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Salubrious Curcumin Fortified Whey Beverage Formulation and Study its Antioxidant Property

[1] Ankitha R, [2] Laxmana Naik N, [3] Ashwani Sharma
[1], [3] Department of Biotechnology, R.V. College of Engineering, Bangalore-560059,
[2] Dairy Chemistry Section, ICAR-National Diary Research Institute, SRS, Bangalore-560030

***Abstract*— In this 21st century, sedentary lifestyle has hampered the eating habits which has resulted in inadequate intake of essential nutrients, thereby giving rise to various health complications. The food and beverage we consume plays an important role on our health and intake of right diet help to overcome diverse health problems. Nutraceuticals provide the required dietary supplements with presence of epigenetic modulators have emerged as potential therapeutic agents. Curcumin; a bioactive compound is a potential epigenetic modulator with beneficial properties to promote health. Whey is a by-product of dairy industry, also shows health beneficial properties, in current trend whey based beverage are sold as nutraceutical. In the present study an attempt was made to fortify the curcumin in to whey in order to develop a curcumin rich whey beverage. Curcumin was extracted from raw turmeric by solvent extraction, it was purified. Curcumin, sweetener and flavor level were optimized in the fortified beverage. The antioxidant activity of curcumin extracts and formulated whey beverage was determined by DPPH assay and it was estimated to be 60.56% and 43.51% respectively. Developed curcumin fortified whey beverage had 2 weeks of shelf life at refrigeration condition. Due to whey and curcumin, developed product can be considered as beneficial and highly nutritional in terms of functional properties.**

***Index Terms*— Nutraceutical, curcumin, curcumin extract, whey beverage, fortification, DPPH assay.**

I. INTRODUCTION

The famous quote "It is health that is real wealth and not pieces of gold and silver" is indeed the main spirit of the present study. In this colossal change in lifestyle and eating habits have paved a way for numerous multiple health problems such as metabolic syndrome, hyperlipidemia, cancer, diabetics, neurodegenerative and cardiovascular diseases etc [1]. Synthetic medicine has been used since a decade to treat various health problems. Owing to rapid urbanization people have started to recognize the adverse effects of synthetic medicine on long term consumption. This has led to the growing interest towards herbal medicine and nutraceuticals to improve overall well-being of an individual. Food is the chief unrivalled key solution to tackle diverse health related problems [2]. A popular Indian spice called 'Turmeric' (Curcuma longa) also known as Haldi, is found to be used in culinary and as ancient traditional medicine. This is mainly because of the presence of a bioactive compound that play an important role for its therapeutic applications [3].

Curcumin(1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), also called diferuloylmethane is a natural bioactive derivative of herbaceous-medicinal plant, yellow colored, polyphenolic compound which is found abundantly in turmeric (Curcuma longa L.) [4]. A typical extract of Curcuma longa L. contains three curcuminoids they are curcumin (~77), Demethoxycurcumin (~17) and Bisdemethoxycurcumin (~3), out of which curcumin is the principle curcuminoid. Curcumin I is present in abundant amount when compared to other 2 curcuminoids making turmeric as the rich source of curcumin [5]. The compound has a molecular weight of 368.37 grams per mol and melting point at 183°C with a chemical formula is C21H20O6. Curcumin is relatively water insoluble but soluble in acetone, methanol, ethyl acetate, ethanol, hexane, isopropanol etc., also forms a reddish-brown colored salt with alkali. It is highly unstable at basic pH and degrades within 30 minutes but at acidic condition. Curcumin is light sensitive; therefore, it is suggested that all biological samples comprised of curcumin should be kept safe from light [6,7]. Due to its unstable property curcumin showed a significant change in its absorption spectrum and is pH dependent [8]. Curcumin holds numerous scientifically entrenched functional and biological attributes which has proven to modulate various functional activities in our body such as cell growth, inflammation and apoptosis thus promote health [9]. Curcumin a vital dietary supplement that has exhibited various pharmacological activities like antioxidant, anti-inflammatory, antibacterial, antifungal, antiprotozoal, antiviral, hypocholesteremic antihypertensive, antidiabetic, antitumor, anti-alzheimeric and so on. Thus making curcumin a promising and a potent candidate treating many chronic health disorders and has been

scientifically illustrated to have promising pharmacological effects [10]. Curcumin – a strong and a powerful antioxidant both in vivo and in vitro, it is involved in the formation of pro-oxidant like reactive oxygen species (ROS), it was believed that the proapoptotic effect is ascribed to pro-oxidant activity. Thus making curcumin a robust antioxidant agent which in turn helps tackle various chronic disease and help in treatment and prevention of it [11]. Current century, Nutraceutical have emerged as a potential therapeutic agent to prevent and treat numerous health related issues. Nutraceutical enriched with epigenetic modulator like curcumin play a vital role in augmenting health [12]. Hence Curcumin is marketed largely worldwide and commercially sold in the forms such as, dietary herbal supplements, tablets, ointments, capsules, soaps and sport energy drinks. Apart from that curcumin is also used as cosmetic ingredient, where it's added as a preservative and a flavoring agent in soaps and as a natural coloring dye in many industries [13].

Whey is a liquid by-product material obtained from the dairy industry during the manufacturing of cheese. It is highly nutritious and contain 50% of the total solids and 93% water, 20% of milk protein (whey protein), 70% of milk sugar (lactose), 50% of total milk solids, 90% of milk minerals and all water soluble vitamins that are present in milk. Whey protein is globular proteins with unique physio-chemical properties which has broad range of solubility, flavorless, act as carrier for aroma compounds, increase viscosity and has neutral pH [14]. Hence, efficiently utilized in food and beverage industries for manufacturing various whey-based value added products and can be marketed as a dietary supplement. Whey protein has many pharmacological properties includes antibacterial, antiviral, antioxidant, antihypertensive, anti- inflammatory, antidiabetic and so on. These beneficial properties help preventing and controlling of various diseases conditions like bacterial and viral infection, type 2 diabetes, heart diseases and inflammatory bowel diseases [15]. Consumption of whey protein also helps reducing hunger to maintain body weight, body and blood fat and assists in enhancement of body's immunity. Commercially, whey is widely being sold as whey-protein energy bars, whey dietary supplement in form of powder and whey protein shakes etc. [16].

A diverse whey-based beverages been successfully developed and marketed all over the world. Different types of whey beverages are present comprising of plain, fruit flavored, carbonated and alcoholic beverages that are widely accepted [17]. For a beverage to be marketable accepted it should satisfy certain criteria's, should be thirst-quenching, optimum quality, affordable with respect to price, good sensory properties and above all should have a positive health benefits. Hence has made a way for many food and beverage industries across the world to utilize whey proteins and whey in a productive way to manufacture whey-based products

[18]. The present study is anticipated to develop a delicious and salubrious curcumin fortified ready-to-serve (RTS) whey based beverage and also study its functional properties.

II. MATERIALS AND METHOD

A. Extraction Procedure

A fresh raw turmeric root was taken, 5 grams of sample weighed, washed, peeled and grated to obtain small segments of sample. Solvent extraction was carried out using water, ethanol, ethanol: water and acetone as per the described [19] [20] methods. Measured 30 mL each of water, ethanol, solvent mixture of ethanol: water (15:15 v/v) and acetone added to grated sample. Samples kept on magnetic stirrer for agitation at 180 rpm for 2 hours at room temperature (27°C). Extract was centrifuged at 3500 rpm for 15 minutes and collected the supernatant and further subjected to vacuum filtration. Filtrate collected was stored at - 4°C for further use. B. Determination of maximum absorbance (λmax)

Extracted curcumin filtrate maximum absorbance was measured by using Labman UV-Vis Spectrophotometer. Maximum absorbance (λmax) of the commercially purchased curcumin standard and extracted curcumin samples was determined by scanning from 200 to 500 nm [21].

C. Qualitative Analysis

Qualitative analysis of the sample performed to check for the presence of the curcumin in the extracted sample by Thin-layer chromatography (TLC). Curcumin extract was spotted on TLC Silica gel 60 F254 pre-coated aluminium sheets (as stationary phase) against the commercially purchased curcumin standard. Chloroform: methanol used as mobile phase, as its reported to show best desirable separation [22]. Chromatographic procedure was carried out as follows; the elution chamber was saturated with the

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solvent system. Sample was spotted with the capillary glass tube and left to dry and placed into the chamber. TLC plate was run until the solvent reaches more than 3/4th of the length of the plate. Detected for yellow spots and retention factor (Rf) calculated [23].

D. Yield Estimation

Extracted sample was poured into a clean petri plate and kept for atmospheric evaporation until weight remained constant and no trace of solvent. Yield percentage was calculated by using the formula: Where, W1 is the weight of the empty petri plate and W2 is the weight of petri plate along with sample.

E. Fortification and optimization process

Extracted curcumin from raw turmeric roots fortified into liquid cheese whey as follows; concentrated curcumin extract added into ~50 mL whey sample with help of glass road scrapped the curcumin residues from petri plate. Similarly, commercial curcumin was fortified into whey. If any sedimentation observed, then the sample was subjected to vacuum filtration and collected the filtrate. The filtrate obtained is simple curcumin fortified whey sample. Sweetener-flavour stock solution was prepared by weighing 10-gram sugar and dissolved in 50 mL cheese whey. Thus making the concentration of sugar stock solution to 20%. To that 1 mL of the citrus flavour was added. Different aliquots of the stock were taken i.e., 2, 4, 6, 8 and 10 mL added into whey sample making up to 10 mL final volume [24]. Further optimization done by taking different aliquots of the stock i.e., 1, 2, 3, 4 and 5 mL added into whey making final volume to 10 mL. Based on sensory evaluation a single optimized parameter was chosen for development of final product. TLC was performed to check for the presence of curcumin in fortified sample.

A. DPPH assay for antioxidant activity

The antioxidant activity of the extracted curcumin and curcumin fortified whey beverage was determined and compared to that of L-ascorbic acid by using DPPH assay method. The antioxidant activities of samples were evaluated according to the DPPH assay described by tuba et al [25] with slight modification. Briefly, 1.5 mL of 0.1mM solution of DPPH was prepared in ethanol added to 1.5 mL of sample and control was prepared by taking 1.5 mL of DPPH with 1.5 mL water, all mixed well and incubated in dark for 30 minutes, the absorbance is measured at 517 nm. The DPPH inhibition percentage (% I) is calculated by:

$$Inhibition\ (\%) = \frac{[Absorbance_{(control)} - Absorbance_{(sample)}]}{Absorbance_{(control)}} \times 100$$

If sample exhibit free radical scavenging activity, then will donate electron or hydrogen atom that combines with DPPH* (free radical) to from a stable DPPH radical. This results in color change from purple to yellow colored solution, absorbance measured used to calculate antioxidant activity as percentage inhibition. A standard calibration curve was prepared using different concentrations of L-ascorbic acid. Antioxidant capacity of samples were expressed as ascorbic acid antioxidant capacity (AEAC).

III. RESULTS AND DISCUSSION

Crude curcumin was extracted from fresh raw turmeric roots by solvent extraction. Solvents used for extraction were distilled water, ethanol, ethanol: water and acetone, among them ethanol and acetone showed better results. Using ethanol: water as solvent for extraction showed charring of the sample and resulted in blackish-brown extract (see fig 1). Hence only distilled water, ethanol and acetone solvent extraction was considered for further analysis.

Figure 1: Flowchart showing curcumin extraction process by solvent extraction

B. Maximum absorbance of curcumin extract

The maximum absorbance (λmax) was measured for standard commercial curcumin along with the ethanol, acetone and enzyme-assisted extracts. As reported in a study that the maximum absorbance depends on the pH and state of the curcumin present in solution. Also it has widely reported to show maximum absorbance between 420 to 425 nm. Maximum absorbance measured for standard curcumin was 424 nm. For the extracted samples, distilled water extract – 274 nm, ethanol extract – 424 nm and acetone extract – 427 nm. Hence 424 nm was considered as optimum and was used for further analysis purpose.

C. Thin-layer Chromatography (TLC) analysis

The presence of the curcumin in extracts from sources by using different solvent systems were analyzed by Thin-layer chromatography (TLC). The TLC plates were visualized as yellow spots (see fig 2) with calculated Rf values (see table 1). The Rf value obtained from extracts was compared with standard commercial curcumin. Using chloroform: methanol

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as mobile phase showed the best desirable separation.

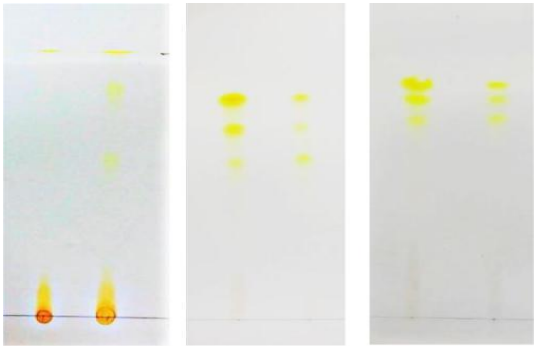

Figure 2: TLC plate image for different curcumin extraction methods (a) Water extract, (b) Ethanol extract and (c) Acetone extract

Curcumin was identified in the extracts of ethanol and acetone extraction. Calculated the Rf value for samples and compared with the curcumin standard as shown in table 1.

Table 1: Calculate Rf value for curcumin standard and extracted sample (Note: Spot on given from top to down)

Sl No.		R_f Value	R_f value
Method	Spot No	STD	Extract
a	1	0.92	-
	2	0.90	-
	3	0.61	-
b	1	0.90	0.89
	2	0.84	0.83
	3	0.75	0.76
c	1	0.89	0.88
	2	0.77	0.76
	3	0.63	0.64

The best result was obtained from ethanol and acetone extraction method, the Rf value of the curcumin extract found to be close to the Rf value of standard curcumin. Hence both the extract was used for further use.

D. Estimation of yield

The yield percentage of the extracted sample was estimated for ethanol and acetone extraction method. The one with the heights yield was selected for beverage fortification purpose. The estimated yield percentage for ethanol and acetone extraction found to be 13.55% and 9.68% respectively. Ethanolic extraction showed high yield percentage, hence was considered for fortification.

E. Developed fortified beverage

Both standard commercial curcumin and ethanol extracted curcumin was fortified into pasteurized liquid whey by direct addition of whey into extract. About 0.1355 g of both sample was added into whey (~50 mL). After 24 hours two of the fortified samples were observed for sediments (see fig 3). Both fortified sample was observed and found sedimentations so centrifuged and filtered the samples by vacuum filtration. Collected the sample filtrate and stored in a closed lid glass jar and it was observed in sample (b) standard curcumin fortified was lost during filtration. Where as in sample (a) extracted curcumin was retained even after filtration (see fig 3). Hence natural extracted unprocessed curcumin has shown to be dissolved in whey, making a way for development of curcumin fortified whey beverage

(a) (b)

Figure 3: Curcumin fortified whey sample
(a) Ethanol extracted curcumin fortified sample
(b) Commercial curcumin powder fortified sample

The presence of curcumin in fortified whey beverage was analyzed by TLC. Chloroform: methanol was used as mobile phase as it showed good separation.

Table 2: Calculated Rf values for curcumin standard and fortified beverage sample

Name	Spot no	R_f Value
Curcumin standard	1	0.895
	2	0.704
	3	0.521
Fortified beverage	1	0.886
	2	0.704
	3	0.513

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Figure 4: TLC plate image for fortified whey beverage

Fortified beverage sample was spotted on the TLC plate and observed for yellow spots with respect to curcumin standard spot (see fig 4) with calculated Rf values as in table 2. Hence, curcumin was identified in developed fortified whey beverage. The sweetener-flavour stock solution (SS) prepared was added in different volumes i.e., 2, 4, 6, 8 and 10 mL of the stock into fresh pasteurized whey. Based on preliminary sensory evaluation, sample conating 2, 4 and 6 mL of the stock solution was selected. Further optimized by taking 2, 3, 4, 5 and 6 mL of the stock into fresh pasteurized whey as seen in figure 5. Different sample code names were given for sensory evaluation purpose. Based on preliminary evaluation for parameter sample conating 4 mL of the stock solution with code name B53 was selected to be the optimum and was further used for shelf-life analysis

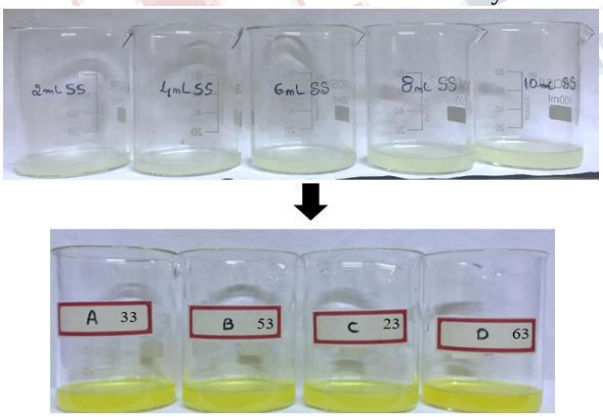

Figure 5: Image shows sweetener and flavour optimization in fortified beverage sample

F. Antioxidant activity

Free radical scavenging activity of the curcumin standard, extract and fortified beverage was estimated by DPPH assay. For positive control ascorbic acid plus DPPH, negative control ethanol plus DDPH and for blank distilled water plus DPPH was used. After addition of reagent kept for incubation in dark for half an hour. Measured the absorbance at 517 nm and calculated the percentage inhibition using standard calibration curve of L-ascorbic acid as seen in figure 6.

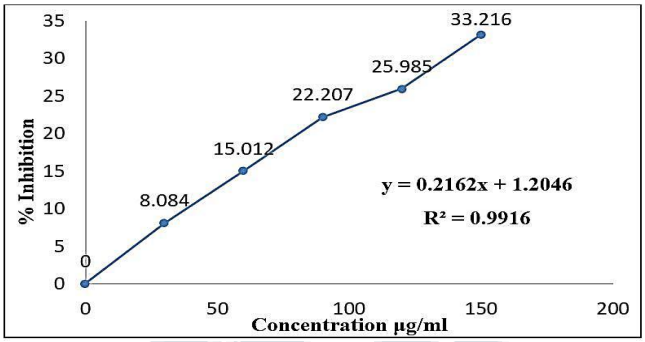

Figure 6: Standard calibration curve of L-ascorbic acid

The % inhibition of curcumin standard and ethanol extract is found to be close. The inhibition activity is high in the extracts when compared to fortified beverage sample indicating that on filtration of fortified beverage essential curcumin was lost. The antioxidant activity of curcumin as shown to increase with increasing concentrations (R2 – 0.9916) as seen in table 3.

Table 3: Shows inhibition percentage calculated for the samples

Sl.No	Sample Name	% I
1	Curcumin standard	61.70
2	Ethanol extracted curcumin	60.65
4	Whey fortified with curcumin standard	17.43
5	Whey fortified with curcumin extract	43.51

G. Estimation of shelf-life

The shelf life of the fortified beverage was estimated to be 16 days which is approximately 2 weeks without addition of any preservatives. After 18th day white coagulates were seen and on 21st day unpleasant odor was observed (see fig 7).

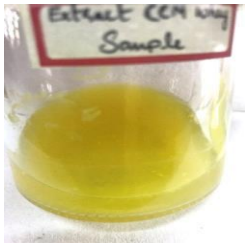

Figure7: Image shows white coagulates on walls of the container indicating spoilage

IV. CONCLUSION

Curcumin is reported to be extracted from commercial curcumin and dried turmeric roots in many studies. Owing to its solubility, in the present study, an attempt was made to extract curcumin from fresh raw turmeric roots. It was found that ethanol extraction was the best solvent extraction method as highest yield obtained when compared to other methods. Curcumin extract from raw turmeric obtained closer Rf value compared to commercial curcumin standard by TLC. The extracted crude curcumin was fortified into liquid whey sample. It was found that curcumin showed better solubility, this indicates that there are factions holding curcumin and helps increase its bioavailability. Further, studies to be carried out to identify the factor. In this beverage, level of curcumin, sweetener and flavour were optimized for consumer acceptance. Developed product exhibited better antioxidant activity as compared with standard commercial curcumin. The shelf-life of the developed fortified beverage was approximately 2 weeks without addition of any preservatives. Thus through this study, an attempt was made to develop and formulate curcumin fortified ready-to-serve (RTS) whey-based beverage.

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