

RESEARCH ARTICLE

Extraction and process optimization of East Indian Red Wood (*Caesalpinia sappan*) in stirred yoghurt

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ABSTRACT

Yoghurt is a fermented dairy product, having several health benefits. Redwood sappan wood (*Caesalpinia sappan*) is a plant yielding bark and stem with water soluble red pigment. Two methods of colour extraction were carried out, namely, Heat Extraction (extraction by aqueous & alcohol) and Cold Extraction (Ambient temperature & Refrigerated temperature) using five different medium such as distilled water, potable water, sodium carbonate solution, sodium hydroxide solution and ethyl alcohol. Among the mediums tried, potable water was found to be the best medium for extraction. The bark was heated in potable water for 2.5 h. The heat extracts had a pleasing pink to red colour. The colour of the extract was pH dependent, it was yellowish to brown at below neutral and pink to dark red at above neutral pH. Stirred type of yoghurt was prepared by standard method using cow milk. Redwood extract level was optimized at three levels viz. 10 mL, 20 mL and 30 mL with skim milk powder @ 40 gm and sugar 20 gm per 100 mL of yoghurt. Redwood extract 20 mL optimized based on 9-point Hedonic scale, textural parameter and storage stability of colour extraction up to 7 days. Physico-chemical analysis of the redwood added stirred yoghurt product such as pH, acidity(%), Lactose (%), Sucrose (%), Protein (%), Ash (%), Fat (%) values are 4.6±0.01, 1.02±0.00, 5.76±0.01, 20.44±0.02, 3.89±0.01, 1.10±0.01 and 2.49±0.10. respectively.

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INTRODUCTION

In today's world, consumers become more health conscious, their demands for functional dairy products are increasing rapidly. Functional dairy products occupy a significant space in the functional food market (Du *et al.*, 2021).

Yogurt, the most popular functional and fermented dairy product consumed worldwide, contains nutritive functions and beneficial actions in the reduction of gastrointestinal discomfort, eliminating symptoms of lactose intolerance, strengthening the immune system, protection against colon cancer and *Helicobacter pylori* infection, and elevating metabolic rates, thereby presenting it as an essential ingredient of healthy diets (Helal *et al.*, 2022).

East Indian Redwood or Sappan wood (*Caesalpinia sappan*) is a natural dye yielding medicinal plant. In India, it is cultivated in gardens and nurseries as a live fence plant in parts of Tamil Nadu, Kerala, Karnataka, Andhra Pradesh, and West Bengal. In traditional Chinese medicine, sappan wood has sweet, salty, and neutral properties, and is associated with the Heart, Liver and Spleen meridians. Its main functions are to invigorate the blood, promote menstruation, and reduce pain and swelling. The bark and pods of the tree contain a red dye, which is used for colouring cakes and alcoholic drinks. In addition, the wood contains several medicinal properties and is used for treating

dysentery, diarrhea, and skin diseases in alternative systems of medicine (Badami *et al.*, 2003).

The yogurt market is projected to generate revenue of USD 181.30 billion in 2024, with an annual growth rate of 6.83% (Compound Annual Growth Rate) expected from 2024 to 2029 (Statistics, 2024). Considering the rising popularity of yogurt and the proven nutritional benefits associated with it, it was decided to add natural redwood extract as a coloring agent in plain stirred yogurt.

MATERIALS AND METHODS

Materials

Cow milk (3% fat) was procured from Institute Cattle Yard / NDRI Milk Parlour and Homogenised milk was procured from Nandini milk parlour (KMF), Yoghurt culture (a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus*,) in 1:1 ratio collected from the Dairy Chemistry & Bacteriology Section of the Institute. Sugar and skim milk powder were purchased from a local supermarket. East Indian Redwood (*Caesalpinia sappan*) procured from medical shop, Trivandrum. The reagents were of analytical grade or authentic standard chemicals.

Redwood Extraction

Extraction of colour from east Indian redwood

The bark was chopped into small pieces and colour was extracted from them using five different mediums viz. Distilled water, Potable water, Sodium hydroxide solution, Sodium carbonate solution and Ethyl alcohol (Kandasamy *et al.*, 2023). Two methods of extraction were carried out, namely, Heat Extraction (extraction by aqueous & alcohol) and Cold Extraction (Ambient temperature & Refrigerated temperature).

The colour extracts were packed in oxygen and light proof packaging material of LDPE and laminated pouch for conducting storage studies and the colour intensity (Fig.1) was checked on 0th day and at the end

of 10th days at 5°C.

Analysis of redwood stirred yoghurt

pH

The electrode assembly of a digital pH meter (Digisun Electronics, Hyderabad, Model: DI 707) was calibrated against a standard buffer of pH 7.0 and 4.0 (Qualigens Fine Chemicals). Then the pH of the samples was determined using the calibrated digital pH meter at 20 °C. (AOAC, 2006).

Acidity

The titratable acidity of yoghurt was determined by titrating 10g of samples against standard alkali.

Colour measurement

The extent of extraction of the colour was monitored by measuring the reflectance using Reflectance meter and colour parameters of the extracts by Adobe Photoshop-Scanner method.

Physico chemical characteristics

Physico chemical characteristics of redwood stirred yoghurt such as Lactose (%), Sucrose (%), Fat (%), Ash (%) and Protein (%) were analyzed (AOAC, 2000).

Sensory evaluation

A panel of 10 semi-trained judges carried out sensory evaluation of the samples. The samples for analysis were presented before the judges after suitable marking. The judges were provided with a room with good lighting and appropriate facilities. The selection of optimum product from all the treatments was done based on the sensory scores obtained. An expert panel of judges evaluated it on 9 point hedonic scale where a score of nine represented “like extremely” and a score of 1 represented “dislike extremely”. The parameters judged were colour and appearance, flavour, body & texture, sweetness and overall acceptability (Amerine *et al.* 1965)

Fig. 1. East Indian redwood and their colour extracts



Texture Analyser

Texture Analyser (TA-XT plus, Stable Micro Systems, Surrey, UK) measured the firmness, consistency, and adhesive force of yogurt. The p/25 aluminium cylindrical probe was used for texture analysis.

Statistical analysis

Data obtained from various experiments during standardization and storage study was statistically analyzed by analysis of variance, employing appropriate computer package analyzed by analysis of variance, employing appropriate computer packages, MS-Office 2007 (MS-Excel), and SPSS Software. Results were analyzed using complete randomized design to test the statistical significance as per Snedecor and Cochran (1994)

Plain stirred yogurt preparation

The cow milk (500 mL) was pasteurized at 85 °C for 15min. Skim milk powder (40g) was added into the pasteurized milk to increase the viscosity of the

milk and cooled to 40 °C and 1% of the yogurt culture was inoculated and incubated for 4hr 30min (Geetha, 2024). Once the yoghurt is set, 100g of yoghurt was taken and 20g of sugar added the entire content was mixed thoroughly and then extract of redwood was incorporated at three levels viz. 10, 20 and 30 mL (Fig.2).

RESULT AND DISCUSSION

Evaluation studies of redwood colour extract:

Effect of type of medium of extraction

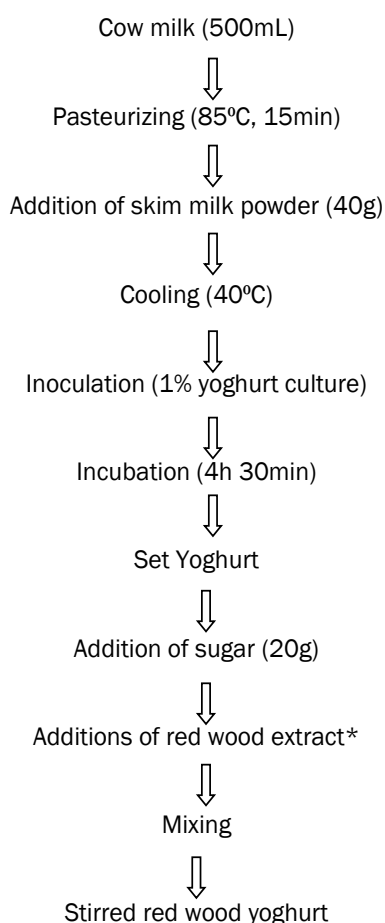
Two methods of extraction namely heat extraction and cold extraction with different timings and temperature and samples were drawn at regular intervals . It was observed that as the extraction time increased, the amount of colour extracted also increased. This was measured by a Reflectance meter. Five mediums were tried for colour extraction viz. distilled water, potable water, sodium carbonate solution, sodium hydroxide solution and ethyl alcohol.

The results of reflectance % of samples showed decreasing trend as shown in Fig.3 (heat extraction) and Fig.4 (cold extraction). In both cases, the initial reflectance of the solution was 100%, which rapidly decreased to below 70 % after 30 min. However, this decrease took place to a lesser extent in cold extraction . This indicates that the colour compound from the bark readily migrated from the interiors part to aqueous medium due to its nature of water solubility. Migration of colour is attributed due to presence of certain dissolved salts in water. After 30 min, the rate of colour migration came down and remains constant thereafter. Results indicated that the samples of potable water extracts displayed least reflectance values indicating more colour extraction into it (100°C for 2hr 30min), followed by sodium carbonate, distilled water and sodium hydroxide (Table 1 and 2). Alkaline medium showed no advantage over the neutral medium. Hence, potable water extraction up to 2hr 30 min is recommended because it is practically easy to use potable water.

Effect of pH on the colour expression

The effect of pH on the red wood colour was checked by adjusting the pH of the extracts using NaOH or citric acid. It was observed that the colour of the redwood extract was pH-dependent, being

Fig.2. Flow chart for preparation of redwood stirred yoghurt



*Parameters optimized



Table 1: Heat extraction of redwood bark colour extracts

Time (min)	POTABLE WATER	DISTILLED WATER	SODIUM HYDROXIDE- DW	SODIUM CARBONATE- DW	ALCOHOL
0	-	-	-	-	-
30					
50					
1.10					
1.30					
1.50					
2.10					
2.20					
2.30					

Fig. 3. Colour extracted from Red wood by Heat Extraction

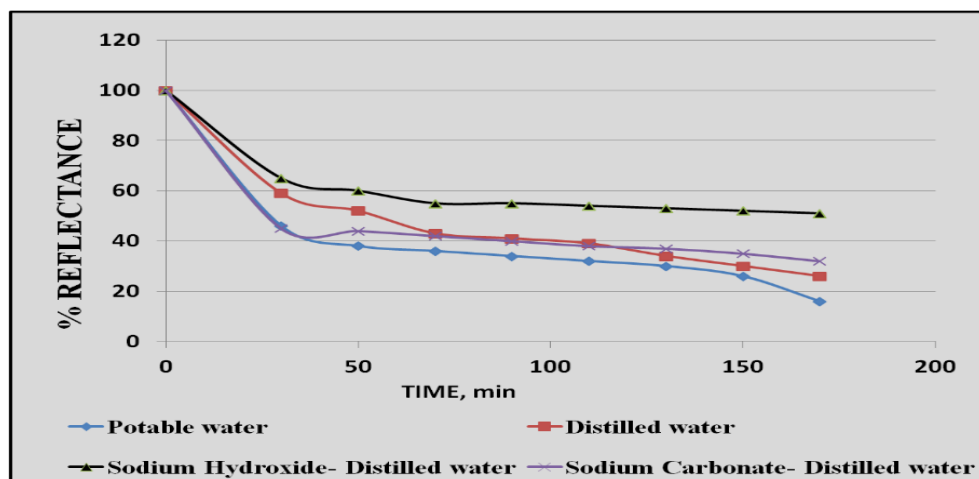


Fig 4. Colour extracted from Red wood by Cold Extraction

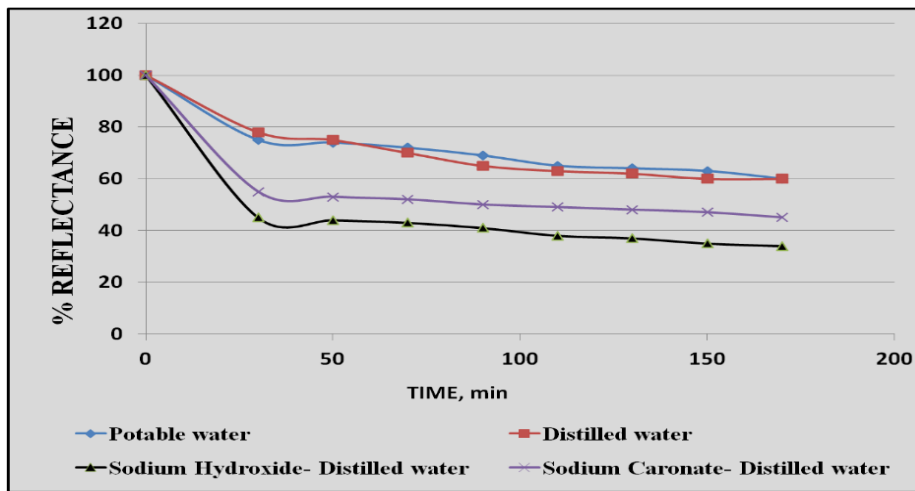

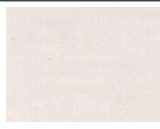
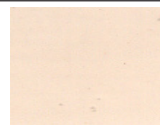





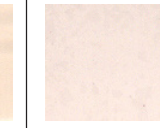

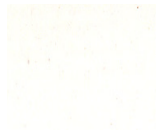




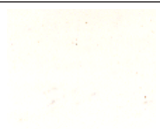
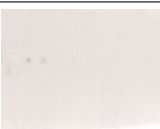



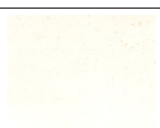
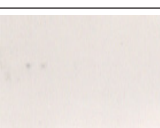

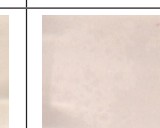
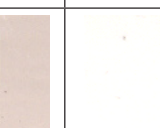

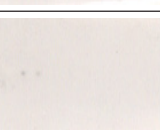
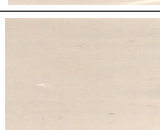

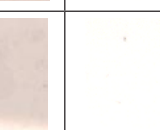

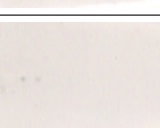
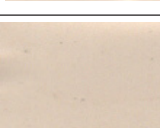

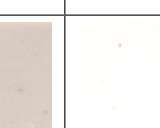


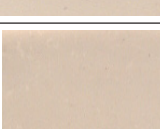
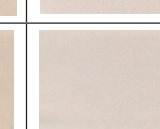



Table 2. Cold extraction of red wood bark colour extracts at ambient temperature

Time (min)	POTABLE WATER	DISTILLED WATER	SODIUM HYDROXIDE-DW	SODIUM CARBONATE-DW	ALCOHOL
0	-	-	-	-	-
30					
50					
1.10					
1.30					
1.50					
2.10					
2.20					
2.30					

intense red at higher pH levels and intense yellow at lower pH levels. From Fig.5, it may be noted that the reflectance value of the colour solution decreased as the pH increased. It indicates that as pH increased the solution darkened, though colour changed gradually from yellowish to dark red. At lower pH values below 7.0 the extract showed yellow to brown colour and above pH 7.0 the extracts turned pink to dark red. At pH 9.87, the solution became so dark red that it appeared deep purple (Fig. 6).

Redwood coloured stirred yoghurt

Redwood extract was used as colouring agent in the preparation of plain stirred yoghurt. Various parameters of yoghurt for the preparation of redwood coloured yoghurt were standardized.

Effect of redwood extract level on the sensory redwood stirred yoghurt

On incorporation of the extract, the yoghurt attained a light yellow colour whose darkness increased with increased incorporation level of the extract (Fig.7). The colour and appearance scores were 7.33, 7.73 and 7.53 for the extract levels of 10, 20 and 30 mL per 100 g yoghurt (Fig.8).

However, body and texture and flavor scores decreased as observed from the table. This may be attributed to the slight dilution caused by the addition of an increased amount of color. This trend was reflected in overall acceptance also. The sweetness also slightly decreased by increasing the extract level. However, in this study the primary aim was to enhance

Fig 5. Effect of pH and Reflectance

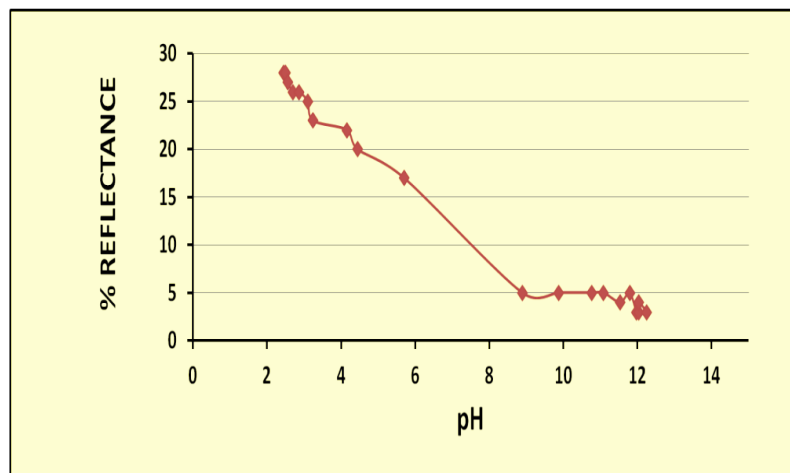


Fig 6. Redwood colour extract by using different medium

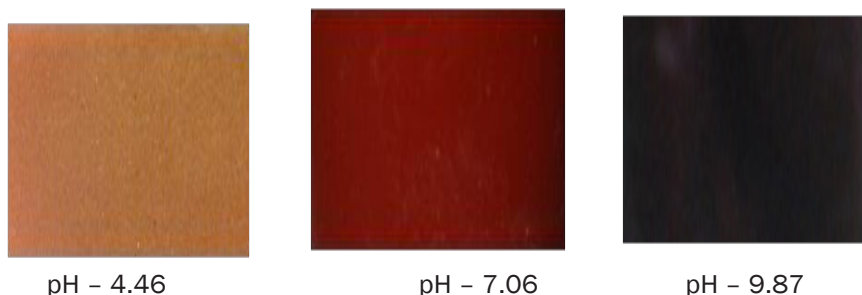
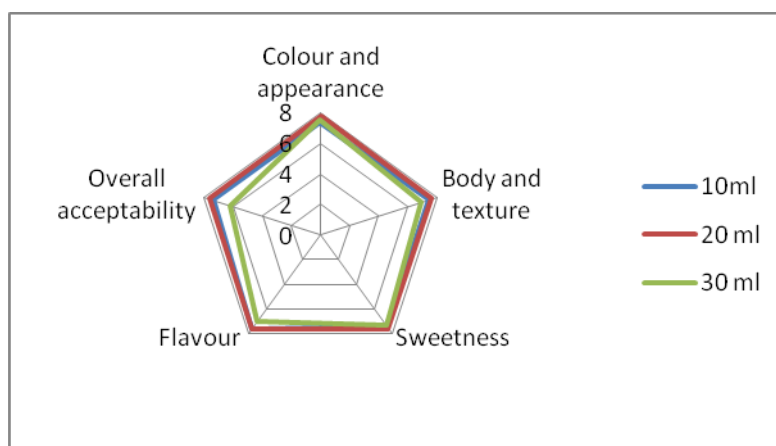


Fig. 7. Stirred yoghurt added with different levels of redwood extract



Fig.8. Effect of redwood extract level on sensory quality of plain stirred yoghurt



the colour, which was imparted successfully to yoghurt by addition of 10 mL of extract. This level of extract was used for subsequent trials. Statistical analysis indicated a significant effect of incorporation of extract on the quality attributes of stirred yoghurt ($P < 0.05$). However, all the yoghurt samples were sensorial acceptable. The colour component of redwood was identified as Brazilien that is water soluble (Lioe, *et al.*, 2012). It has dissolved in water in yoghurt and imparted yellowish colour to the product.

Effect of redwood extract level on the textural redwood stirred yoghurt

The textural quality results of yoghurt were presented in Table 3. The yoghurt with 10 mL extract had acceptable firmness of 19.42 g, consistency 111.66g.sec and adhesive force of 9.78 g whereas the value of textural quality was decreased to 16.93g, 81.59g.sec and 5.62g respectively when 20 mL extract was used. As can be seen from the Table 3, these values further decreased when 30 mL of the extract was used as colouring agent. This decrease could be attributed to the dilution factor. ANOVA indicated a statistically significant effect of addition of extract on the textural attributes of yoghurt ($P < 0.05$).

Storage stability of redwood colour added stirred yoghurt

The natural colours gradually fade during storage whose rate depends on a number of factors. In yoghurt, the stability of redwood colour was checked by adding the colour at three levels and storing the product at refrigerated temperature. Since the colour intensity depended on pH, this parameter was also measured. The colour of surface of yoghurt was measured by reflectance value every day. The results were presented in Table 4. The study was limited to one week because yoghurt is normally stored up to 7 days at refrigerated temperatures. It was observed that the reflectance of the yoghurt increased during storage, from 95 to 99 for 10 mL level, 89 to 97 for 20 mL and from 87 to 96% for 30 mL level of the extract. It shows that higher the concentration, higher was the fading of colour. The increase in reflectance value indicated fading of colour. This could be attributed possibly to structural changes or oxidation of the pigments. It is known that the natural colours are good antioxidants, so they get oxidized themselves resulting in lightening of colour intensity (Rosenberg, 2008).

Table 3: Effect of redwood extract level on textural quality of plain stirred yoghurt

Textural attribute	Level of redwood extract, mL per 100g yoghurt		
	10	20	30
Firmness, g	19.42±0.57 ^c	16.93±1.83 ^b	16.14±2.24 ^a
Consistency, g.sec	111.66±2.58 ^c	81.59±3.75 ^b	70.42±3.74 ^a
Adhesive force, g	-9.78±0.26 ^a	-5.62±0.39 ^b	-5.35±0.26 ^c

Note: Means with different superscripts in a row are significantly different $P < 0.05$



Table 4. Changes in colour of redwood added stirred yoghurt during storage

DAY à	0	1	2	3	4	5	6	7
Extract vol. 10 mL								
pH	4.50	4.32	4.33	4.27	4.22	4.21	4.20	4.19
Reflectance, %	95	97	97	98	98	98	98	99
Colour								
Extract vol. 20 mL								
pH	4.45	4.26	4.25	4.25	4.23	4.22	4.20	4.15
Reflectance, %	89	90	90	93	96	96	97	97
Colour								
Extract vol. 30 mL								
pH	4.50	4.42	4.42	4.41	4.39	4.30	4.28	4.25
Reflectance, %	87	89	92	94	94	96	96	96
Colour								

Table 5. Physico-chemical characteristics of redwood colour incorporated yoghurt

Particulars	Control yoghurt	Redwood yoghurt	t test
pH	4.49±0.02	4.6±0.01	3.628**
Acidity (%)	1.0±0.01	1.02±0.00	1.177 ^{NS}
Lactose (%)	5.54±0.08	5.76±0.01	14.982**
Sucrose (%)	20.48±0.02	20.44±0.02	2.055 ^{NS}
Fat (%)	2.3±0.11	2.49±0.10	10.597**
Ash (%)	1.0±0.008	1.10±0.01	6.932**
Protein (%)	3.41±0.05	3.89±0.01	31.127**

Physico-chemical characteristics of redwood colour incorporated yoghurt

Physico-chemical characteristics of redwood flavoured yoghurt and control yoghurt was presented in Table 5. It may be noted that there is no influence of redwood extract on the compositional and other attributes of the yoghurt.

CONCLUSION

The East Indian red wood extract was successfully extracted by using heat extraction method using potable water. The colour extracts were sensitive to

light and oxygen, so recommended to be stored in oxygen and light-proof packaging materials. Based on sensory, textural attributes and storage studies, 20 mL of redwood extract was optimized and incorporated to stirred yoghurt. The redwood extract incorporated stirred yoghurt samples shown no effect on pH, acidity, lactose, sucrose, protein, ash, and fat content. It was observed that the reflectance of the yoghurt increased during storage of 7 days at refrigerated temperatures. This study highlights the potential of natural plant-



based colorants like East Indian Red wood extract in developing value-added dairy products with extended shelf life and consumer acceptability.

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