Development and Characterization of Biocolour Fortified Yogurt: A New Pathway towards Functional Foods

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Abstract: *At present, the demand for functional foods is increasing worldwide due to the increased awareness on therapeutic and medicinal properties and their benefits among public because they provide health benefits beyond the provision of essential nutrients. Numerous plant foods or physiologically active ingredients derived from plants have been investigated for their role in disease prevention and health. Natural dyes are those derived from plants, insects, animals and minerals. In this study, color extracted from purple cabbage was used for the development of fortified yogurt, since dairy products or the milk derived products can be considered as functional foods as they are health beneficial for human. The physiochemical and rheological properties of fortified and plain yogurt were checked. Plain yogurt is considered as control. The rheological properties were measured using rheometer which shows a similar flow behavior of Bingham plastic nature. The color stability in terms of anthocyanin content was determined at 560nm at different pH. The polyphenol content (in terms of mg GAE/ gm dry sample) of both fortified and control yogurt were determined spectrophotometrically which shows an increase in values of 1.78 times than control. Thus it can be concluded that the overall acceptability of fortified yogurt is better than the control in terms of sensory analysis, syneresis as well as functional properties.*

Keywords: *fortified yogurt, biocolour, purple cabbage, anthocyanin, functional foods.*

I. Introduction

Functional foods can be obtained in whole or fortified, which provide health benefits by enriching essential nutrients (e.g., vitamins and minerals).Various functional foods have the potential to perform role in disease prevention. But, only a small number of these have had substantive clinical documentation of their health benefits [1]. Dairy products or the milk derived products can be considered as functional foods as they are health beneficial for humans [2]. Functional dairy foods, the probiotics already have positive health image by their traditional use for centuries [3]. The market of functional food increased rapidly [2].

In plant foods, it is found that fruits and vegetables are reach in bio-active compound viz. phenolic compound, carotenoids, flavonoids, anthocyanin, vitamins, higher content of antioxidant and an antimicrobial compounds. In fact the waste peels, seeds can be used as a source of nutraceutical. Thus higher intake of fruits and vegetables offers an effective tool for preventing serious disease like cancer, cardiovascular disease [4].
Colour is one of the most important characteristic of food. Main objective for the addition of colour in food is to make the food more appealing and recognizable. Everyone is sensitive to the colour of the food as it can stimulate or suppress one's appetite [5]. Natural colorants have received increased acceptability, mainly because of the apparent lack of toxicity. Natural colours are sometimes rich sources of antioxidants [6].

Purple cabbage (*Brassica oleracea var. capitata F. rubra*) is a vegetable with good antioxidant capacity. The leaves of this cabbage contain biologically potent anthocyanins [7]. It has anticancerous [8], anti-inflammatory [9] antibacterial [10], anti-diabetic properties[11] as well as it possesses antioxidant, and antihyperglycemic [12]. It is also very rich in minerals, vitamins, anthocyanins, polyphenols, and glucosinolates. It was observed that it contains about 196.5 mg of polyphenols per 100g of raw purple cabbage. It is more unique among the cruciferous vegetables as it provides a good quantity of anthocyanins, which acts as antioxidant as well as anti-inflammatory nutrients. The antioxidant richness of cabbage is partly responsible for its cancer prevention benefits [13] [14].

Anthocyanins are water soluble pigment and they are also known as flavonoids. They provides the bright and attractive orange, red, purple, and blue colours of most fruits, vegetables, flowers and some cereal grains. There are More than 600 structurally distinct anthocyanins have been identified in nature [15].

They are one of the important groups of visible plant pigments. Anthocyanins also have some health-promoting benefits, as they can perform as natural antioxidant [16].

For the presence of carbonyl and a hydroxyl group, anthocyanin can attach the dye with TiO_2 surface. It stabilizes the excited states of anthocyanin and resulted the maximum absorption by having lower energy. Anthocyanin exhibit broad region of the visible light spectrum and attributed to charge transfer transitions [17, 18].

In this recent study the pigment of the purple cabbage was fortified in yogurt for making one functional food of increased anthocyanin as well as polyphenol content.

II. EXPERIMENTAL PROCEDURES

A. *Extraction of Colour of Purple Cabbage:*

Purple cabbage was collected from local supermarket (Kolkata, India.). The cabbage was then cleaned with running distilled water. The cabbage is then sliced finely by using mechanical slicer. Then the sample was grounded with 200 ml 0.1 (N) citric acid buffer solutions. Then the colour was extracted by distilled water at room temperature. The solution was then filtered and centrifuged (Remi C 24) at 12000 r.p.m. for 10 minutes at -5°C. Then the extracted colour was dried by using freeze drier (Eyela FDU1200 freeze dryer).

B. *Preparation of Yogurt:*

Homogenized and Pasteurized cow's milk (milk fat 3.6%, protein 3.3%, SNF 8.6%, pH 6.6-6.7) was brought from local super-market (Kolkata, India). Pasteurized toned milk was heated to 90-95°C till its volume reduces to 1/3rd of its original volume and then it was cooled to about 37°C. The milk was separated in two parts by volume. One part was separated for control yogurt (C) preparation and another part was for fortified yogurt (D) preparation. In fortified yogurt 0.2% (W/W) freeze dried colour was added to the milk. . Then, 2% of yoghurt (purchased from local super-market of Kolkata, manufactured by a reputed dairy industry of India) is being mixed thoroughly to both the samples individually for incorporation of starter culture. The samples were then incubated at 37°C for 7 hours and refrigerated at 4°C till use. The colour of the bio colour fortified yogurt was observed pink.

C. *Analysis of the stability of Extracted Pigment from Purple Cabbage:*

Stability of the extracted colour was analysed by following the procedure of Dasgupta *et al*, (2019) [14]. pH of the colour was changed several time by addition of 0.1 (N) HCl and 0.1 (N) NaOH to the extracted pigment. The pH was measured by the digital pH meter (pH meter CL 46+, Toshcon Industries Pvt., Ltd.). The amount of anthocyanin present in the extracted colour with varying pH was determined by observing the absorbance at 560nm wavelength by using UV-Vis spectrophotometer (Jasco V 630 Spectrophotometer).

D. *Analysis of Total Polyphenol of the Extracted Colour:*

The total polyphenol content of colour extract with varying pH was determined UV-Vis spectrophotometer (Jasco V 630 Spectrophotometer) at 765nm by standard method of Mcdonald *et al.*, (2001) [19] and expressed in terms of gallic acid equivalent (GAE).

E. *Physico-Chemical Analysis of the Yogurt Samples:*

Degree of syneresis or the amount free whey of the both yogurt sample (C and D) were determined by a slightly modified process of Al-Kadamany *et al*., (2003) [20]. In this process 50 gms of samples were filtered by vacuum filtration, by using Buckner funnel and Whatman 1 filter papers for 10 minutes.

% of synersis= Free whey (gm/100gm)
= 100 x (weight of initial sample - weight of sample after filtration) /weight of initial sample

The rheological analysis of the yogurt samples were done by using Brookfield Rheometer (model DV2T) with spindle no. 27 where 9 rotational speeds (20, 30, 40, 50, 60,70,80,90 and 100 rpm). The temperature was kept constant at 30±2°C. All data were taken after 1 min between each 9 rotational speeds in each sample, with a resting in time between the measurements at the different spindle speeds. The flow curves, shear stress vs. shear rate, of the samples were plotted.

The flow curves of the yogurt samples were established by using the following models, which are the most frequently used for engineering applications:

Newton's model

The shear stress versus shear rate data was fitted to Newton's model (Equation. 1) by linear regression.

$$\tau = \eta \gamma$$

Where τ is the shear stress (mPa), η is dynamic viscosity (mPa.s) and γ is the shear rate (s^{-1}).

Herschel-Bulkley model

The Herschel-Bulkley model shows the flow behavior of a material with a yield stress and shears thinning or shear thickening at stresses above the yield

$$\tau = \tau_0 + K \gamma n$$

Where:

τ is shear stress (mPa), τ_0 is the yield stress (mPa), K is the consistency index (mPa s^n), γ is shear rate (s-1) and n is the flow behavior index (n = 1 for Newtonian fluids, n < 1 for shear thinning fluids).

Data was analyzed by using software of Brookfield Engineering Labs, Inc. Rheocalc V3.2 Build 47-1 where, Herschel-Bulkley math models provide a numerically and graphically analyze for the behavior of data sets.

Moisture content, ash content, acidity of both the yogurt samples (C and D) was determined by AOAC (1999) [21] official methods.

F. Sensory analysis:

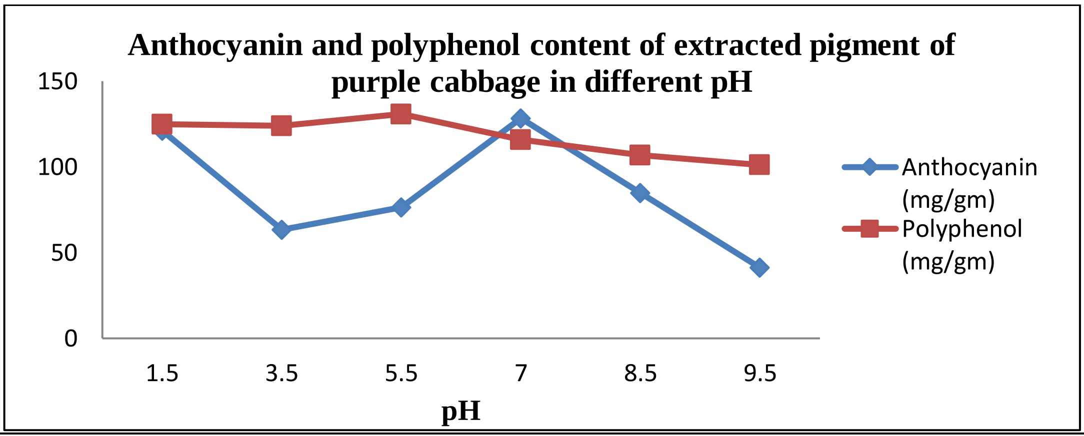
Sensory analysis of both the control and fortified yogurt were done for by eight trained panel members of food technology department by using 9 point hedonic scale [22].

III. RESULT AND DISCUSSION:

A. Anthocyanin and Polyphenol content of the pigment of Purple cabbage:

The colour has been extracted at pH 1.5-1.56. pH of extracted colour was adjusted by addition of acid and alkali and it ranges between 1.5-9.5 with changing in colour from Purplish red to olive green. The anthocyanin content (mg/gm) and the polyphenol content (mg GAE/gm) in varying pH are presented in Fig.1.

Fig. 1: Anthocyanin and polyphenol content of extracted pigment of purple cabbage in different pH



It was observed that anthocyanin (mg/gm) content from pH 3.5 to 5.5 varies between 63.33 and 74.416. The polyphenol content (mg GAE/gm) in the same range of pH varies from 123.93 to 130.85. As the pH of yogurt ranges from 4 to 4.6, the biocolour of the purple cabbage can increase the oxidative stability and the nutraceutical properties of the fortified yogurt.

B. Physico-Chemical Analysis of the Yogurt Samples:

Proximate analysis of the yogurt samples are presented in table I.

Syneresis is one of the most important factors for consumer acceptance and shelf life of yogurt. It measures the quantity of whey separated from the yogurt. According to Aprodu *et al.*, (2012) [23] excess syneresis indicates low quality yogurt. This study revealed that the syneresis of control yogurt was 53.46% which was greater than the syneresis of fortified yogurt i.e. 22.6%. The syneresis of the prepared yoghurt samples were affected significantly ($P<0.05$) by both G*undelia tournefortii* concentration and storage time [24]. Water-holding capacity of yoghurt also reflects the stability of coagulation condition [25].

It was observed that the pH of the samples is 4.4.and 4.6 respectively for C and Das well as the acidity (%L.A.) for fortified yogurt is less than C. Similar analysis was reported by Okoye *et al*. (2009) [26]. This might be due to variation in the growth of lactic acid bacteria during fermentation.

The polyphenol content of D is about 73.74% more than sample C. It indicates that the fortified biocolour extracted from purple cabbage enhanced the polyphenol content of D.

Table I: Proximate analysis of yogurt samples:

Sl. No.	Sample	pH (Avg±s.d.)	Acidity (% LA) (Avg±s.d.)	Ash Content (%) (Avg±s.d.)	Moisture Content (%) (Avg±s.d.)	Polyphenol Content (mg GAE/ gm dry solids) (Avg±s.d.)	Syneresis (%) (Avg±s.d.)
1	Control (C)	4.4±0.051	1.08±0.032	0.2±0.075	52.08±0.051	78.65 ±1.38	53.46±0.077
2	Fortified(D)	4.6±0.027	0.89±0.035	0.7±0.509	53.01±0.053	136.08±0.762	22.6±0.053

(Avg±s.d.)= Average± standard deviation, n=5

C. Rheological Analysis of Yogurt Samples:

The flow behaviour of both the samples was Bingham Plastic in nature. For, both sample the flow behaviour index (n) = 1 and yield stress (τ_0) > 0. The formula used to characterize the flow behaviour is as follows: $\tau = \eta (du/dy)^n + \tau_0$

Where, τ= Shear Stress; η = Kinetic Viscosity; du/dy = Shear Rate; n= flow behaviour index; τ_0= Yield Stress;

The flow behaviour of the samples were presented graphically in fig. 2 and fig. 3 where the curves are plotted shear rate (1/sec) vs. Shear stress (Dyne/cm^2).

Fig. 2: Flow behaviour of control yogurt (C)

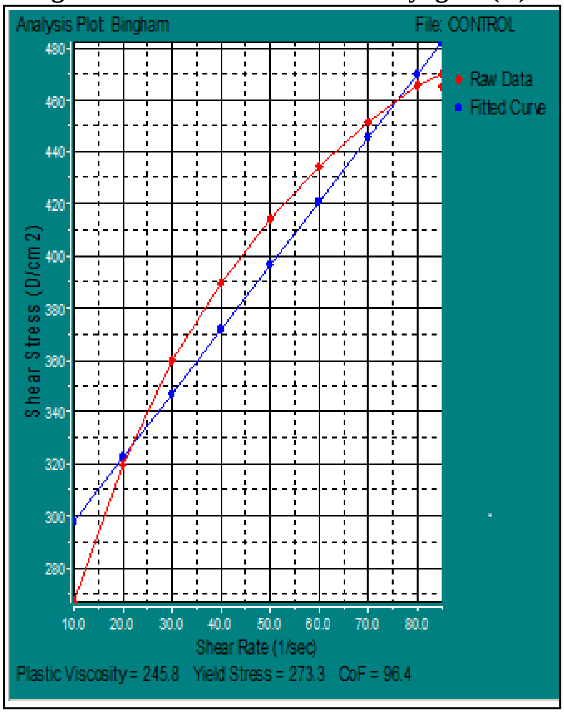
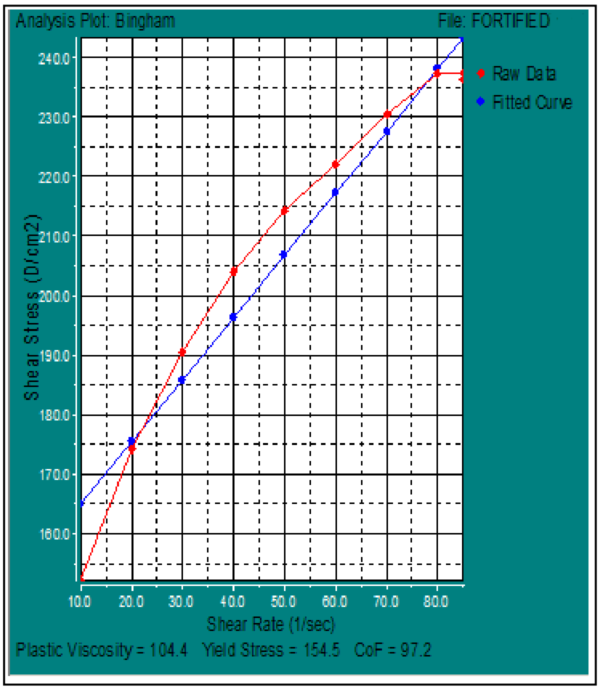


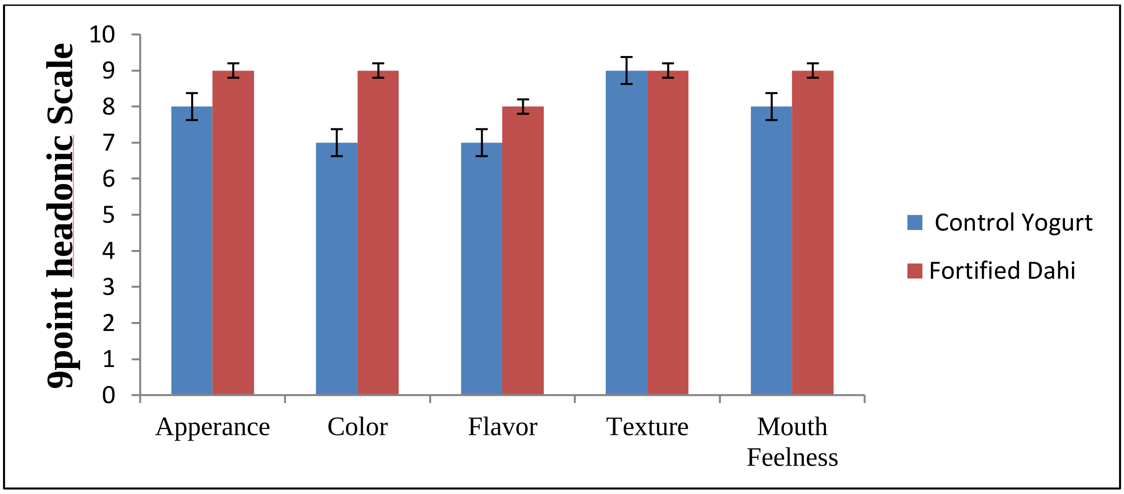
Fig. 3: Flow behaviour of fortified yogurt (D)



D. Sensory Analysis of Yogurt:

Sensory analysis of samples is evaluated in 9 point hedonic scale. It is found that in case of the purple cabbage colour fortified yogurt, the colour and mouth-feelness are about 9.0, body texture and flavour are about 8.5 and overall acceptance is about 9 more than the control. The sensory analysis of the samples was present in fig. 4.

Fig. 4: Sensory Analysis of Yogurt Samples:



IV. CONCLUSION

As we know regular intake of rare vegetable rich diet or their extract fortified rich diet plays an important role in human nutrition. They have positive effects on health as phytonutriceuticals of vegetables can protect the human body from different types of chronic diseases. In this project, many trials have been done with different modifying processes. Fermented dairy products such as yogurt have long been known for its functional value, particularly managing intestinal disorders such as lactose intolerance or acute gastroenteritis. The application of each treatment (addition of probiotics, different food ingredients) particularly influences rheology and textural properties of yogurt. In terms of health although technology has been applied with almost complete success to produce functional yogurt with sufficient rheological properties still there is need for product optimization. From the values of overall acceptability and other physiochemical and rheological analysis it can be concluded that fortified yogurt with enhanced functional properties can get easily accepted by the consumers in the worldwide market.

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