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Studies on Physico-chemical properties of flavoured cow milk blended with annatto (*Bixa orellana*) seed extract as a colouring agent

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Abstract

The present study evaluated the physico-chemical properties of flavoured cow milk prepared with different levels of annatto (*Bixa orellana*) seed extract. The study was conducted during 2025-26 at the Department of Animal Husbandry and Dairy Science, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. Five treatments were used: T₁ (0%), T₂ (3%), T₃ (5%), T₄ (7%) and T₅ (9%) annatto seed extract, with 8 per cent sugar constant. The experiment was conducted in Completely Randomized Design with four replications. Fat, protein, total solids, moisture, total sugar and ash were analysed. Fat, protein, total solids and total sugar decreased gradually with increasing extract level, while moisture and ash increased. Treatment T₄ (7%) showed a desirable physico-chemical composition and was considered the most suitable level under the conditions of the study.

Keywords: Annatto, flavoured milk, colouring agent

Introduction

Milk is a nutritious food and is widely consumed. Flavoured milk is a popular value-added dairy product prepared by adding sugar and suitable flavouring ingredients to milk. It provides the nutritional benefits of milk along with better taste and variety. Aneja *et al.* (2002) ^[1] reported the importance of flavoured milk as a value-added dairy product.

There is increasing interest in the use of natural ingredients in food products. Annatto obtained from the seeds of *Bixa orellana* is an important natural colouring material used in food products. Levy and Rivadeneira (2000) ^[2] reported the use of annatto as a natural food colour in different food applications. Hirko and Getu (2022) ^[3] also described annatto as a useful source of natural colouring material.

The addition of plant-based extracts may affect the chemical composition of milk products. The level of extract added can influence the amounts of fat, protein, total solids, moisture, total sugar and ash in the final product. De Souza *et al.* (2019) ^[4] reported the use of water-based extraction for obtaining colouring material from *Bixa orellana* seeds.

Several studies have reported changes in the physico-chemical properties of flavoured milk after adding natural ingredients. Repate *et al.* (2010) ^[5] studied flavoured milk prepared from cow milk and safflower milk, while Dhande *et al.* (2016) ^[6] studied lemongrass flavoured milk.

Therefore, the present study was undertaken to evaluate the effect of different levels of annatto seed extract on the physico-chemical properties of flavoured cow milk.

Materials and Methods

The investigation was conducted during 2025-26 at the Department of Animal Husbandry and Dairy Science, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra. Fresh and clean cow milk was collected from the Livestock Instructional Farm of the department. Annatto seeds were procured from the local market at Akola. Cane sugar was used at 8 per cent in all treatments and cardamom was kept constant. The experiment consisted of five treatments and four replications in Completely Randomized Design (CRD). Preliminary

trials were conducted to select suitable levels of annatto seed extract for preparation of flavoured cow milk.

Preparation of Annatto Seed Extract

Annatto seeds were cleaned and dried before extraction. The cleaned seeds were mixed with distilled water at a ratio of 1:10 (w/v). The mixture was heated at 70-90 °C for 30-60 minutes with stirring. After extraction, the mixture was cooled and filtered to remove the seed residues. The filtered aqueous annatto seed extract was collected and used as a natural colouring agent in the preparation of flavoured cow milk.

Flow Chart for Preparation of Annatto Seed Extract

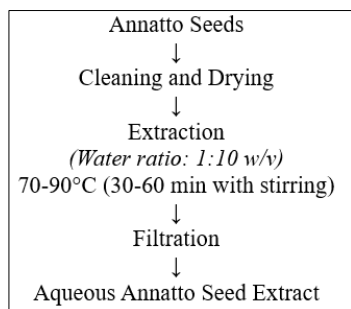


Fig 1: Flow diagram of annatto seed extraction

Treatment Details

Five treatments were used in the experiment.

Treatment	Treatment details
T ₁	Sweet milk (Control)
T ₂	T ₁ + annatto seed extract @ 3%
T ₃	T ₁ + annatto seed extract @ 5%
T ₄	T ₁ + annatto seed extract @ 7%
T ₅	T ₁ + annatto seed extract @ 9%

Sugar was added at 8 per cent in all treatments was kept constant.

Preparation of Flavoured Cow Milk

Fresh cow milk containing approximately 4.0 per cent fat was pre-heated to 35-40 °C and filtered. Sugar was added at 8 per cent and mixed thoroughly until completely dissolved. The milk was then blended properly and pasteurized at 72 °C for 15 seconds. After pasteurization, the milk was cooled to room temperature. Annatto seed extract was then added according to the respective treatment level and mixed thoroughly for uniform distribution. The prepared flavoured milk was filled into 200 ml bottles, sealed and stored under refrigerated conditions for further analysis.

Flow Chart for Preparation of Flavoured Cow Milk

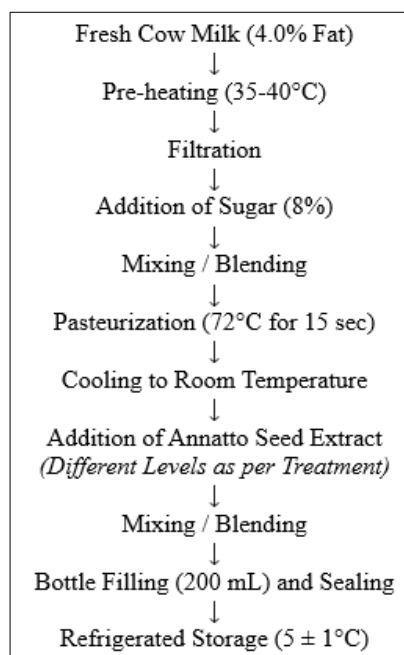


Fig 2: Flow diagram for preparation of flavoured milk with addition of different levels of aqueous annatto seed extract (Adapted from Aneja *et al.*, 2002, with slight modifications)

Chemical Analysis

The prepared flavoured cow milk samples were analysed for fat, protein, total solids, moisture, total sugar and ash. Fat content was determined by the Gerber method as described in IS:1224 (Part I), 1977 [7]. Total solids were determined by the gravimetric method according to IS:1479 (Part II), 1961 [8]. Protein was estimated by determining nitrogen using the Micro-Kjeldahl method. Ash content was determined according to the standard method described by A.O.A.C. (1995) [9]. Total sugar was determined according to the standard procedure used for milk products. Moisture content was determined from the total solids values.

Statistical Analysis

The experiment was conducted in Completely Randomized Design (CRD) with five treatments and four replications. The data obtained for different physico-chemical parameters were subjected to analysis of variance (ANOVA). Statistical analysis was carried out according to the procedure described by Panse and Sukhatme (1978) [10]. Treatment means were compared at the 5 per cent level of significance.

Results and Discussion

Physico-Chemical Properties of Flavoured Cow Milk

The mean values of fat, protein, total solids, moisture, total

sugar and ash of flavoured cow milk prepared with different levels of annatto seed extract are presented in Table 1.

Table 1: Physico-chemical properties of flavoured cow milk as affected by different levels of annatto seed extract

Treatment	Fat (%)	Protein (%)	Total solids (%)	Moisture (%)	Total sugar (%)	Ash (%)
T ₁ (Control)	3.68	3.24	20.02	79.98	12.48	0.61
T ₂ (3% A.S.E.)	3.56	3.14	19.92	80.08	12.39	0.67
T ₃ (5% A.S.E.)	3.48	3.08	19.85	80.15	12.33	0.73
T ₄ (7% A.S.E.)	3.40	3.02	19.79	80.21	12.26	0.78
T ₅ (9% A.S.E.)	3.32	2.96	19.71	80.29	12.21	0.82

A.S.E. = Annatto seed extract

The results indicated that incorporation of annatto seed extract influenced the physico-chemical composition of flavoured cow milk. Fat, protein, total solids and total sugar showed a decreasing trend, whereas moisture and ash showed an increasing trend with increasing level of annatto seed extract.

Fat

The fat content decreased gradually with increasing level of annatto seed extract. The highest fat content was recorded in T₁ (3.68%), followed by T₂ (3.56%), T₃ (3.48%), T₄ (3.40%) and T₅ (3.32%). Thus, the lowest fat content was recorded in T₅ containing 9 per cent annatto seed extract.

The decrease in fat content may be mainly due to the dilution effect caused by the addition of aqueous annatto seed extract and the corresponding reduction in the proportion of milk in the final product. Similar changes in the chemical composition of flavoured milk with the incorporation of plant-based ingredients have been reported by Dhande *et al.* (2016) [6].

Protein

Protein content also showed a gradual decreasing trend with increasing level of annatto seed extract. Protein content decreased from 3.24 per cent in T₁ to 3.14, 3.08, 3.02 and 2.96 per cent in T₂, T₃, T₄ and T₅ respectively.

The decrease may be associated with the replacement of a portion of milk with aqueous annatto seed extract, which resulted in a lower proportion of milk protein in the final product. The change was gradual as the level of extract increased.

Total Solids

Total solids showed a gradual decreasing trend with increasing levels of annatto seed extract. The highest total solids content was recorded in T₁ (20.02%), followed by T₂ (19.92%), T₃ (19.85%), T₄ (19.79%) and T₅ (19.71%).

The reduction in total solids may be due to the higher moisture content of the aqueous annatto seed extract. As the quantity of extract increased, a greater amount of water was incorporated into the product, resulting in a slight reduction in total solids.

Moisture

Moisture content increased gradually with increasing levels of annatto seed extract. The moisture content increased from 79.98 per cent in T₁ to 80.08, 80.15, 80.21 and 80.29 per cent in T₂, T₃, T₄ and T₅ respectively.

The increase in moisture may be attributed to the aqueous nature of the annatto seed extract. Higher levels of extract resulted in the incorporation of more water into the flavoured milk. The highest moisture content was observed in T₅ containing 9 per cent extract.

Total Sugar

Total sugar content showed a slight decreasing trend with increasing level of annatto seed extract. The total sugar content was highest in T₁ (12.48%) and decreased to 12.39, 12.33, 12.26 and 12.21 per cent in T₂, T₃, T₄ and T₅ respectively.

Although sugar was added at a constant level of 8 per cent in all treatments, the slight variation in total sugar may be associated with the replacement of a portion of milk by aqueous annatto seed extract. The decrease was small across the treatments.

Ash

Ash content showed an increasing trend with increasing level of annatto seed extract. The ash content increased from 0.61 per cent in T₁ to 0.67, 0.73, 0.78 and 0.82 per cent in T₂, T₃, T₄ and T₅ respectively.

The increase in ash content may be associated with the mineral constituents contributed by the annatto seed extract. The gradual increase observed with increasing extract level indicates that the incorporation of annatto seed extract influenced the mineral fraction of the flavoured milk.

Overall Physico-Chemical Performance

The incorporation of annatto seed extract resulted in gradual changes in the physico-chemical composition of flavoured cow milk. Fat, protein, total solids and total sugar decreased gradually, whereas moisture and ash increased with increasing levels of extract.

Among the treatments, T₄ containing 7 per cent annatto seed extract showed a desirable physico-chemical composition. It also corresponded with the treatment that showed the best overall sensory acceptance in the present investigation. Therefore, T₄ was considered the most suitable treatment under the conditions of the study.

Conclusion

The present study showed that annatto seed extract can be used as a natural colouring agent in the preparation of flavoured cow milk. Increasing the level of annatto seed extract resulted in a gradual decrease in fat, protein, total solids and total sugar, while moisture and ash increased. Among the treatments, T₄ containing 7 per cent annatto seed extract was considered the most suitable level based on its desirable physico-chemical composition and overall product performance. The use of annatto seed extract therefore provides a suitable approach for developing a naturally coloured value-added flavoured cow milk.

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