Journal of Postharvest Technology
2024, 12 (1): 49-59
www.jpht.in

RESEARCH ARTICLE

Development of flavoured milk by adding extracted curcumin from turmeric

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ARTICLE INFO

Received : 16.12.2023
Accepted : 27.12.2023

ABSTRACT

India's iconic and treasured spice is turmeric. Turmeric known "Indian Saffron" as well. The main and most effective component of turmeric is curcumin. Turmeric contains a potent plant component that is thought to have potent anti-inflammatory and antioxidant properties. In this study, ethanol was used as a solvent to extract curcumin from turmeric. Different concentrations, including 0%, 25%, 50%, 75%, 85%, and 95%, were used. The ethanol solvent produced a 4.1% yield at a 95% concentration. Curcumin was characterized and identified using the HPLC technique, UV spectroscopy, and IR spectroscopy. Milk is an essential food. It provides a variety of nutrients, including protein, carbohydrates, calcium, vitamins A and B, fats and minerals. For humans, it is difficult to digest. Full-fat milk cannot be effectively digested by infants or those who are ill. It is possible to solve these issues by producing flavored milk. Because it is well-liked by consumers as a refreshing and energizing milk product, flavor milk is quickly becoming an important part of the market milk sector. Therefore, milk that has been flavor-infused with curcumin has a unique flavor. Curcumin functions as a flavoring, fragrant, and nutritive substance. On the basis of sensory and proximal investigation, the F1 sample was chosen. Ash was 0.038%, fat was 3.1%, and the energy per 100 grams was 74 Kcal. According to the microbiological investigation, the TPC of the flavored milk increased on the sixth day. So curcumin increased the shelf life of curcumin..

Keywords: Curcumin, Ethanol, Extraction, Flavoured milk, HPLC, IR.

Citation: Lad, B.R., Ingale, V.M., More, G.G., Wategaonkar, S.B., Prathapan, K., and Khot, J.A. 2024. Development of flavoured milk by adding extracted curcumin from turmeric. *Journal of Postharvest Technology*, **12** (1): 49-59.

INTRODUCTION

Turmeric is a traditional and valued spice in India. It is also called as “Golden spice of India”. It is derived from rhizomes of Curcuma longa. It is perennial crop and grown in tropical as well as subtropical region of Asia. The name derives from Latin word ‘Terra merita’ i.e. meritorious earth referring to the colour of ground turmeric which resemble a minerals pigments. It is nature’s most powerful healers. It is universal herbal medicinal plant. Turmeric plantation done two times in the year according to variety of turmeric (Nasri et al., 2014).

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ISSN: 2348-4330

Turmeric was used at back years 4000 years ago in Ayurveda. Turmeric was used in ayurveda to balance all three doshas vata, pitta and kapha. Turmeric has a particularly beneficial effect for rasa dhatu and raktdhatu, i.e. the tissue of the blood, plasma and lymph. Turmeric is a flowering plant. The plant only grows to a height of two to three feet, and it produces both flowers and underground stems called rhizomes. The rhizomes like ginger in form. The stem-like root from which yellow turmeric spice is produced. It requires 20˚C to 30˚C temperature. Turmeric used for daily cooking as a curry powder and gives colour to product (Ansari et al., 2020). The production of turmeric in world wide is around 11 lakh tones per annum. India dominates in world production scenario contributing 79% to 80%. Then china (8%), Myanmar(4%), Nigeria(3%), Bangladesh(3%). In current year 2023 the highest yield taken by india is 5.13 lakh metric tones. In India the state of maharshtra with over 367 thousand metric tones was the leading producer of turmeric during year 2022. Telgana and Karnataka were second and third respectively in the ranking during that year. In Maharashtra state, Sangli is to be the largest and most important trading center for turmeric in Asia. Popularly known as “Saffron City”. Sangli has been cultivating its world famous turmeric since the 1900s. Sangli turmeric has a curcumin percentage 4% to 6% which is very high. This deep saffron coloured turmeric received the Geographical Indication Tag (GI) in 2018 (Turmeric Outlook June 2021).

Chemical Constituents of turmeric: The main active curcuminoid in Curcuma longa is curcumin (diferuloylmethane). Desmethoxycurcumin and bisdes methoxycurcumin are the other two curcuminoids, along with a number of volatile oils such tumerone, atlantone, and zingiberone. Sugars, proteins, and resins are among additional ingredients. Turmeric's yellow color is a result of polyphenols called curcuminoids. Demethoxycurcumin, bis-demethoxycurcumin, 5′-methoxycurcumin, Dihydrocurcumin, and cyclocurcumin are all derivatives of turmeric (Koccadam et al., 2015). Curcumin is the major and most active component of turmeric. It is a yellow pigment found primarily in turmeric. It is a hydrophobic phenolic compound with the chemical name 1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6- heptadiene-3,5-dione. Curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin are three main chemical compounds that belong to the "curcuminoid" family. The powder form of pure curcumin is typically a yellow-orange crystalline material. Curcumin has a lightly bitter taste, poor water solubility, poor chemical stability, particularly in alkaline conditions, and low bioavailability. These is attributed to low bioaccessibility and chemical transformation because of metabolic enzymes in the gastrointestinal (GI) tract. These factors make direct application of curcumin much less effective. According to toxicity studies, curcumin is safe to consume even at comparatively greater quantities. It has undergone substantial research as a nutraceutical ingredient for use in functional foods due to its broad-spectrum biological activity and low toxicity. Such curcumin-incorporated food products will improve the immunity of the human body and may combat the viral infections including coronavirus (Tripathy et al, 2021). Because of this, anything that can be done to combat chronic inflammation may be useful in avoiding and treating these illnesses. Curcumin is a bioactive chemical that has anti-inflammatory properties. (Tao Yang et al., 2023). Curcumin has also anti-cancer effect. Several studies have demonstrated that curucmin is able to inhibit carcinogenesis at three stages: angiogenesis, tumor promotion and tumor growth (Rathore et al., 2020).

Since ancient times, milk has been referred to as the wonder beverage with miraculous health effects (Moore et al., 2000). Numerous kids aren't getting the necessary amounts of calcium and potassium, which has led to their identification as nutrients of concern around the world. One of the recommended strategies to increase consumption of milk including flavoured milk (Khedkar et al., 2016). The modified form of this milk is known as flavoured milk if other ingredients such as color, flavor, and sugar are added. Full-fat milk cannot be effectively digested by young children or the ill. Milk with flavorings can be produced to address all of these issues. Flavoured milk is becoming an integral part of market milk industry because it has accepted by people of all ages and gender. Flavored milk is a nutritious snack that is marketed to children for consumption at home or school. The crucial period of adolescence is when strong bones and muscles can be developed, preventing fractures later in life. Teenagers who drink flavor-enhanced milk at least twice a week had a five-fold higher likelihood of continuing to

consume enough dairy products throughout adolescence. The consuming flavoured milk improves diet quality by providing nutritions like protein, fat, vitamins and minerals etc. It is not affected on weight due to have some sugar in the flavoured milk (Tiwari et al., 2017). So, requirement of manufacturing the flavoured milk is flavor, aroma and visual attraction and curcumin has all these attributes. Curcumin gives yellow color and flavor also.

MATERIALS AND METHODS

Extraction of turmeric

Solvent extraction of solids, which is commonly known as “solid- liquid” extraction, is a well understood and widely used method. An Extensive range of solvents including non polar organic solvents and a combination of organic solvents and water has been used to extract curcumin from plants carried out a comparison of extraction solvents. In this study curucmin from turmeric was extracted in ethanol (80 mL) at 60 ˚C following filtration and collection.

Identification and Characterization of Curcumin:

IR Spectroscopy: In this system, we detect the functional groups of any compound. The alcohol, ketones, carboxylic group, aldehydes etc functional group are recognized and give information about it. The Y-axis of an IR spectroscopy graph represents the frequency or wavelength, and the X-axis is the absorbed infrared radiation. The dipole moment of the particular molecule changes in tandem with the change in vibrational energy. The polarity of the connection affects how much absorption occurs. Symmetrical non-polar bonds in N≡N and O=O do not absorb radiation, as they cannot interact with an electric field (Theophanides, 2012).

UV Spectroscopy: From each particle size 2 g of sample was taken and mixed with 30 ml of ethanol and 30 ml of water respectively, separately and then filtered. The concentration of each of the filtrate was kept same and then the absorbance was measured using spectrophotometer at 425nm (Anamika Baghi 2012).

HPLC Method: HPLC system operates under high pressure but the efficiency of separation is not related to pressure. A solvent is in the mobile phase in this system's reservoir after passing through a filter and being pushed by a solvent pump into a column containing the stationary phase. The sample containing the elements meant for separation is introduced via an injector situated between the column and the pump. As the column's elute passes over a detector, signals are produced. These signals are stored on a recorder and analyzed by a data processor.

Standard Preparation:

At a temperature of 40˚C, the elution was performed using a gradient solvent system with a flow rate of 1 ml/min (Table 1). Methanol (23%), acetonitrile (41%) and water (36%) made up the mobile phase on a v/v basis. A chromatographic analysis was performed using an HPLC system with a 10 ml loop and a 7225i Rheodyne injector. The injection rate was 10 ml each loop. For the purpose of detecting curcumin, a PDA detector from the Prominence modular series was employed at a wavelength of 425 nm. The chromatograms were then analyzed by the Chromeleon Chromatography Management System, and software was used to quantify the curcumin in each sample (Kamble et al., 2011).

Table 1: Chromatographic Condition

Mobile Phase	Acetonitrile : Methanol: Water 41 : 23 : 36
Column	C18, 250 mm column over compartment
Wavelength	425 nm
Retention Time	5.3 min
Flow Rate	1 ml/min
Temperature	40˚C
Detector	PDA (Photo Diode Array)

Development of curcumin added flavoured milk

The development of curcumin added flavoured milk was prepared by the following standard procedure. It prepared chocolate flavoured milk by UHT treatment. The flavoured milk was prepared by adding different concentration of sugar (7, 9 and 11 %), cocoa powder @1.5 % and carrageenan (0.00, 0.01, 0.03, 0.05 and 0.10 %) in reconstituted whole milk. After proper mixing, mixture is preheated to 95˚C for 9 s and then final heating to 145˚C for 6 s followed by cooling to ≤ 35˚C.

Proximate analyses

The proximate analysis in which protein, fat, ash and calorific value were estimated. The protein estimation was done with the help of Kjeldahl method. The fat estimation by Gerber's method. The ash content of flavoured milk was estimated by using muffle furnace. The energy estimated by Bomb calorimeter. All the guidelines following from FSSAI manual.

Microbial analysis

The microbial activity test such as TPC and MBRT also done under the guidelines of FSSAI Manual for milk and dairy products.

RESULTS AND DISCUSSION

Extraction of curcumin by using Ethanol as solvent

The green solvent i.e. ethanol was used for the extraction process. The table 2 shows that the highest yield of curcumin i.e. 4.1% was obtained at 95% concentration of ethanol. Generally turmeric contains 3 to 7 % of curcumin. The similar results were observed by Anamika Baghi (2012). From the "Mega" variety we obtained 4.1% of curcumin.

Table 2: Data for extraction yield of curcumin

Ethanol Conc. (%)	0%	25%	50%	75%	85%	95%
Yield (%)	0.03%	1.37%	2.58%	3.41%	3.48%	4.16%

Table 3: Statistical Analysis of Extraction of curcumin by using Ethanol as solvent in ANOVA Software

Conc.	Yield 1	Yield 2	Yield 3	Total	Mean
0	0.003	0.002	0.003	0.008	0.0027
25	0.137	0.134	0.139	0.41	0.1367
50	0.256	0.259	0.261	0.776	0.2587
75	0.341	0.342	0.342	1.025	0.3417
85	0.385	0.383	0.386	1.154	0.3847
95	0.426	0.419	0.423	1.268	0.4227
Total	1.548	1.539	1.554	4.641	

cf = 1.1966

Table 4: ANOVA

SV	Df	SS	MSS	F cal	F tab	Result
Rep	2	1.9E-05	9.5E-06	2.567568		
Conc.	5	0.3902305	0.078046	21093.54	5.6363262	H. Sig.
Error	10	3.7E-05	3.7E-06			
Total	17	0.3902865				

The experiment was conducted with six concentrations from 0 to 95%. The mean yield is presented in table 3 and 4 and dissipate in fig. 1. It is clear from table that as the concentration increases yield increased sigificantly. The highest yield was observed (0.4227 gm) was observed when conc. Was 95%.

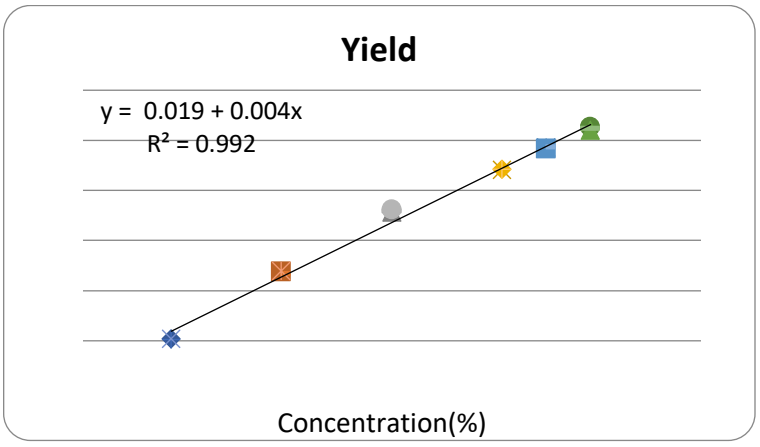

Fig. 1: The graph of concentration vs yield

Further Regression Analysis was used and the equation obtained is $Y= 0.019^{**} + 0.004^{**}$ (Concentration), $R^2 = 0.992^{**}$. Now it is clear that as from this equation as solvent conc. Increases by 1% yield increases by 0.004 gm. The coefficient of determination $R^2= 0.992^{**}$ indicates that 99.2 % increase in yield is due to solvent concentration.

IR Spectroscopy

The every compounds shows functional group as well as structure, so characterization was done by IR spectroscopy (Table 5; Fig. 2).

Table 5: Characterization of curcumin functional group by IR Spectroscopy

Functional Group	Standard Values (cm^{-1})	Absorbed frequency (cm^{-1})
Alcohol (-OH)	3650 – 3600	3600
Aromatic (C- H)	3050 – 3000	3050
Ketone (C= O)	1725 – 1700	1700
Ether (C- O – C)	1300 – 1000	1150

IR spectrum of curcumin showed that graph of frequency wavelength. The characteristic of alcohol (OH) stretching peak observed at 3600 cm^{-1}. The aromatic SP^2 =C-H stretching appeared at 3050 cm^{-1}. The characteristic of ketone (C=O) stretching observed at 1700 cm^{-1}. The ether stretching appeared at 1150 cm^{-1} .

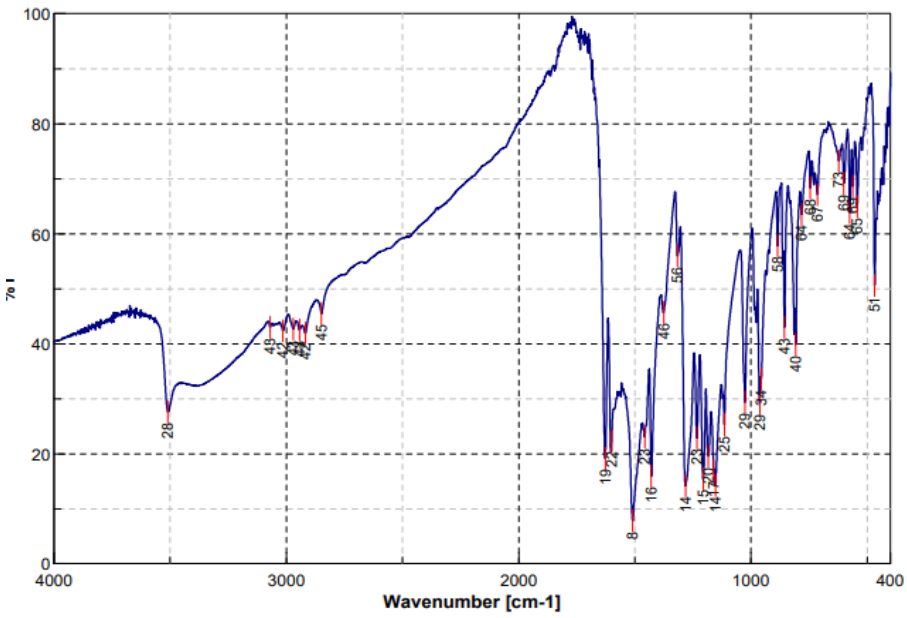

Fig. 2: Frequency Wavelength of Curcumin compound

UV Spectroscopy

Curcumin is a highly conjugated compound. It is a diketone tautomer that exists in water as a keto form and in enolic form in organic solvents UV spectroscopic study shows that λ_{max} at 424 nm (Fig. 3) it matches with the literature (Baghi, 2012).

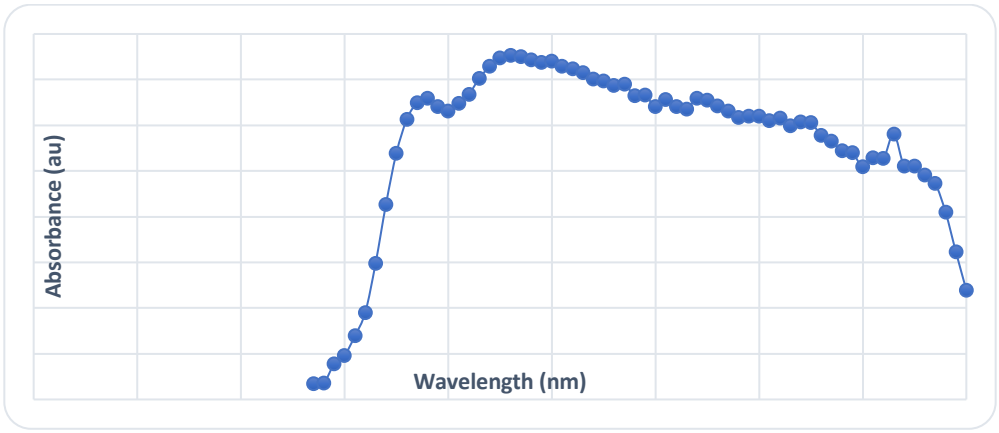

Fig. 3: Wavelength and Absorbance of curcumin in UV spectroscopy

HPLC method

The identification of curcumin by HPLC method is shown in Table 6 and Fig. 4. The chromatogram indicated the peak value and this is called curcumin. The curcumin availability in extracted sample was 97%.

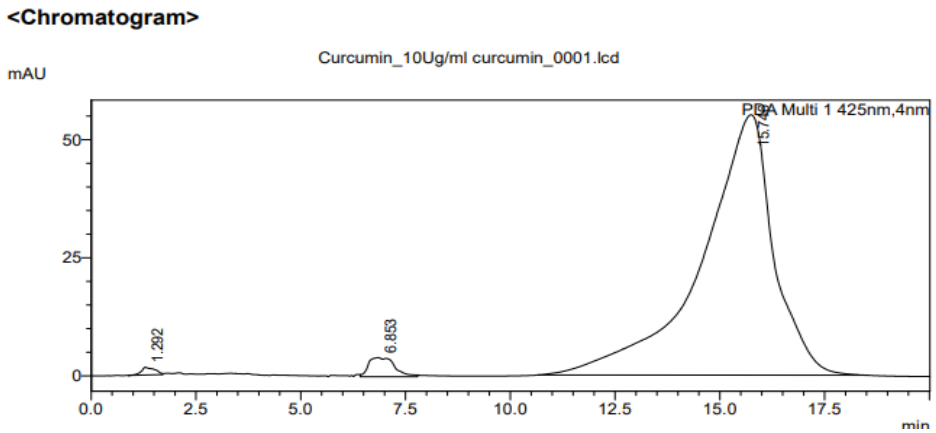

Fig. 4: Chromatogram

The remaining two peaks indicated about Dimethoxy curcumin and Bis- dimethoxy curcumin with 2.5% and 0.5% respectively. Ingale et al. (2011) observed the curcumin percentage in the range of 2.2 to 4.5% .

Table 6: Peak Value of Curcumin

Peak	Name	Ret. Time	Area	Area %
1		1.292	37197	0.549

2		6.853	175090	2.584
3	**Curcumin**	**15.746**	**6564626**	**96.867**
Total			6776914	100.00

Optimization of flavoured milk with varying concentration of curcumin

On the basis of sensory evaluation F1 (Curcumin 4.34%, milk 86.95% and sugar 8.60%) treatment getting highest score than other three treatments, Tiwari and Asgar (2017). The sensory evaluation was carried out by 9 point hedonic scale.

Table 7: Optimization of flavoured milk

Sample Code	Curcumin (%)	Milk (%)	Sugar (%)
F0	4.67	86.65	8.66
F1	4.34	86.95	8.60
F2	4.00	87.25	8.75
Control	0	90	10

Analysis of final product

The Table 8 indicates the nutritional values of sample and control in which F0 was superior than F1 and F2 sample. But the final selection was done on the basis of sensory evaluation of sample.

Table 8: Data of different nutritional parameter of flavoured milk

Parameter	**F0**	**F1**	**F2**	**Control**
Energy Kcal/100g	75	74.5	72	70
Protein %	4.12	4	3.8	3
Fat %	3.5	3.1	2.8	4.6
Carbohydrates %	13.3	12.8	11.4	4.9
Ash %	0.048	0.038	0.035	-

Sensory evaluation of flavoured milk

The sensory evaluation of flavoured milk was done for the attributes such as color, appearance, taste, odor, flavour and overall acceptability of samples (Table 9). Evaluation was done on the basis of 9 point hedonic scale. Milk sample used as a control for sensory evaluation. The F1 sample had high overall acceptability. Similar sensory evaluation was also reported by Kisore et al. (2020).

Table 9: Data for Sensory analysis of developed flavoured milk

Sample	Color	Odor	Flavour	Appearance	Taste	Overall Acceptability
F0	7.7	7.9	8	8	7.8	7.48
F1	9	8	8.5	8.4	8.5	**8.48**
F2	7.5	8	7.8	7.9	7	7.64
Control	7	6.5	6.8	7	6.3	5.6

Microbial analysis

The TPC indicates that microbial load carrying by sample. The TPC range for the milk and flavoured milk was 30 x 10^3 cfu/ml to 50 x 10^3 cfu/ml. The table 10 indicates that the shelf life of milk increases after adding curcumin in it. The TPC for the 1st day of developed flavoured milk was 32 x 10^3 cfu/ml. This was a normal microbial range for milk and milk product. This was due to packaging material. The TPC for the 3rd day was increased with 5 x 10^3 cfu/ml. The microbial load increased after two days. On the 6th day it was highly increased and 8th day it was gone near to expired condition. The control sample i.e. milk have 2 days shelf life with refrigeration. Thus Curcumin increased the shelf life of milk.

Table 10: TPC of developed flavoured milk on different days

Days	1st	3rd	6th	8th
TPC (cfu/ml)	32 x 10^3	37 x 10^3	44 x 10^3	**49 x 10^3**
Control	30 x 10^3	**52 x 10^3**	-	-

The MBRT was carried out for given sample as per FSSAI Manual for milk and dairy product. The reduction (decolorization) was not observed after 30 min of incubation time that indicated good quality of flavoured milk sample.

CONCLUSION

The ethanol was the best solvent for extraction of curcumin from “Mega” variety. The Ethanol solvent gave highest yield 4.1% at 95% concentration. The percentage curcumin recovery (97%) was done by HPLC method. The F1 sample was selected on the basis of nutritional as well as sensory evaluation. The shelf life of low fat milk was 2 days under the refrigeration. The curcumin added flavoured milk have 7 to 8 days shelf life. So the curcumin adding in milk increases shelf life. It is healthy as well as pleasure to drink.

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