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Process for the Extraction and Encapsulation of Curcumin in Nanoemulsion using Edible oils

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Abstract

A green technique for the isolation of curcumin from turmeric is developed in this study instead of the conventional method of using organic solvents. Curcumin was extracted using edible oils under different process conditions. The quality parameters of edible oils such as free fatty acid value, iodine value, saponification value, unsaponificable matter and peroxide values were analysed and the fatty acid profile was estimated using gas chromatography (GC). Extraction of curcumin in bench scale showed that percentage of curcumin was higher in sesame oil making it suitable for preparation of nanoemulsion. The hydrophilic lipophilic balance (HLB) of emulsifier was derived and stable emulsions were prepared. Transmission Electron Microscopy (TEM) confirmed the particle size of prepared nanoemulsion as less than 200 nm. The emulsion size and properties were further confirmed by Differential Light Scattering (DLS). Poly Dispersity Index (PDI) of 0.237 corresponds to homogeneous particle size and stability of curcumin nanoemulsion. Curcuminoids are water insoluble and its applications are limited. Hence, a water soluble form of curcuminoids having medical and nutraceutical application was developed.

Keywords: *FFA: Free Fatty Acid, HLB: Hydrophobic Lipophilic Balance, GC: Gas Chromatography, O/W: Oil in Water, W/O: Water in Oil*

1. Introduction

Curcumin (diferuloylmethane) is the yellow pigment in turmeric and is used extensively in food and chemical industry, used as coloring, flavoring and Chemically, curcumin is a bis-α, β-unsaturated β-diketone exhibiting keto-enol tautomerism (Sharma *et al.*, 2005) having a predominant keto form in acidic and neutral solution and stable enol form in alkaline medium (Wang et al., 1997).

1.1 Biological properties of curcumin

The pharmacological safety and efficiency of curcumin makes it a potential compound for treatment and prevention of a wide variety of human diseases. Curcumin has been found to have a wide spectrum of biological and pharmacological activities such as antioxidant, anti-inflammatory (Ruby *et al.*, 1995), antimicrobial, anticarcinogenic (Anand *et al.*, 2007) hepato and nephroprotective (Bartine, 1997), antifungal (Barthelemy, *et al.*,1998), antiviral and enhanced wound healing have been assumed as other benefits of curcumin.

Curcumin has been studied in multiple human carcinoma condition like melanoma, head and neck, breast, colon, pancreatic, prostate and ovarian cancer (Barthelemy *et al.*, 1998, Anand, *et al.*, 2008). Multi action of curcumin and its anti-cancer effect are diverse and comprehensive, targeting many levels of regulation in the process of cellular growth. Curcumin molecule modulates an array of targets involved in the cell signaling pathways such as transcriptional factors, cytokine, kynase, receptors and growth factors which results in neoplasia, inflammation, unregulated apoptosis or a combination of these (Arbiser *et al.,* 1998). A nanoemulsion is thermodynamically stable liquid dispersion of an oil phase a water phase, and a surfactant. Small size of nanoemulsion leads to useful properties such as high surface area, stability, transparent appearance, and tunable rheology. Nanoemulsions have applications in areas like drug delivery, food, material synthesis, pharmaceuticals and cosmetics (Gupta *et al.,* 2016). The dispersed phase is composed of small droplets and has very low oil/water interfacial tension. The size of droplet is less than 25% wavelength of visible light, making it transparent. Nanoemulsion is also known as mini-emulsion submicron emulsion and ultrafine emulsion.

2. Materials and Methods

All chemicals and solvents used were from Ranbaxy fine chemicals Ltd., Sigma Aldrich standards and MERCK. Turmeric powder and edible grade oils procured from local market.

2.1 Physicochemical characteristics of lipid solvents

Physical and chemical characteristics of edible oils like Coconut oil, Palm oil, Sunflower oil and Sesame oil were estimated. The Free Fatty Acid (FFA), iodine value, peroxide value, saponification value and unsaponifiable matter were determined by standard methods (AOCS, 1997). The fatty acid profile of lipids was estimated by gas chromatography.

2.2 Extraction of curcumin from turmeric

Curcumin was extracted from turmeric powder using four different types of oils Coconut oil, Palm oil, Sunflower oil and Sesame oil under four different process conditions like 1) magnetic stirring at room temperature 2) magnetic stirring at temperature 50°C 3) ultrasonication at room temperature 4) ultrasonication at temperature 50°C.

2.3 Estimation of curcumin: By Spectrophotometric method

The curcumin contents in turmeric powder and oil extracts were estimated using spectrophotometer. The absorbance at 425 nm of a dilute solution of alcohol extract of turmeric is taken and the percentage of curcumin is calculated using the standard value of absorbance 0.42 for a solution containing 0.0025 g of curcumin/ liter. The absorbance measured at 425 nm in 1 cm cell against an alcohol blank.

2.4 Preparation of oil in water nanoemulsion

Based on hydrophilic lipophilic balancing (HLB) value of all ingredients like emulsifiers and oil, the proportion of the component calculated and prepared a stable nano emulsion.
Components: Curcuminated sesame oil, Water, Emulsifiers-Tween 80 and Span 85

The proportion of constituents in emulsion preparation was 2.5 g of oil and 7.5 g of water. The surfactant constitution varied in the following order and 5 samples were prepared, to which 0.1g (0.06 g span 85 & 0.04 g tween 80), 0.2 g (0.12 g span 85 & 0.08 g tween 80), 0.3g (0.18 g span 85 & 0.12 g tween 80), 0.4 g (0.24 g span 85 & 0.16 g tween 80) and 0.5 g (0.3 g span 85 & 0.2 g tween 80) of emulsifiers added, respectively and sonicated for 1 hour at room temperature. After sonication, it was allowed to stand for two hrs for layer separation. Curcumin loaded sesame oil in water nanoemulsions was prepared by dissolving 16 g of the aqueous emulsifier, Tween 80 in 750 ml of water and stirred for half an hour. 24 g of Span 85 which was soluble in oil phase was added to 250 g oil and stirred for half an hour. After thorough mixing these two phases are mixed together and passed through high pressure microfluidizer at 35000 psi. The microfludization cycles were repeated until the droplet size reached nano range. After preparation of emulsion it was stored at 20°C for further studies. The nanoemulsion obtained was dissolved in acetone and analyzed using UV-VIS spectroscopy. The acetone was kept as blank and compared to curcumin content in oil and emulsion.

2.5 Determination of particle size of nano emulsions

The average particle size (hydrodynamic diameter, Z average) and polydispersity index (PDI) analyses of the prepared nanoemulsions were determined by using Zetasizer NanoZS (Malvern, UK) by the principle of Dynamic Light Scattering (DLS).

2.6 Transmission Electron Microscopy (TEM)

TEM analysis was performed to determine the morphology, size and shape of the nanoemulsions. TEM measurements were done by HITACHI 226 H-7650, operating at 120 kV. The TEM grid was prepared by placing a drop of the bio-reduced diluted solution on a carbon-coated copper grid and later drying it under a lamp.

3. Results and Discussion

The physiochemical properties like FFA, iodine value, peroxide value, saponification value and unsaponifiable matters of the four oil samples are given in Table.1.

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The quality parameters of four different oils showed that these were edible grade and analytical results are comparable to the standard specifications. Iodine value is higher in the case of sunflower oil. That means, unsaturation is higher in sunflower oil and iodine value is very low for coconut oil because it is saturated. From the table it is seen that peroxide value is higher for sesame oil and is very low for coconut oil. Peroxide value is the measure of extent to which an oil sample can undergo primary oxidation. Saponification value is higher in the case of coconut oil.

Table .1: Quality parameters of oils

Sample	Fatty acid composition							
	C8	C10	C12	C14	C16	C18	C18:1	C18:2
Coconut oil	3.52	0.26	45.96	21.95	10.39	3.69	8.14	2.07
Sesame oil					9.27	5.71	41.66	43.29
Palm oil				0.185	49.32	0.18	38.43	11.13
Sunflower oil			1.14	0.58	6.63	3.57	26.61	61.17

3.1 Fatty acid composition of edible oil estimated using GC

The coconut oil contains about 45% lauric acid. The next major fatty acid is myristic acid (C14). Other fatty acids found in coconut oil are C: 8, C: 10, C: 16, C18:1 and C18:2.*ie.* Coconut oil contain higher amount of saturated fatty acids. Four fatty acids are mainly found in sesame oil. In sesame oil, 43.29 % is C18:2 which is the major fatty acid. C18:1 is about 41.66 %; C18 is about 5.71% and C16 about 9.27 %. Palm oil mainly contains C18:2, C18:1, C16 and very trace amount of C14 and C18.
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Table.2: Relative percentage of fatty acid composition of different oils

Samples	Parameters				
	Free Fatty Acid (FFA) value	Iodine Value	Peroxide value	Saponification value	Unsaponifiable matter
Coconut oil	0.82	7.10	0.19	256.18	0.86
Palm oil	0.62	108.60	2.11	196.80	0.20
Sesame oil	0.70	110.70	2.23	189.90	0.61
Sunflower oil	0.50	136.30	0.993	187.30	0.95

C16 in major amount, about 49.32%. C18 is about 0.18% only. Palm oil also contains unsaturated fatty acids C18:1, having about 38.43% and C18:2, about 11.13%. In sunflower oil C18:2 is the fatty acid found in higher amount having about 61.17% and contains C14, C12 in very trace amount. It contains unsaturated fatty acids C18:1 and C18:2 which are higher than palm oil. The relative fatty acid compositions of the different oils are given in table 2 and the GC chromatograms shown in Fig1-4.

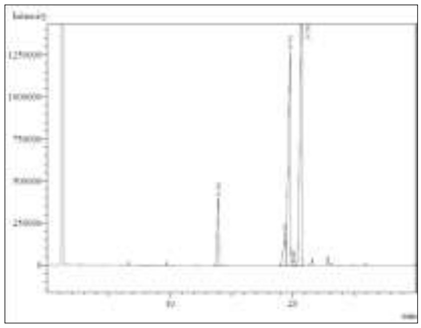

Fig.1 Gas chromatogram of coconut oil

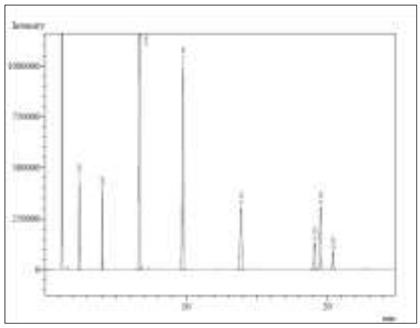

Fig 2 Gas chromatogram of sesame oil

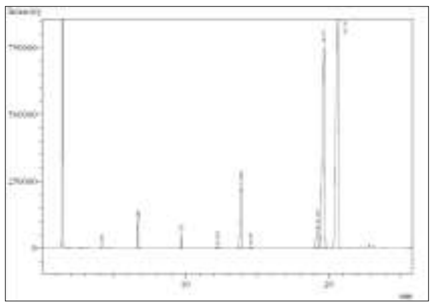

Fig.3 Gas chromatogram of palm oil

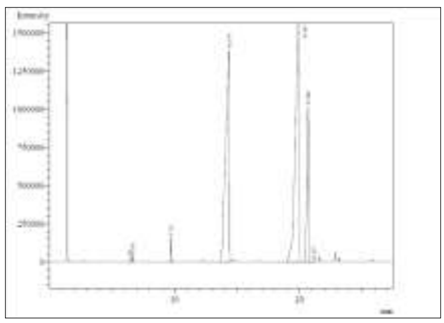

Fig.4 Gas chromatogram of Sunflower oil

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3.2 Spectrophotometric analysis of curcumin extracted oil

The curcumin content was higher in sesame oil (0.29%) followed by coconut oil (0.22%). In sunflower oil, curcumin solubility is low compared to other three oils. Details of curcumin content in different oil samples are given in Table.3.

Table.3. % of curcumin in different oils

Type of oils	% of curcumin			
	Magnetic stirring at room temp	Magnetic stirring at 50˚C	Sonication at room temp	Sonication at 50˚C
Coconut oil	0.17	0.21	0.21	0.22
Sesame oil	0.28	0.29	0.18	0.18
Palm oil	0.16	0.20	0.17	0.19
Sunflower oil	0.13	0.19	0.15	0.17

3.3 Preparation of stable emulsion

2.5 g of curcuminated sesame oil, 7.5 g of water, 0.4 g and 0.5 g emulsifier proportions showed good stability. There was no layer separation after two hours. In all other combinations, oil and water layers separated. The different combination of the emulsions is given in Fig.5. The samples with emulsifiers of concentration 0.4 g and 0.5 g are more stable. This combination was used for further studies.

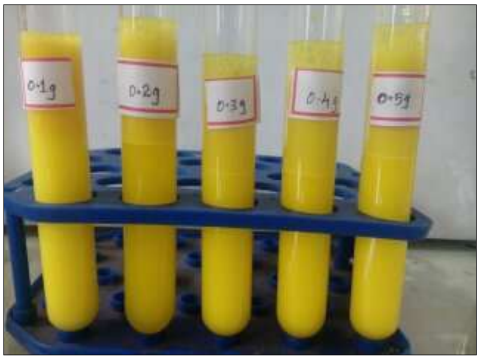

Fig.5 Emulsions with different proportions of emulsifier

3.4 Nanoemulsion by Microfluidizer

After first cycle of microfluidization, the sample was turbid and opaque microemulsion was obtained. Fig.6 is the micro emulsion of curcumin loaded sesame oil in water. The microfluidization process was continued and after 5th cycle samples were collected. The colour of samples reduced and visibly transparent emulsions were obtained. Nanoemulsions from 6th cycle to 10th cycle was collected after micro fluidization and labeled as 6-10 (Fig.6). Nanoemulsions appear visibly different from microemulsions since the droplets can be much smaller than optical wavelength of visible spectrum.

Fig.6 Nanoemulsions

3.5 Characterization of nanoemulsion Particle size analysis by DLS

Analysis of the average particle size and size distribution is essential to use nanoparticles in many applications (Salata, 2004; Zook *et al.*, 2011). The DLS measurements of polydisperse nanoparticles were expected in the range of 100 – 300 nm with a PDI of 0.4 or below generally.

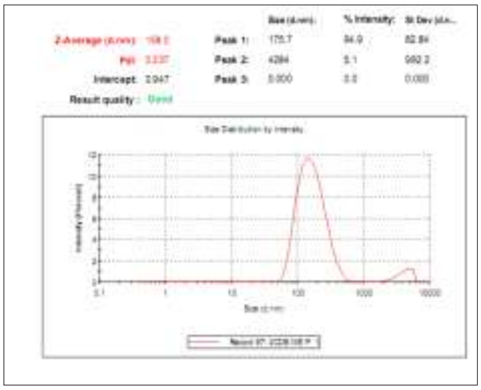

Fig.7a Particle size distribution of curcumin nanoemulsion

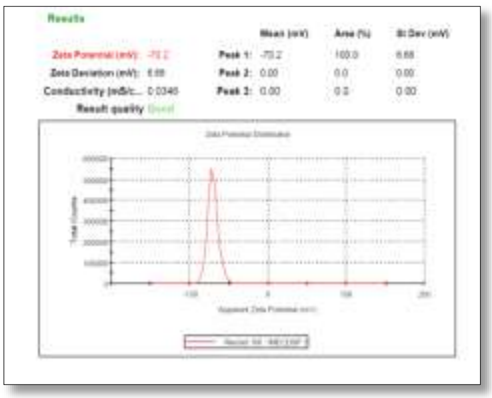

Fig.7 b Zeta potential of curcumin nanoemulsion

The particle size analysis of curcumin nanoemulsion showed that more than 90 % particles are less than 200 nm. A low value of poly dispersity Index (PDI) 0.237 corresponds to homogeneous particle size of the nanoemulsion. Low particle size of 158 nm increases the bioavailability of curcumin. The zeta potential of -70.2 mV was obtained for curcumin nanoemulsion.

3.6 Transmission Electron Microscope (TEM) Analysis

TEM analysis confirmed the particle size of prepared curcumin nanoemulsion in the range of 100-150 nm.

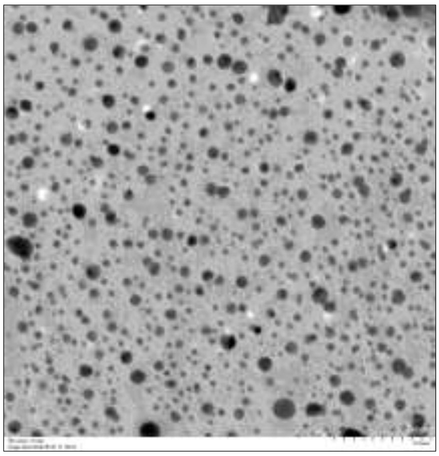

Fig.8. TEM image of curcumin nanoemulsion

6. Conclusions

The quality parameters of coconut oil, palm oil, sunflower oil and sesame oil such as free fatty acid value, iodine value, saponification value, unsaponifiable matter and peroxide value were comparable with edible grade oils. The major fatty acids in the four oil samples were estimated by GC. The percentage of curcumin was higher in sesame oil compared to other oil samples. Therefore curcuminated sesame oil was used as the oil medium for the preparation of emulsion. Nowadays water soluble curcuminoids have many health and nutraceutical applications. In modern food formulations, water soluble curcuminoids are added to enhance the antioxidant and nutritional value. In this study a green technique for the isolation of curcuminoids from turmeric powder was developed.

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References

[1] Anand, P., Kunnumakkara, A. B., Newman R.A., Aggarwal. B.B. Bioavailability of curcumin: Problems and promises. Molecular Pharmaceutics. **4**(6): 807-818 (2007).

[2] Anand, P., Sundaram, C., Jhurani, S., Kunnumakkara, A.B., Aggarwal, B.B. Curcumin and cancer: An “old-age” disease with an “age-old” solution. Cancer Letters., **267**(1): 133-164 (2008).

[3] AOCS, Official Methods and Recommended Practices of American Oil Chemists’ Society, 5th edn., edited by D. Firestone, AOCS Press, Champaign, Methods Ca 5a-40, Cd 1-25, Ca 2c-25, Ca 6a-40, Cd 3d -63, Ca 14 -56 (1997).

[4] Arbiser, J.L., Klauber, N., Rohan, R., van Leeuwen, R., Huang, M.T., Fisher, C., Flynn, E., Byers, H.R. Curcumin is an in vivo inhibitor of angiogenesis. Mol Med., **4**(6): 376-383(1998).

[5] Barthelemy, S., Vergnes, L., Moynier, M., Guyot, D., Labidalle, S., Bahraoui, E. Curcumin and curcumin derivatives inhibitTat-mediated transactivation of type 1 human immunodeficiency virus long terminal repeat. Research in Virology., **149**(1): 43-52 (1998).

[6] Bartine, H. and Tantaoui-Elaraki, A. Growth and toxigenesis of *Aspergillus flavus* isolates on selected spices. J Environ Pathol Toxicol Oncol. **16**(1): 61-65 (1997).

[7] Gupta, A., Eral, H.B., Hatton T.A, Doyle, P.S., Nanoemulsions: formation, properties and applications. Soft Matter., **12**(11):2826-41 (2016).

[8] Ruby, A.J., Kuttan, G., Babu, K,D., Rajasekharan, K.N., Kuttan, R. Antitumor and Antioxidant Activity of Natural Curcuminoids. Cancer Letters. **94**(1): 79-83 (1995).

[9] Salata, O.V., Applications of nanoparticles in biology and medicine. Journal of Nanobiotechnology., **2**(3) :(2004).

[10]Sharma, R.A., Gescher, A. J., Steward, W.P. Curcumin: The story so far. European journal of cancer, (Oxford, England: 1990) **41**(13): 1955-1968 (2005).

[11]Wang, Y.J., Pan, M.H., Cheng, A.L., Lin, L.I., Ho, Y.S., Hsieh, C.Y., Lin, J.K. Stability of curcumin in buffer solutions and characterization of its degradation products. Journal of Pharmaceutical and Biomedical Analysis. **15**(12), 1867-1876 (1997).

[12]Zook, J. M., Rastogi, V., MacCuspie, R. I., Keene, A. M. and J. Fagan, Measuring agglomerate size distribution and dependence of localized surface plasmon resonance absorbance on gold nanoparticle agglomerate size using analytical ultracentrifugation. ACS Nano, vol. **5**(10): 8070-8079 (2011).