESULTS AND DISCUSSION

PHYSICOCHEMICAL CHARACTERISTICS OF CONFECTIONERY JELLY

Chewy and cohesive texture is characteristic of confectionery jelly. It was observed that the confectionery jelly prepared with sugar had excellent chewy texture up to 5 days (163 N to 278 N) after which it was retained up to 10 days on storage. Fig 1 shows that after the 10th day confectionery jelly lost the characteristic chewy texture (93N) and crumbled. Confectionery jelly prepared with FOS showed similar characteristics but did not become crumbly after 10 days of storage (170 to 267 N); it retained the chewy texture (230 N) and was stable up to 15 days. Addition of the herb did not affect the textural characteristics of the product. Texture profile analysis (TPA) indicated that chewiness, the most desirable property in confectionery jelly increased significantly in

DNA PROTECTION ASSAY

The DNA protective effect of phenolic fractions was determined electrophoretically (Submarine electrophoresis system, Bio-rad, India) using calf thymus DNA (Molyneux P, 2004). Calf thymus DNA (1mg) was subjected to oxidation by Fenton’s reagent (30 mM H₂O₂, 50mM ascorbic acid and 80mM FeCl₃). Relative difference in the migration between the native and oxidized DNA was ensured on 1% agarose gel electrophoresis after staining with ethidium bromide.

EVALUATION OF ANTIOXIDANT POTENTIAL OF EXTRACTS

DPPH Radical Scavenging Assay or the free radical scavenging activity of the extract was measured in vitro by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay [18]. Various concentration of the extracts adjusted in a final volume of 2.5 ml were mixed with 5 ml of 0.1 mM DPPH solution. The tubes were shaken properly and incubated for 20 min in the dark. The changes in the absorbance of the samples were measured at 517 nm using a spectrophotometer. The DPPH radical scavenging activity was given as

\[ \% \text{ DPPH radical scavenging} = 100 \left( \frac{A_0 - A_t}{A_0} \right) \]

Where A₀ is absorbance of control and Aₜ is absorbance of sample. All samples were run in triplicate and radical scavenging activity was reported as mean ± SD (Palani S, et al, 2010, Subba Rao MV, and Muralikrishna G, 2002).

CHARACTERIZATION OF PHENOLICS BY HPLC

The active phenolic components of both herbal extract and confectionery jelly prepared with sugar and FOS were characterized by HPLC (model LC-10A, Shimadzu Corporation, Tokyo, Japan) analysis on a reverse phase Shimpak C18 column (4.6 X 250 mm) using a diode array UV-detector (operating at 280 nm). A solvent system consisting of water: acetic acid: methanol (isocratic; 80:5:15, v/v) was used as mobile phase at a flow rate of 1.0 mL/min. Phenolic acids such as gallic, protocatechuic, and chlorogenic acid were used for identification of phenolic components present.

ESTIMATION OF TOTAL PHENOLICS

Different concentrations of extracts were mixed with 1.0 ml of 10 fold diluted Folin-Ciocalteu reagent and 1 ml of saturated sodium carbonate solution. After allowing it to stand for 30 min at 30°C, the absorbance was measured at 765 nm using a UV-visible spectrophotometer (model UV 160, Shimadzu, Japan). Total phenolics were calculated using a standard Gallic acid curve and results were expressed as mg gallic acid equivalent/g of extract.

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INSTRUMENTAL COLOR MEASUREMENT

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FOS incorporated confectionery jelly when compared to control sugar based jelly. The instrumental colour measurement of the products indicated that lightness values \( L^* \) or lightness of both the products decreased with increasing herbal powder in the sample, along with \( b^* \) values i.e., yellowness as expected (Table 1). FOS being naturally light yellow in colour, the product prepared with it had higher \( b^* \) value of 16.3, when compared to the control sugar product (SHC) with a value of 10.9. \( \Delta E \), which represents the total colour difference increased with an increase in the herbal powder. Confectionery jelly has a characteristic translucent property and that prepared with FOS had the lowest opacity value of 89.8 indicating higher level of translucency, while that of control sugar had 93.9 higher than that of FOS and hence less translucent. The opaqueness of the product increased with increasing the level of added borage powder. At 1.5% level of addition of the herbal powder the product was opaque (Table 1).

### Table 1 Colour characteristics confectionery jelly prepared with sugar and FOS

<table>
<thead>
<tr>
<th><strong>HE</strong> (%)</th>
<th><strong>Instrument colour values</strong></th>
<th><strong>Confectionery Jelly</strong></th>
<th><strong>Opacity (Y)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar Jelly</td>
<td>0</td>
<td>40.95±0.12²</td>
<td>0.39±0.02³</td>
</tr>
<tr>
<td>0.5</td>
<td>29.13±0.72d</td>
<td>0.83±0.041e</td>
<td>10.78±0.64a</td>
</tr>
<tr>
<td>1.0</td>
<td>27.49±0.66f</td>
<td>1.07±0.11f</td>
<td>10.81±0.73a</td>
</tr>
<tr>
<td>1.5</td>
<td>22.90±0.91d</td>
<td>0.34±0.02d</td>
<td>10.57±0.82a</td>
</tr>
<tr>
<td>FOS Jelly</td>
<td>0</td>
<td>41.24±1.02²</td>
<td>0.32±0.01b</td>
</tr>
<tr>
<td>0.5</td>
<td>27.60±0.076e</td>
<td>0.36±0.01bc</td>
<td>19.33±0.89d</td>
</tr>
<tr>
<td>1.0</td>
<td>23.12±0.18b</td>
<td>0.22±0.02a</td>
<td>15.11±0.91bc</td>
</tr>
<tr>
<td>1.5</td>
<td>20.95±0.23e</td>
<td>0.34±0.01b</td>
<td>15.07±0.42b</td>
</tr>
</tbody>
</table>

**HE - Herbal extracts**

Means followed by the same superscripts in a column are not significantly different at \( p \leq 0.05 \) (n=3)

### TOTAL PHENOLIC CONTENTS OF BOTH HERBAL POWDER AND CONFECTIONERY JELLY

The amount of total phenolics, measured by Folin–Ciocalteu method, is expressed as GAE/100 g. The herbal extract showed the highest value of 243 mg/100g, while that added to confectionery jelly (with sugar) at 0.5, 1.0 and 1.5% were 6.9, 9.05 and 10.47 respectively. Changing the sweetener to FOS did not show significant differences in the total polyphenol content. Jelly prepared with FOS at same levels of addition showed 6.67, 9.11 and 10.76 mg/100g (Table 2). The values were similar that of the phenols extracted from jelly made with sugar.

### Table 2 Total polyphenol content of confectionery jelly prepared with sugar and FOS

<table>
<thead>
<tr>
<th><strong>HE</strong> (%)</th>
<th><strong>Gallic acid (mg/100g)</strong></th>
<th><strong>Protocatechin acid (mg/100g)</strong></th>
<th><strong>Chlorogenic acid (mg/100g)</strong></th>
<th><strong>Total Polyphenols (mg/100g)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar Jelly</td>
<td>0</td>
<td>4421.2 ± 5.19²</td>
<td>3960.5 ± 4.57²</td>
<td>7320.1 ± 5.21²</td>
</tr>
<tr>
<td>0.5</td>
<td>57.75 ± 3.22³</td>
<td>33.05 ± 3.67³</td>
<td>54.25 ± 3.15³</td>
<td>6.90 ± 3.60³</td>
</tr>
<tr>
<td>1.0</td>
<td>116.1 ±3.47⁴</td>
<td>41.91 ±3.31⁴</td>
<td>82.19 ±3.64⁴</td>
<td>9.05 ± 3.52⁴</td>
</tr>
<tr>
<td>FOS Jelly</td>
<td>1.5</td>
<td>198.71 ±2.18⁵</td>
<td>171.39 ±2.95⁵</td>
<td>132.23 ±3.01⁵</td>
</tr>
<tr>
<td>0.5</td>
<td>60.27 ±2.93⁴</td>
<td>31.2 ±3.11⁴</td>
<td>52.6 ±2.57⁴</td>
<td>6.67 ±3.08⁴</td>
</tr>
<tr>
<td>1.0</td>
<td>118.3 ±3.12⁵</td>
<td>42.68 ±3.84⁵</td>
<td>83.37 ±3.62⁵</td>
<td>9.11 ±3.11⁵</td>
</tr>
<tr>
<td>1.5</td>
<td>204.19 ±2.96⁶</td>
<td>169.3 ±2.92⁶</td>
<td>138.2 ±3.18⁶</td>
<td>10.76 ±2.91⁶</td>
</tr>
</tbody>
</table>

**HE - Herbal extracts**

Means followed by the same superscripts in a column are not significantly different at \( p \leq 0.05 \) (n=3)

### ANTIOXIDANT POTENTIAL OF HERBAL EXTRACT IN CONFECTIONERY JELLY

DPPH is used widely to evaluate the antioxidant property of plant extracts (Kumar SG, et al, 2006, Rodrigues H, Akman SA, 1998). DPPH is a stable nitrogen-centered free radical, the color changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation. Substances, which are able to perform this reaction, can be considered as antioxidants and therefore radical scavengers (Stone H, et al, 1974).

The results in the Fig 2 shows that the IC\(_{50}\) values of herbal powder are extremely low indicating high potential of antioxidant activity. Addition of 0.5% of herbal extract in confectionery jelly showed the least activity while that at 1.5% level showed increased.

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activity (Fig.2). Similar trend was observed in both the confectionery jellies made with sugar and that prepared with FOS.

No significant differences were observed in the IC 50 values by changing the sweetener in the product. Kumaran et al. (Duncan DB, 1955) have reported that the leaf extract Indian Borage (Coleus aromaticus) exhibited good antioxidant potency and antibacterial activities with significant reducing power, superoxide scavenging ability, nitric oxide scavenging and also ferrous ion chelating ability.

Figure 1 Texture (Chewiness) of confectionery jelly prepared with sugar and FOS

Figure 2 IC 50 Values of confectionery jelly with added herbal powder

*HE = Herbal extract; Sug = Sugar; FOS = Fructo-oligosaccharide

Figure 3 DNA protection ability of A- Herbal extracts (HE) and B- added Coleus aromaticus in confectionery jelly at 0.5, 1.0 and 1.5% levels

Sensory analysis revealed that the confectionery jelly prepared with sugar showed that it was highly acceptable up to 5 days but after that it lost its characteristic chewiness and translucent appearance (Table 3) also observed in the instrumental colour measurements. Similar results were observed in the confectionery jelly prepared with added herbal powder at all three levels, the confectionery jelly at 0.5% and 1.0% levels were more acceptable. At 1.5% level of addition the characteristic herbal flavour was slightly more and thus decreased the overall acceptability score, which was 10 and 8 at lower levels of addition (Table 3). Chewiness decreased with an increase in the days of storage in products prepared with sugar this could be attributed to crystallization of sugar on storage. It was also observed that there was a slight decrease in the sweetness perception with an increase in the level of addition of the herb at 1.5% level; this is mainly due to the strong characteristic flavour of the herb. After 10 days of storage, the products prepared with sugar broke into pieces, losing its characteristic texture. Thus confectionery jelly prepared with sugar was stable and acceptable only up to 5 days after which the textural qualities declined. The confectionery jelly with added herbal extract of 0.5% and 1% were highly acceptable, whereas confectionery jelly with extract added at 1.5% had strong flavour of Coleus aromaticus and hence reduced overall acceptability.

Sensory analysis of confectionery jelly prepared with FOS (Table 4) showed higher overall acceptability score when compared to that of sugar (13.0 and 12.5). No significant difference in chewiness was observed on storage indicating that they were acceptable up to 10 days of storage. The products were analysed after 15 days also and found only slight differences in sensory quality. Marginal changes were observed in the chewiness and hardness of the product on the 10th day. The results of the sensory study showed that 1.0% level of addition of herb powder was acceptable and also that FOS confectionery jelly scored higher than above that of the control confectionery jelly prepared with sugar in all sensory attributes. Confectionery jelly with FOS was found to be more acceptable than that prepared with sugar; the product with FOS also scored higher with respect to other attributes such as translucency, hardness, chewiness and springiness thus higher OQ score.

**DNA PROTECTION ASSAY**

Recent research focuses on various strategies to protect crucial DNA from oxidative damage induced by free radicals. This assay was based on the ability of extracts to protect DNA against damage caused by hydroxyl (‘OH) radicals. Hydroxyl radicals generated by the Fenton reaction are known to cause oxidative induced breaks in DNA strands. Exposure of DNA to Fenton’s reagent ultimately results in strand breaks, mainly due to the generation of reactive species-hydroxyl radical and the subsequent free radical-induced reaction on DNA. Hydroxyl radicals react with nitrogenous bases of DNA producing base radicals and sugar radicals. The base radicals in turn react with the sugar moiety causing breakage of sugar phosphate backbone of nucleic acid, resulting in strand break (Ebrahimzadeh MA, et al, 2008). In most metabolic disorders generation of free radical is high leading to pathophysiological conditions which can be reduced so by intervention of bioactive compounds in

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CONCLUSIONS

As the demand for nutraceutical rich foods is increasing at a fast pace one of the popular traditional sweet confectionery jelly was prepared using prebiotic FOS and further enriched with a herb rich in polyphenols, antioxidants and carotenoids to provide improved the health benefits. Texture profile analysis (TPA) indicated that chewiness, the most desirable sensory property of confectionery jelly increased significantly in FOS confectionery jelly compared to that of sugar confectionery jelly. The colour of the products prepared with the herb was darker due to the natural colour. Sensory analysis indicated that FOS confectionery jelly can be stored up to 10 days without losing its desirable “chewiness” property.

The confectionery jelly prepared with addition of herbal powder was thus enriched with polyphenols and antioxidants, indicating the potential of the herb *coleus aromaticus* extract as a source of natural colour, antioxidants or nutraceuticals that could be of use in confectionery and traditional Indian confections with a potential application to reduce oxidative stress in living system.

REFERENCES


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