

Optimized Method for Curcumin Separation from Turmeric Oleoresin

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ABSTRACT: *In this work, a novel and modified method for the solvent purification of Curcumin is presented, a composite of normal hexane & 2-propanol solvents was used to separate the fat content and other impurities. The process was modeled considering the response surface methodology as described by the central composite design. The adjusted R-squared was 0.9443 indicating the variation between the regressors (solvent composition, solvent-to-oleoresin ratio, and temperature) in relation to the selected responses (product purity and process yield) were well described by the constructed model. Optimum conditions were 90% w/w 2-propanol in the solvent, 1.5 solvent-to-oleoresin weight ratio and 5°C temperature with predicted 95.94% purity and 35.77% yield, F values were significant for both models. Therefore, the development of high purity products is achievable with this novel method with economic advantages.*

KEYWORDS *Curcumin; Purification; Response surface methodology; FT-IR; NMR; Curcumin gelatin complex.*

INTRODUCTION

Turmeric, the dried and powdered root of the *Curcuma Longa* plant is rich in Curcuminoids. Turmeric is a commonly used food additive and colouring agent [1], especially in Asia, it is also used as a preservative. It is obtained by drying and powdering the rhizomes of the plant *Curcuma Longa* L., Zingibraceae. Curcuminoids are a family of compounds among the main components of Turmeric, namely Curcumin, Demethoxycurcumin and Bisdemethoxycurcumin. These compounds, especially Curcumin itself, have been proven to have many medicinal applications as anti-inflammatory [2-4] and antibacterial [5] drugs. They have also been proven to have anti-diabetic, anti-cancer [2; 6] and immunomodulatory

effects and also been shown to have positive effects for the prevention of Alzheimer's disease [7] and HIV alongside AZT [8] among many other possible health benefits [9;10].

Curcumin, however, is not soluble in water and as a result, less than 2% of the curcumin available (which makes up about only 4-6% of turmeric) can be absorbed by the body through direct consumption [11-13] and a range of methods have been employed to improve the bioavailability of curcumin [14]. Therefore to use Curcumin as a drug or for other applications, it is necessary to extract Curcumin from turmeric and also purify Curcumin [15; 16]. Many methods have been proposed

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for extraction [17-19] which have been studied in depth and optimized, such as maceration [20; 21] and microwave assisted extraction [22; 23] as well as innovative technologies such as molecularly imprinted polymers [24;25].

Purification processes, however, can also be very time consuming and expensive and require large amounts of solvents. In addition, with regards to the specifications of the product required, operating conditions of a single process may be changed for the process to be carried out optimally. Therefore, it is of great importance to study the effects of operating conditions on the product obtained and also the interaction between the different parameters.

In this work, the three factors; solvent composition, solvent to oleoresin ratio, and operation temperature were studied and their effects on the purity of curcumin as product and process yield further investigated. Using a central composite design, optimal conditions in terms of both product purity and process yield were obtained. In addition, Curcumin obtained in optimum conditions was utilized in the production of a Curcumin-Gelatin complex, a product with high aqueous solubility and many applications in food and pharmaceutical industries, and compared with product specifications in the existing literature.

EXPERIMENTAL SECTION

Materials

Acetone and 2-propanol were obtained from Merck, n-Hexane was obtained from Oxford Laboratories. Dried and powdered Curcuma Longa rhizomes were obtained from local suppliers. Pure Curcumin for reference was obtained from Merck.

All materials and intermediary products were stored below -10°C and sheltered from moisture and light.

Oleoresin Extraction

Turmeric extract was obtained from the powder using a Soxhlet apparatus Tecator (Soxtec System HT 1043 Extraction Unit) with acetone as a solvent and used after extraction as is.

Design of Experiments:

Solvent extraction of impurities using a defatting solvent was the method of purification used, whereby the oleoresin was stirred in direct contact with the solvent

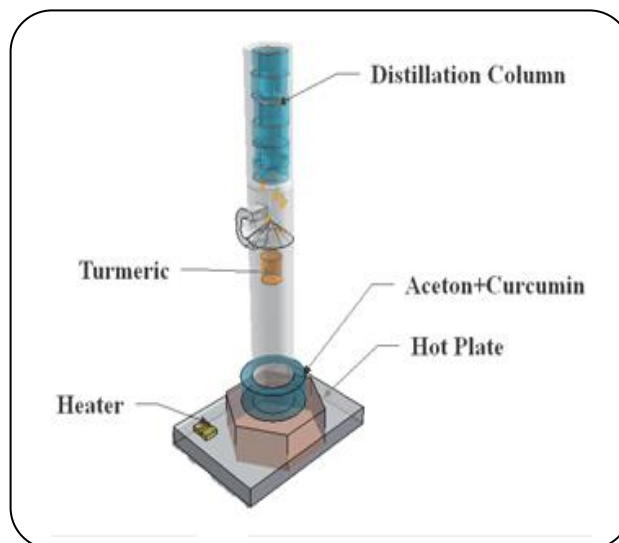


Fig. 1: Schematic of the extraction elements through the Soxhlet apparatus Tecator.

for 3 hours. The temperature was kept constant using an incubation system. The solution was then immediately suction filtered using equipment that was also maintained at the same temperature. Subsequently, the filter cake dried under vacuum at 50°C . The experiments were conducted in vessels protected from light with amber glass and aluminum foil. The purification solvent was composed of 2-propanol and n-Hexane.

The experiments were designed with the response surface methodology Central Composite Design (CCD), Design Expert 7.0.0, the independent Factors are 2-propanol in solvent (g/g), Solvent to Oleoresin weight ratio (g/g), and Temperature ($^{\circ}\text{C}$).

These Factors were selected based on preliminary experiments. The upper limit to 2-propanol in solvent composition was chosen as 0.8 since, at higher ratios, very little product was obtained and sharp decreases in yield were observed. Higher temperatures resulted in low purity and temperatures above 20°C were found to result in purities too low to be valid for consideration. On the other hand, very low temperatures resulted in viscous products due to poor oleoresin separation, this caused process difficulties and imperfect filtration.

The dependent Factors were the Curcumin composition of the product, which was analyzed using a Unic2100 Spectrophotometer at 419nm, and purification yield which was calculated as Curcumin Mass in product per Curcumin Mass in oleoresin precursor.

$$\text{Purity}(\%) = \frac{\text{currcumin content in product} \left(\frac{\text{g}}{\text{g}} \right)}{\text{Oleoresin precursor}(\text{g})} \times 100\% \quad (1)$$

$$\text{Yield}(\%) = \frac{\text{pure curcumin obtained}(\text{g})}{\text{Oleoresin precursor}(\text{g})} \times 100\% \quad (2)$$

Design Expert[®] 7.0.0 (Stat-Ease Inc., Minneapolis, MN, USA) was used for the experimental design and analysis of variance by RSM. The significance of the parameters effective in the results was analyzed using the F-test and only factors with a P-Value below 0.05 were deemed significant.

Analysis

The standard curve for pure Curcumin (purchased from Merck) was obtained using Unic2100 Spectrophotometer. The peak of maximum absorption in acetone solution was found at 490nm and, at low enough concentrations, a linear relation between concentration and absorption number was found:

$$\text{ABS} = 0.1911 \cdot C \quad (3)$$

Where *ABS* (a.u) is the absorption number and *C* (mg/L) is the concentration of Curcumin in an acetone solution. This method was used to analyze the purity of products. Validation of Curcumin as the major component in the product was conducted with FT-IR and NMR.

Gelatin-Curcumin Complex for increased aqueous solubility

The optimized product was used for the production of Curcumin Gelatin Complex, gelatin being a well-researched substrate for the increase of Curcumin solubility [26-28], by dissolving Curcumin and 100 bloom gelatin in a 75% acetic acid / 25% water solution. Separate solutions were prepared with 5% and 10% weight ratio of Curcumin to gelatin respectively, by adding 0.15g (0.3g) Curcumin and 3g gelatin to 6mL solution and stirring at 80°C. The product was then dried under vacuum and ground using a mortar and pestle. 0.01g of the product was dissolved in 100mL water and the amount of Curcumin loaded on gelatin was obtained using spectrophotometry in aqueous solution at 420nm according to the method presented in literature [2].

RESULTS AND DISCUSSION

Using FTIR and NMR techniques, as we see in the following results shown in Fig. 2, the major component in the final product is Curcumin for sure.

The results of the purification experiments are presented in Table 2, all three parameters were found to be effective in the purity and yield of purification. Other factors such as purification time and purification pressure were determined to have very little influence on the results from preliminary experiments, however, these parameters should be considered in the design and scale-up of this process.

The ANOVA tables for both dependent variables are presented in Table 3. As can be seen, all three factors influence the purity and yield of the product significantly. This shows that the model is able to accurately predict the *Purity* of the product and *Yield* of the process, the following equations were :

$$\text{Purity} = -1042.50219 + 1341.21857 \cdot \text{IWR} + 541.68276 \cdot S - 0.27898 \cdot T - 642.40667 \cdot \text{IWR} \cdot S - 3.57813 \cdot S \cdot T + 0.29132 \cdot T^2 \quad (4)$$

$$\text{Yield} = -414.20488 + 607.75309 \cdot \text{IWR} + 183.08805 \cdot S - 0.41636 \cdot T - 218.20064 \cdot \text{IWR} \cdot S - 1.30001 \cdot S \cdot T - 91.44581 \cdot \text{IWR}^2 + 0.083722 \cdot T^2 \quad (5)$$

In which *IWR* is the isopropanol weight ratio in solvent (g/g), *S* is the solvent to oleoresin weight ratio (g/g) and *T* is the Temperature (°C).

The Response Surface plots for the effects on the independent variables on the dependent variables are presented in Fig. 3.

As is evident from Fig. 3 (a), the effect of 2-propanol concentration on purity is positive for lower amounts of solvent to oleoresin, but negative for higher ratios. This is a result of phase equilibria between solvent and solid residue, showing there is an optimum composition of solvent to achieve minimum oily residue in solid sediment. The surface plot in Fig. 3 (c) shows similar behavior for the yield of extraction. In other words, purity plots and yield plots show there is a positive correlation between these two. A very sharp drop in yield can be seen when increasing solvent amount at high 2-propanol composition, this is attributed to the relative solubility of curcumin in 2-propanol. Curcumin will be dissolved at relatively higher concentrations when the solvent has higher 2-propanol in composition; effectively increasing lost curcumin and decreasing yield sharply as more solvent is used. The independent effect of solvent to oleoresin ratio

Table 1: NMR Analysis of purified Curcumin.

ppm shift	
2.5, 3.84, 6.05, 6.71, 6.75, 6.81, 6.83, 7.13, 7.15, 7.30, 7.52, 7.55	H NMR
55.66, 111.4, 115.66, 121.05, 122.98, 126.27, 140.56, 147.94, 149.31, 183.07	C NMR

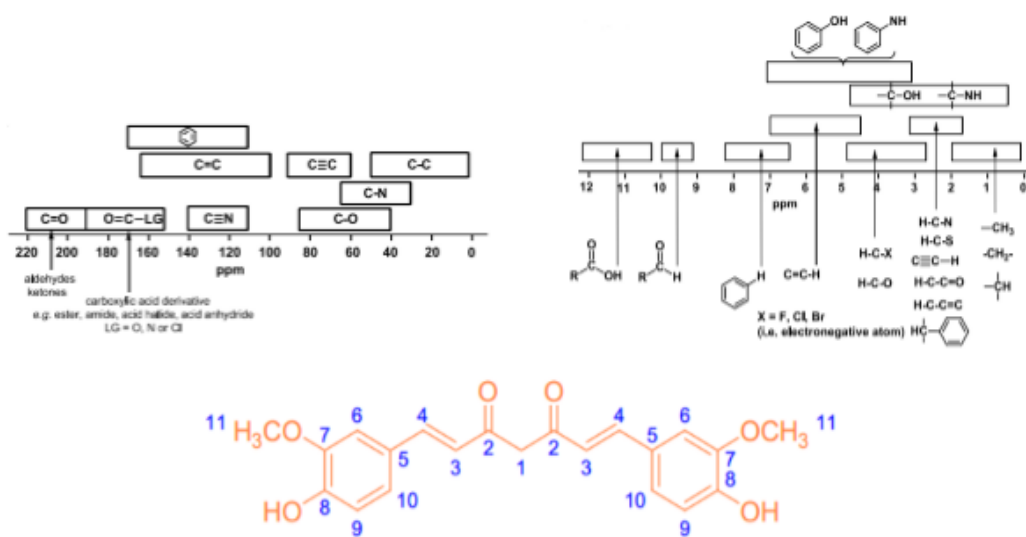
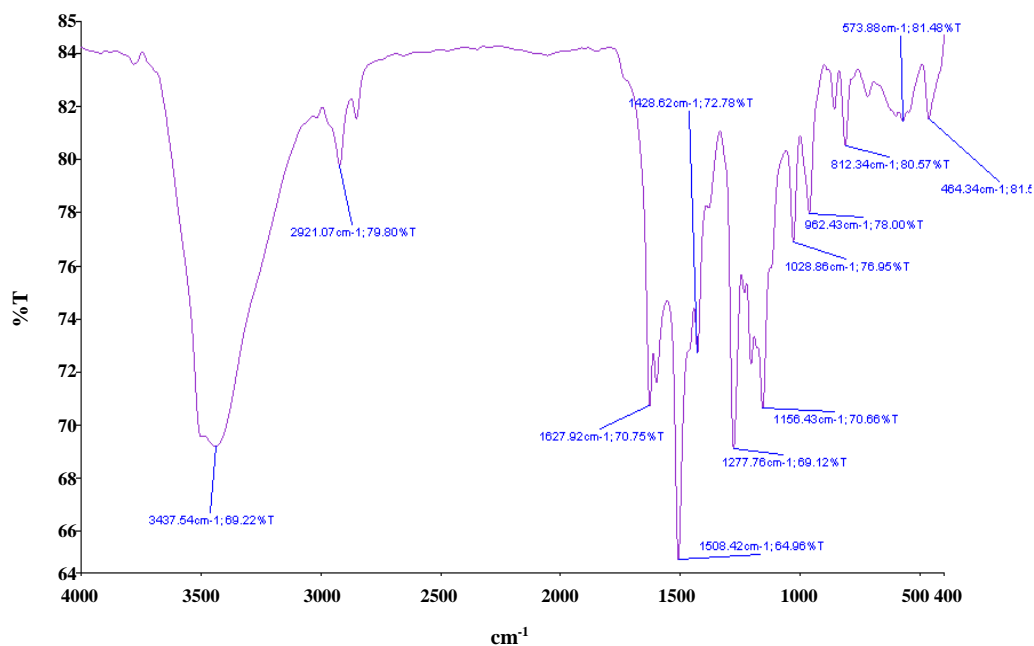


Fig. 2: (a) FTIR and (b) H-NMR spectra peaks for Curcumin.

Table 2: Design matrix for a face-centered cube CCD⁽¹⁾.

Experiment number	Coded level of factors			The actual level of factors			Responses			
	x ₁	x ₂	x ₃	IWR (g/g)	S (g/g)	T (°C)	Purity%		Yield%	
							Actual	predicted	Actual	predicted
1	+1	0	-α	0.8	2	0	86.60	85.99	30.71	30.52
2	0	+1	+1	0.7	2.5	15	51.16	64.87	19.43	18.61
3	+1	0	0	0.8	2	10	46.75	45.48	15.53	17.06
4	+1	0	0	0.8	2	10	41.59	45.48	16.31	17.06
5	+1	0	+α	0.8	2	20	54.34	56.89	18.98	20.33
6	+α	0	+1	0.9	2	15	45.81	55.30	21.29	18.19
7	+1	-α	0	0.8	1	10	49.57	52.19	22.23	21.53
8	0	-1	-1	0.7	1.5	5	13.40	11.57	6.36	6.36
9	+1	0	0	0.8	2	10	38.44	44.83	15.70	17.06
10	+α	0	0	0.9	2	10	48.21	50.18	17.05	18.65
11	+1	+α	0	0.8	3	10	35.89	32.44	12.47	12.58
12	+1	0	0	0.8	2	10	44.98	45.48	16.62	17.06
13	-1	0	0	0.6	2	10	25.73	26.72	8.58	8.39
14	+α	+1	-1	0.9	2.5	5	32.18	28.72	14.21	13.39
15	+1	0	0	0.8	2	10	39.17	34.58	18.22	17.06

(1) Alpha (α) was selected equal to 2; Three levels, Three factor model used.

Table 3: Analysis of variance (ANOVA) for the statistical models⁽¹⁾.

Source of variation	Purity%		Yield%	
	F	Prob > F	F	Prob > F
Model	40.59	<0.0001	20.51	0.0004
x ₁	19.81	0.0021	18.04	0.0038
x ₂	11.01	0.0106	15.80	0.0054
x ₃	40.29	0.0002	18.66	0.0035
x ₁ x ₂	132.3	<0.0001	70.38	<0.0001
x ₂ x ₃	10.26	0.0126	6.25	0.0410
x ₁ ²	-	-	6.85	0.0346
x ₃ ²	98.49	<0.0001	35.86	0.0005
Residual	-	-	-	-
Lack of Fit	1.18	0.4394	4.91	0.0792
R-Squared	0.9682		0.9535	

(1) Significant at P value < 0.05

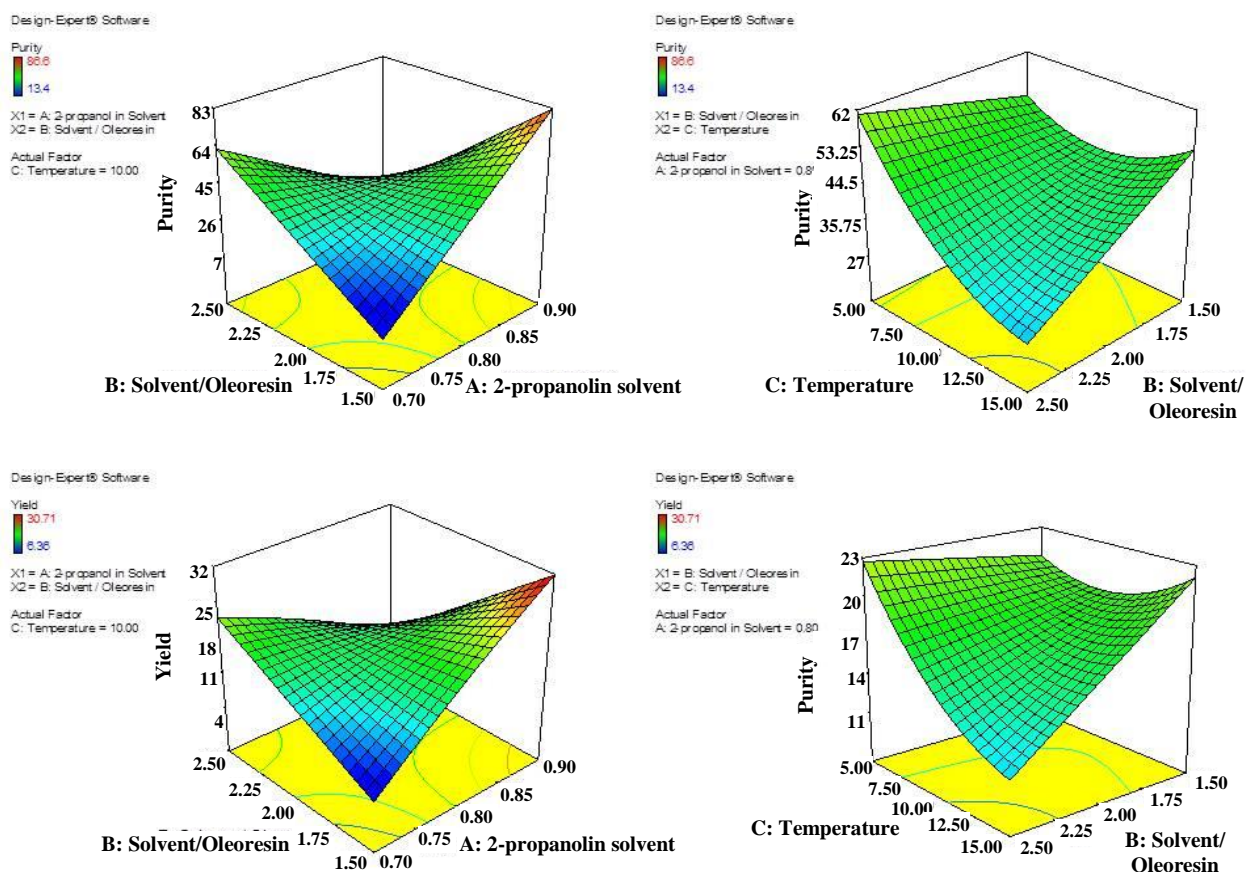


Fig. 3: (a) Surface plot of the effects of 2-propanol concentration in solvent and solvent to oleoresin ratio on purity at constant temperature 10 C° (b) Surface plot of the effects of temperature and solvent to oleoresin ratio on purity at constant 2-propanol concentration in solvent equal to 0.8 (c) Surface plot of the effects of 2-propanol concentration in solvent and solvent to oleoresin ratio on yield of extraction at constant temperature 10 C° (d) Surface plot of the effects of temperature and solvent to oleoresin ratio on yield of extraction at constant 2-propanol concentration in solvent equal to 0.8.

also differs for solvents with low and high 2-propanol concentration. In Fig. 3 (b, d) it is apparent that the yield and purity of the extracted Curcumin increase with an increase in the solvent to oleoresin ratio and decrease of temperature; it is noteworthy that these graphs are at constant solvent composition consisting of 0.8 w/w ratio 2-propanol. The difference between the morphologies of surface plots in Fig. 3 (a, c) and Fig. 3 (b, d) demonstrate there is an optimum point for the process at each constant temperature.

A clear interaction can be seen between parameters x_1 and x_2 , and a second degree dependence on temperature is observed for both purity and yield. Furthermore, R-squareds for both responses were above 0.95 and F values were significant for both models which indicate

they are promising models for the prediction of desired purity and yield.

Optimization

With the RSM results obtained, the purification process was optimized with Design Expert 7.0.0 to obtain the parameters resulting in maximum purity and yield. By defining a desirability function with absolute maximums of 100% purity and 100% yield, 30 solutions were found and the highest desirability obtained was 56%. The optimum parameters selected for this condition were 2-propanol weight ratio of 0.9 in solvent, solvent to oleoresin ratio of 1.5 and temperature of 15°C which would result in a product of purity 90.70% with a yield at 34.59%. This procedure was repeated experimentally

with a yield of 36.1% and the product obtained was found to have a purity of 89.93%.

Gelatin-Curcumin Complex with increased aqueous solubility

The product was used to produce two water-soluble Gelatin Curcumin complex products with 5% and 10% powder to gelatin ratio, respectively. The spectrophotometric absorbance was measured at 420nm in 1cm path length cells. Standard Curve was obtained in the water at the measured peak of absorbance of 420nm. Pure Curcumin (Merck) was used to produce a gelatin complex using 1% powder to gelatin ration (resulting in complete loading of the amount of Curcumin used and known composition), this product in low concentrations was used to create a standard curve. Gelatin has low absorption in 420nm, even this small effect was eliminated by using a similar Gelatin solution as a reference in spectrophotometry.

For the first sample (5%), an absorbance of 0.112 and for the second sample, absorption of 0.205 was obtained. Several samples were prepared using 20% to 25% Curcumin to gelatin in preparation with maximum loading. The absorbance numbers obtained were similar, constant in relation to the Curcumin to gelatin ratio and used to normalize data accordingly to compare to literature. The results show a 28% increase in the amount of loading using 5% Curcumin and an increase of 6.77% using 10% Curcumin with respect to the literature [2].

CONCLUSIONS

The effects of three parameters on the purification of Curcumin from Curcuma Longa Extract (Oleoresin) were studied with the design of the experiment. ANOVA showed that all three factors had a significant influence on the purity of the product and yield of the process. The response surface method was successfully employed to find optimum parameters for the process. Solvent composition and Solvent amount have a significant influence on the characteristics of the product and impact the efficiency of the process. Temperature also impacts the yield of purification and purity of the product compounds. This work presents the impact of purification process parameters on the product along with scientific evidence in its support. This work also can provide applicable data in the process design and development for the

purification of Curcumin and proves high purity products can be achieved in this method, which would be economically advantageous.

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REFERENCES

- [1] El-Shishtawy R.M., Shokry G., Ahmed N.S., Kamel M., [Dyeing of Modified Acrylic Fibers with Curcumin and Madder Natural Dyes](#), *Fibers and Polymers*. **10**(5): 617-624 (2009)
- [2] Neven P., Seretein D., Delarge J., Kiss R., Mathieu V., Cataldo D., Rocks N., ["Water Soluble Curcumin Compositions for Use in Anti-Cancer and Anti-Inflammatory Therapy"](#), in Google Patents(2014)
- [3] Patil M., Taralkar S., Sakpal V., Shewale S., Sakpal R., [Extraction, Isolation and Evaluation of Anti-Inflammatory Activity of Curcuminoids from Curcuma Longa](#), *International Journal of Chemical Sciences and Applications*. **2**(3): 172-174 (2011)
- [4] Rao T.S., Basu N., Siddiqui H., [Anti-Inflammatory Activity of Curcumin Analogues](#), *The Indian Journal of Medical Research*, **137**(4): 841-845 (2013).
- [5] Ghoreishian S.M., Maleknia L., Mirzapour H., Norouzi M., [Antibacterial Properties and Color Fastness of Silk Fabric Dyed with Turmeric Extract](#), *Fibers and Polymers*. **14**(2): 201-207 (2013).
- [6] Duvoix A., Blasius R., Delhalle S., Schnekenburger M., Morceau F., Henry E., Dicato M., Diederich M., [Chemopreventive and Therapeutic Effects of Curcumin](#), *Cancer Letters*, **223**(2): 181-190 (2005).
- [7] Lim G.P., Chu T., Yang F., Beech W., Frautschy S.A., Cole G.M., [The Curry Spice Curcumin Reduces Oxidative Damage and Amyloid Pathology in an Alzheimer Transgenic Mouse](#), *The Journal of Neuroscience*. **21**(21): 8370-8377 (2001).
- [8] Richman D.D., Fischl M.A., Grieco M.H., Gottlieb M.S., Volberding P.A., Laskin O.L., Leedom J.M., Groopman J.E., Mildvan D., Hirsch M.S., [The Toxicity of Azidothymidine \(AZT\) in the Treatment of Patients with AIDS and AIDS-Related Complex](#), *New England Journal of Medicine*. **317**:192-197 (1987).
- [9] Aggarwal B.B., Sung B., [Pharmacological Basis for the Role of Curcumin in Chronic Diseases: an Age-Old Spice with Modern Targets](#), *Trends in Pharmacological Sciences*, **30**(2): 85-94 (2009).

- [10] Comlekcioglu N., Aygan A., Kutlu M., Kocabas Y.Z., **Antimicrobial Activities of Some Natural Dyes and Dyed Wool Yarn**, *Iranian Journal of Chemistry and Chemical Engineering (IJCCE)*, **36**(4): 137-144 (2017).
- [11] Anand P., Kunnumakkara A.B., Newman R.A., Aggarwal B.B., **Bioavailability of Curcumin: Problems and Promises**, *Molecular Pharmaceutics*, **4**(6): 807-818 (2007).
- [12] Shehzad A., Wahid F., Lee Y.S., **Curcumin in Cancer Chemoprevention: Molecular Targets, Pharmacokinetics, Bioavailability, and Clinical Trials**, *Archiv der Pharmazie*.
- [13] Esmaili M., Ghaffari S.M., Moosavi-Movahedi Z., Atri M.S., Sharifzadeh A., Farhadi M., Yousefi R., Chobert J.-M., Haertlé T., Moosavi-Movahedi A.A., **Beta Casein-Micelle as a Nano Vehicle for Solubility Enhancement of Curcumin; Food Industry Application**, *LWT-Food Science and Technology*, **44**(10): 2166-2172 (2011).
- [14] Shaikh J., Ankola D., Beniwal V., Singh D., Kumar M.R., **Nanoparticle Encapsulation Improves Oral Bioavailability of Curcumin by at Least 9-Fold When Compared to Curcumin Administered with Piperine as Absorption Enhancer**, *European Journal of Pharmaceutical Sciences*, **37**(3-4): 223-230 (2009).
- [15] Chearwae W., Wu C.-P., Chu H.-Y., Lee T.R., Ambudkar S.V., Limtrakul P., **Curcuminoids Purified from Turmeric Powder Modulate the Function of Human Multidrug Resistance Protein 1 (ABCC1)**, *Cancer Chemotherapy and Pharmacology*, **57**(3): 376 (2006).
- [16] Inoue K., Nomura C., Ito S., Nagatsu A., Hino T., Oka H., **Purification of Curcumin, Demethoxycurcumin, and Bisdemethoxycurcumin by High-Speed Countercurrent Chromatography**, *Journal of Agricultural and Food Chemistry*, **56**(20): 9328-9336 (2008).
- [17] Dandekar D.V., Gaikar V., **Hydrotropic Extraction of Curcuminoids from Turmeric**, *Separation Science and Technology*, **38**(5): 1185-1215 (2006).
- [18] Nagarajan J., Wah Heng W., Galanakis C.M., Nagasundara Ramanan R., Raghunandan M.E., Sun J., Ismail A., Beng-Ti T., Prasad K.N., **Extraction of Phytochemicals Using Hydrotropic Solvents**, *Separation Science and Technology*, **51**(7): 1151-1165 (2016).
- [19] Dandekar D.V., Gaikar V.G., **Precipitation of Curcuminoids from Hydrotrope Solution: Crystal Nucleation and Growth Kinetics from Batch Experiments**, *Separation Science and Technology*, **38**(15): 3625-3644 (2003).
- [20] Paulucci V.P., Couto R.O., Teixeira C.C., Freitas L.A.P., **Optimization of the Extraction of Curcumin from Curcuma Longa Rhizomes**, *Revista Brasileira de Farmacognosia*, **23**(1): 94-100 (2013).
- [21] Sogi D.S., Sharma S., Oberoi D.P.S., Wani I., **Effect of Extraction Parameters on Curcumin Yield from Turmeric**, *Journal of Food Science and Technology*, **47**(3): 300-304 (2010).
- [22] Wakte P., Sachin B., Patil A., Mohato D., Band T., Shinde D., **Optimization of Microwave, Ultra-Sonic and Supercritical Carbon Dioxide Assisted Extraction Techniques for Curcumin from Curcuma Longa**, *Separation and Purification Technology*, **79**(1): 50-55 (2011).
- [23] Dandekar D.V., Gaikar V., **Microwave Assisted Extraction of Curcuminoids from Curcuma Longa**, *Separation Science and Technology*, **37**(11): 2669-2690 (2002).
- [24] Suwanwong Y., Kulkeratituyut S., Prachayasittikul V., Boonpangrak S., **Effects of Polymerization Methods and Functional Monomers on Curcumin Imprinted Polymer Properties**, *Separation Science and Technology*, **49**(7): 1086-1095 (2014).
- [25] Shamsipur M., Bahrami Adeh N., Hajitarverdi M.S., Zarei F., Yazdimamaghani M.Y., **Synthesis and Properties of Plasticized Sulfur-Montmorillonite Nanocomposites by Melt-Blending**, *Iranian Journal of Chemistry and Chemical Engineering (IJCCE)*, **36**(6): 1-9 (2017).
- [26] Cao F., Ding B., Sun M., Guo C., Zhang L., Zhai G., **Lung-Targeted Delivery System of Curcumin Loaded Gelatin Microspheres**, *Drug Delivery*, **18**(8): 545-554 (2011).
- [27] Cao F., Xi Y., Tang L., Yu A., Zhai G., **Preparation and Characterization of Curcumin Loaded Gelatin Microspheres for Lung Targeting**, *Zhong yao cai= Zhongyao cai= Journal of Chinese medicinal materials*, **32**(3): 423-426 (2009).
- [28] Todd Jr P.H., "Curcumin Complexed on Water-Dispersible Substrates", in Google Patents (1991).