Process Optimization for the Production of Nanoencapsulated Curcumin and Analysis for Physicochemical Characteristics and Antioxidant Mechanism

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Abstract

Polyphenols are compounds present in foods of plant origin that act as major antioxidants in a balanced diet. Curcumin, a polyphenolic phytochemical has been identified as a potent natural agent by ancient Indian and Chinese systems of medicine. The aim of the present study was to optimise the process for preparation of nanoencapsulated curcumin in different edible vegetable oils and to validate the physicochemical properties and antioxidant effect in vitro. Curcumin could successfully encapsulate in palm oil, olive oil and butter oil by emulsion technique. The physicochemical properties of the prepared emulsions showed a strong dependence with the composition of the system. The highest solubility was obtained with oil rich in short chain fatty acid and increased with heating to 80°C for 30 min. The curcumin encapsulated nanoparticles were able to withstand different processing temperature, change in pH (5-7) and ionic strength (0.1-1M). The total antioxidant activity of the encapsulated curcumin is slightly lower than that of unencapsulated one, determined by 2, 2 Diphenyl-1-picryl hydrazyl radical scavenging assay. The study suggests that nanoencapsulation can be a better platform to increase the solubility and stability of highly lipophilic and unstable phytochemicals and to protect them from harsh processing and environmental conditions.

Keywords: Curcumin; Antioxidant; Nanoencapsulation; zeta potential.
1. Introduction
Curcumin is the most prominent polyphenolic component of turmeric (Curcuma longa) plant, a perennial herb belonging to ginger family has been used widely as a dietary spice and in traditional medicine. It has been reported to have many beneficial biological and pharmacological activities like antioxidant, anti-inflammatory, antitumourogenic, anticoagulant, antibacterial and anticarcinogenic etc., (Babu et al, 1997). Curcumin is a unique antioxidant, which contains a variety of functional groups, including the B-diketo group, carbon–carbon double bonds, and phenyl rings containing varying amounts of hydroxyl and methoxy substituents which enhance the antioxidant properties of curcumin (wright, 2002). Curcumin could attenuate free radical-mediated peroxidation of membrane lipids and oxidative damage of DNA and proteins (Sudheer et al, 2005). The antioxidant activity of curcumin has been proposed to be associated with most of its pharmacological effects.

The pharmacological safety of curcumin is shown by the non-toxic consumption up to 100 mg/day in humans and up to 6 mg/day in rats (Commandeur and Vermeulen, 1996). But its application as a functional ingredient is limited because of its poor water-solubility, bioaccessibility and degradation at physiological pH. The major portion of ingested curcumin is excreted un-metabolized through the faeces and a small portion which absorbed is extensively converted and excreted as its water-soluble metabolites. This seriously limits curcumin to reach targets and exert its function (Christopher et al, 2002). The alkaline degradation of curcumin is another potential limitation for its therapeutic use (Tonnesenand Karlsen, 1985).

Several approaches in synthesis of curcumin derivatives have been taken in order to enhance the uptake and distribution (Shehzad et al, 2010). The encapsulated polyphenols can overcome the drawbacks of their instability, unpleasant tastes or flavors, as well as improve the bioavailability of the compound in vivo and in vitro instead of free compounds (Mozafari et al, 2008). The high surface area and kinetic stability of nanoencapsulated molecules further improves bioavailability within the gastrointestinal tract. So the present study was aimed to establish methods which can be scaled up for preparation and characterization of nanoemulsions encapsulating curcumin as bioactive component and to evaluate the antioxidant potential of prepared emulsions.

2. Materials and Methods
2.1 Chemicals
Curcumin (95% pure, Curcuma longa) was purchased from Plant Lipids Pvt. Ltd. (Kerala, India). Palm oil (Raag Gold Refined Palmolein) was purchased from local Karnal market. Butter oil was prepared in the Experimental Dairy, National Dairy Research Institute, Karnal according to the procedure given by De, (1976). Olive oil was purchased from local Karnal market. Tween 80 and DPPH were purchased from MERCK (Merck Specialities Private Limited, Worli, Mumbai). Whey protein concentrate (WPC-70) was procured from Modern Dairy Pvt. Ltd. (Karnal, India).
2.2 Solubility of curcumin in various oils
The solubility of curcumin at room temperature and 80°C for 30 min has been determined using different oils including butter oil (rich in short chain triglycerides), palm oil (rich in medium chain triglycerides), and olive oil (rich in long chain triglycerides). Different concentrations of curcumin was added to 5 ml of oils in test tubes and mixed using vortex mixer to dissolve curcumin properly. The mixture was then centrifuged at 1,300 × g for 10 min to find visible undissolved particles. The maximum concentration at which no undissolved sediments of curcumin appeared on centrifugation was termed as solubility (mg/ ml).

2.3 Preparation and characterization of curcumin encapsulated emulsions
Oil-in-water emulsions of curcumin were prepared by solubilizing curcumin in oil and homogenizing this phase with ultra sonicator with an aqueous phase containing a water soluble emulsifier whey protein concentrate 70 (WPC-70)and surfactant tween 80. 40 mg of curcumin was added to 100 ml of emulsion along with different oils (0.5-2%), surfactant (2-10% w/w) and emulsifiers (0-1% w/w) in millipore water and sonified for 15 min at 5°C by using ultrasonicator. The stability of the emulsions were evaluated by centrifugation (1,300 × g for 30 min) and heating (80°C/30 min). Particle size was determined by dynamic laser light scattering method using Malvern Zetasizer Nano ZS90 (Malvern Instruments, UK). Zeta potential, the electrical charge on the oil droplets in the emulsions was determined by the same instrument.

2.4 Effect of processing conditions on emulsion stability
The physical and chemical stability of curcumin nanoemulsion to processing conditions typically encountered by food products were tested at different pH, ionic strength and processing temperature. Emulsion samples were adjusted to the desired final pH (3-7) using either 0.1 NNaOH or HCl solution, ionic strength (0.1-1M) and temperature 72°C for 15 sec and 95°C for 10 min and subjected tocentrifugation at 1,300 × g for 10 min in order to find aggregation of particles.

2.5 In vitro antioxidant assay
Free radical scavenging activity of the curcumin nanoemulsions were determined by DPPH (2, 2 diphenyl-1-picryl hydrazyl) method given by Williams et al.(1995) and the results were expressed as trolox equivalent antioxidant capacity (TEAC) values i.e. μM of trolox equivalent/ ml of emulsion. All values reported are mean±S.E.M.

3. Results and Discussion
The solubility of curcumin before heating in butter oil, palm oil and olive oil were 0.5, 0.4, and 0.2 mg/ ml respectively. While solubility increased to 0.7, 0.6 and 0.3 mg/ ml respectively on heating. The solubility of curcumin was higher in butter oil followed by palm oil and olive oil. Similar observations were made by Ahmed et al, (2012) that the solubility of curcumin is increased as the average molecular weight of the carrier
molecule is decreased. Short chain triglycerides (SCT) have more polar groups per unit mass which may favour solubilisation of curcumin. Wang et al, (2008) also reported that heating curcumin in lipid phase to 60°C for 10 min increases its solubility.

For the fabrication of nanoemulsions, different combinations of inner oilphase and outer aqueous phase at appropriate ratio were tried to optimize stable compositions. The characterization of emulsions showed a strong dependence with the composition of the system. The particle size of nanoemulsion increases from 639.1 ± 43.8 to 828 ± 32.9 nm as the concentration of oil phase (palm oil) increases from 1- 4% (Table 1). Rao and McClements, (2012) observed that the mean particle diameter remained relatively low (<30 nm) from 0 to 0.2 wt% lemon oil, increased steeply from 0.3 to 0.6 wt% lemon oil, and then increased more gradually at higher lemon oil concentrations. The higher value (>0.5) of poly dispersity index (PDI) of nanoemulsions can be correlated with less stability on storage especially for emulsions with butter oil (rich in SCT) as lipid phase. Ahmed et al., (2012) found similar observations on PDI of curcumin nanoemulsion formulated with SCT. The nanoemulsions which were stabilized only by Tween-80 showed a zeta potential value towards zero which explains the role of emulsifiers in stabilizing the emulsions.

Table 1: Particle size, zeta potential and PDI of nanoemulsions.

<table>
<thead>
<tr>
<th>Nanoemulsion composition</th>
<th>Size(nm)</th>
<th>Zeta potential (mV)</th>
<th>PDI</th>
</tr>
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<tbody>
<tr>
<td>1% Palm oil + 10% Tween 80</td>
<td>639.1 ± 43.8</td>
<td>-0.4 ±0.01</td>
<td>0.624</td>
</tr>
<tr>
<td>4% Palm oil + 10% Tween 80</td>
<td>828 ± 32.9</td>
<td>-1.3 ± 0.1</td>
<td>0.566</td>
</tr>
<tr>
<td>1% Olive oil + 10% Tween 80 + 0.1% WPC-70</td>
<td>609.4 ± 25.1</td>
<td>-10.4 ±0.9</td>
<td>0.826</td>
</tr>
<tr>
<td>1% Butter oil + 7% Tween 80 + 1% WPC-70</td>
<td>318.7 ± 17.3</td>
<td>-10.9 ±1.3</td>
<td>1.394</td>
</tr>
</tbody>
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The stability of the colloidal delivery systems to various environmental stresses were tested by changing various parameters like pH (3-7), ionic strength (0.1 - 1 M); and processing temperature (pasteurization and boiling). The nanoemulsions were stable to a pH range of 5-7, ionic strength of 0.1-0.75 M and pasteurization temperature. The higher rate of aggregation at low pH and high temperature may be attributed to the denaturation of WPC. Rao and McClements, (2011) studied the impact of micro/ nanoemulsions on various environmental stress and found that relatively stable nanoemulsions were formed at pH 6 and 7 and stable microemulsions at pH 5 and 6, but extensive particle growth/aggregation occurred at lower and higher pH values, which was attributed to either chemical (hydrolysis) or physical (electrical charge) effects. The reduction in the electrical charge on the particles at lower pH value would reduce the electrostatic repulsion between them, thereby leading to aggregation (Rao and McClements, 2012).
Table 2: Antioxidant activity of nanoemulsions.

<table>
<thead>
<tr>
<th>Nanoemulsion composition</th>
<th>TEAC /ml</th>
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<tbody>
<tr>
<td></td>
<td>Before encapsulation</td>
</tr>
<tr>
<td>1% Palm oil + 10% Tween 80</td>
<td>1.42 ± 0.01</td>
</tr>
<tr>
<td>4% Palm oil + 10% Tween 80</td>
<td>1.33 ± 0.03</td>
</tr>
<tr>
<td>1% Olive oil + 10% Tween 80 + 0.1% WPC</td>
<td>1.43 ± 0.10</td>
</tr>
<tr>
<td>1% Butter oil + 7% Tween 80 + 1% WPC</td>
<td>1.39 ± 0.01</td>
</tr>
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</table>

The antioxidant activity of the encapsulated curcumin was slightly lower than that of unencapsulated one (Table 2). Nanoemulsion with 1% Palm oil + 10% Tween 80 showed 1.33 ± 0.03 μM Trolox equivalents/ ml before encapsulation while after encapsulating in nanoemulsions the activity reduced to 1.20 ± 0.01 μM Trolox equivalents/ ml. Similarly in case of olive oil, the activity reduced from 1.43 ± 0.04 μM Trolox equivalents/ ml to 1.27 ± 0.01 μM Trolox equivalents/ ml after encapsulation. To exert the bioactivity, antioxidants have to be bio-accessible, i.e., released from the food matrix and solubilized (Bouayed et al, 2011). Although the antioxidant activity of the encapsulated compound is lower than unencapsulated, the fact is that, the encapsulated curcumin not only protects curcumin from degradation but may also preserve a good measure of the antioxidant activity. Donsi et al, (2011) evaluated the effect of the delivery systems of curcumin by comparing the antioxidant activity of the encapsulated compound with that of unencapsulated one. The antioxidant activity of curcumin encapsulated in solid lipid nanoemulsion using FRAP assay was 0.996 ± 0.07 while that of unencapsulated curcumin shows a value of 2.504 ± 0.06 at 593 nm.

4. Conclusion
The present study proposed that nanoencapsulation can meet the challenges of delivering hydrophobic polyphenols for various commercial applications. The curcumin nanoemulsion has a suitable size distribution and stability against aggregation which can be employed in numerous drugs and therapeutic applications.

References


