Protective effects of accompanying proteins on light- and water-mediated degradation of Curcumin

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ABSTRACT

Curcumin is a natural polyphenolic compound with anti-cancer, anti-inflammatory, and anti-oxidation properties. Low water solubility and rapid hydrolytic degradation are two challenges limiting use of curcumin as therapeutic agent. In the current study, the role of the Bovine Serum Albumin (BSA), β-lactoglobulin and casein, as food-grade biopolymers and safe drug delivery systems, on the physical activity of curcumin were surveyed. It appears that BSA and casein as protein vehicles are useful tools to increase stability of curcumin, as a health promoting agent.

Keywords: Curcumin; albumin; casein; Light; Stability

INTRODUCTION

According to the International Food Information Council (IFIC) "functional food" is defined as "food that has health promotion properties beyond basic nutrition". In this way, different phytochemicals especially polyphenols have attracted the attention and extensive research has been carried out to devise novel methods to incorporate the functional ingredients into foods. Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is the best characterized plant-derived polyphenol showing chemopreventive and safety activities against malignancy [1].

This yellow-orange natural compound which is the main component of *Curcuma longa* rhizomes (Figure. 1), has been consumed as dye, food additive and potential therapeutic (Figure. 2) for a long time period in Asia [2]. In the past decade, scientific studies have revealed other medicinal effects of curcumin including anti-proliferative, anti-angiogenic, anti-inflammatory, anti-cystic fibrosis, anti-oxidant and wound healing properties [3, 4] (Figure. 2). Due to low intrinsic toxicity of curcumin for healthy (normal) cells, several clinical trials are either underway or have been completed with an aim to develop curcumin into a treatment agent [2, 5, 6]. Curcumin is extremely safe even at very high doses of 8–12 g/day [5, 6]. But, from the chemical viewpoint, there are several challenges that must be overcome for curcumin to become a routine treatment agent: Despite the safety, efficacy and well-tracked mechanisms of action, curcumin has a low aqueous solubility (approximately 11 ng/mL), which significantly limits its bioavailability *in vivo* [3, 5-7]. On the other hand, in aqueous medium, curcumin undergoes rapid degradation by hydrolysis (even at physiological pH) followed by molecular fragmentation within 30 min [7-9] (Figure. 3). Various approaches have been applied to increase water solubility, stability and bioavailability of curcumin such as emulsification, chemical modification, and encapsulation in polymer nanoparticles, cyclodextrins, hydrogels and nanogels, polymeric and surfactants micelles, lipid bilayers, liposome/phospholipid, solid lipid nanoparticles, polymer conjugates, self-assemblies, and vesicles and other delivery systems [10]. For instance, the water solubility of curcumin in cyclodextrin-based or chemically modified systems can reach about 2-200 mg/ml [11-13]. However, each delivery system may have cytotoxic effects on some normal tissues and cells and/or display limited influence on curcumin stability.

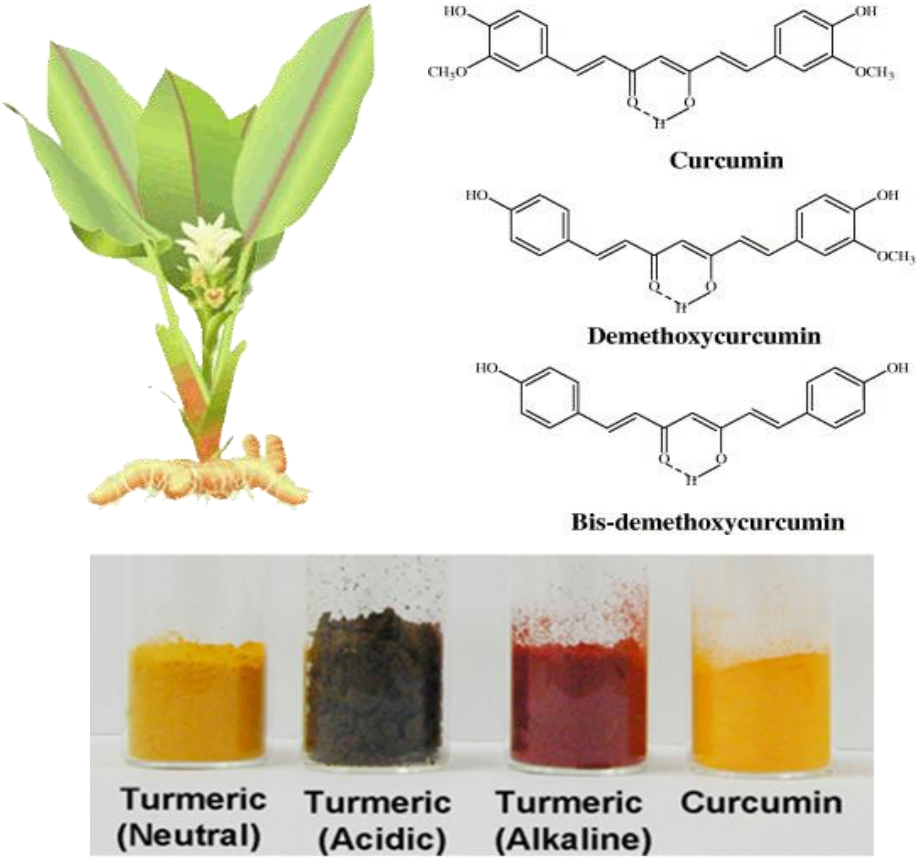

Figure 1. (Top, Left) *Curcuma Longa* with flower and rhizome. Turmeric (*Curcuma longa* L.), belonging to the family of *Zingiberaceae*, is a perennial herb native to India where its rhizome is used as a yellow colorant curry spice and traditional medicine. (Bottom) Turmeric color at different conditions and dried pure curcumin. (Top, Right) Chemical structure of turmeric constituent compounds. The active principle in turmeric was identified as a group of polyphenolic compounds, namely curcumin (74-78%), demethoxycurcumin (15-18%) and bisdemethoxycurcumin (4-6%) commonly referred to as "curcumin".

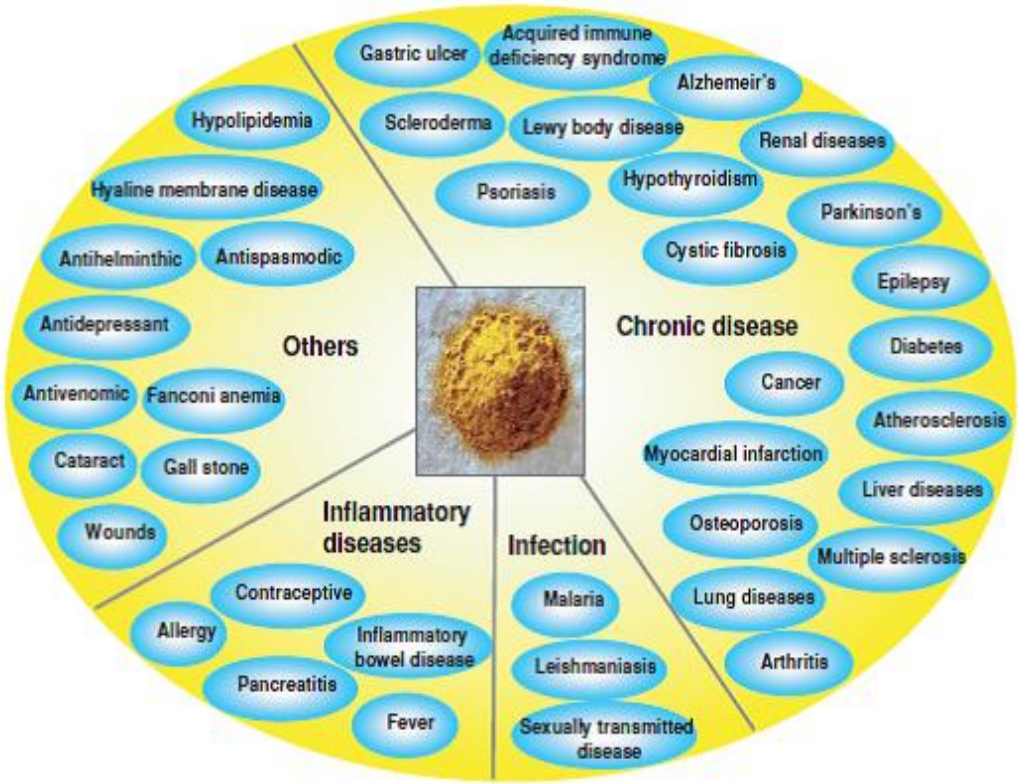

Figure 2. Curcumin is a potential therapeutic for various types of clinical diseases.

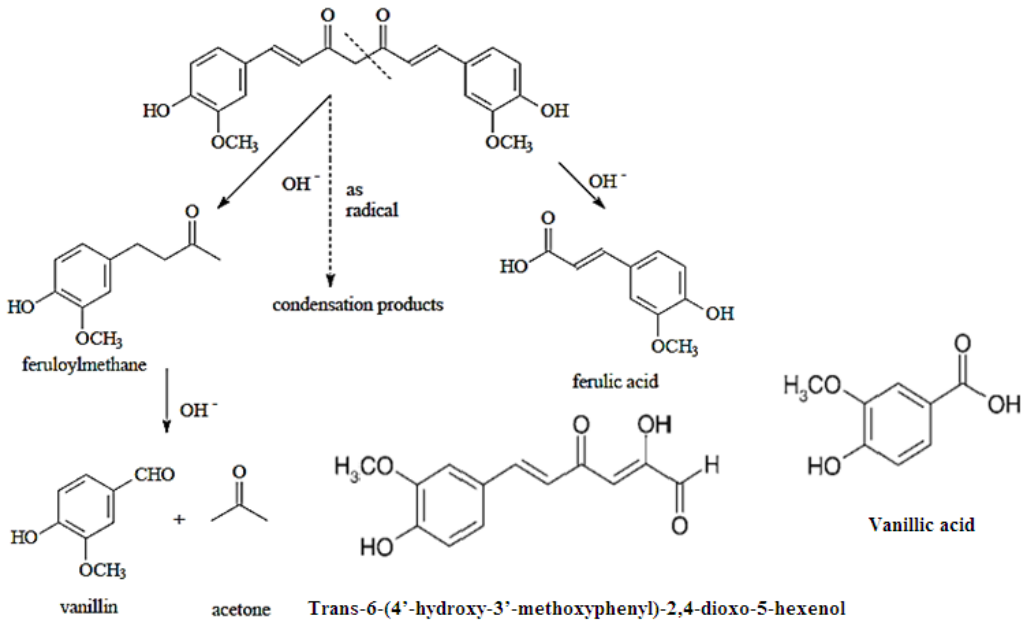

Figure 3. Chemical structures of curcumin and its degradation products. Due to low stability, curcumin degrades under physiological conditions. The degradation products have been identified as trans-6-(40-hydroxy-30-methoxyphenyl)-2,4-dioxo-5-hexenal, ferulic aldehyde, ferulic acid, feruloyl methane and vanillin.

Drug delivery systems based on food proteins hold much promise because of their high nutritional value, low toxicity and excellent functional properties as well as their applications as ingredients in the food industry. The main aim of the current study is to introduce β-lactoglobulin, albumin and casein (Fig. 4) as food-grade amphiphilic materials to interact with curcumin, since these proteins (especially serum albumins and caseins) are commonly used transporting vehicles for proteins, hormones, drugs, and diagnostic agents [14].

Moreover, for several years, *Haldi doodh* (turmeric milk and/or warm milk mixed with some turmeric powder) is commonly used in India as a home remedy when someone is suffering from fever. Turmeric paste is often used in India as an antiseptic in open wounds, while *chun-holud* (turmeric with slaked lime) is used to stop bleeding as home remedies [15].

Casein micelles (major proteins in milk) are relatively easy to prepare, biodegradable, and have potential for high drug loading capacity. Serum albumin is the most abundant of the proteins, circulated several times in the blood [16]. Both casein micelles and albumin delivery systems have been previously employed for administration of several hydrophobic drugs [17, 18]. Beta-casein is also an amphiphilic self-assembling protein that helps to solubilize curcumin more efficiently in aqueous solution [11]. β-lactoglobulin, a small globular protein with 162 amino acid residues is also the major whey protein in bovine milk. It is classified as a member of the lipocalin-protein family because of its high affinity to small hydrophobic ligands [19]. Taking both traditional medicine and literature into account, there is possibility that stability characteristics of curcumin improves upon binding to mentioned proteins. To test this possibility, the current study has focused on the protective effects of accompanying proteins on light- and water-mediated degradation of curcumin. This work is particularly novel and significant because to the best of our knowledge, no previous similar studies have been reported about protective effect of proteins against light-mediated degradation of curcumin. We found that proteins slow down the rate of curcumin degradation compared to the free curcumin. We report that while no curcumin stabilization takes place in the presence of β-lactoglobulin, it is greatly stabilized in the presence of BSA. The results also imply that the association of curcumin to casein contributes significantly to its stability in Indian (home) therapeutic preparations.

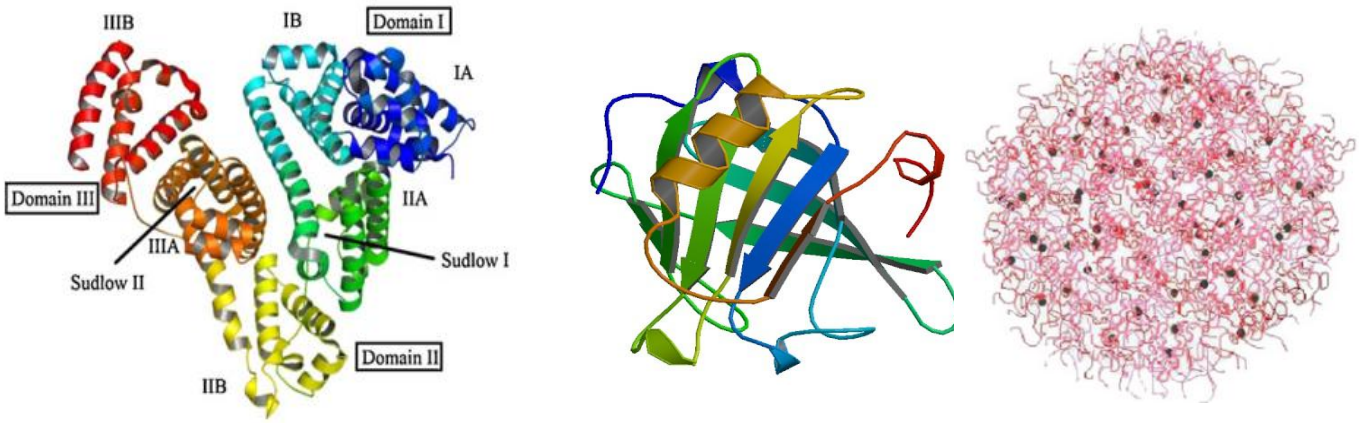

Figure 4. Ribbon representation of BSA (left, ID code 2BXB)) and β-lactoglobulin (middle, ID code 1BEB) as well as a proposed model of casein micelles (right). For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.

MATERIALS AND METHODS

Materials

Bovine serum albumin, β-lactoglobulin and casein provided from Sigma and phosphate buffer, curcumin and the other reagents were analytical grade and purchased from Merck. All experiments were repeated at least 3 times.

Methods

Water-mediated degradation. After excitation at 420 nm, fluorescence emission spectra of curcumin (10μM) in the absence and the presence of each protein (40μM) in phosphate buffer (20mM and pH=7) at 37 °C were recorded from 440 to 700 nm during 60 min with 3 min intervals. The background fluorescence was subtracted from each emission spectrum.

Light-mediated degradation. Absorption of curcumin (10μM) in the absence and the presence of each protein (40μM) were recorded at 420 nm in darkness and front of the direct light during 9 hours.

RESULTS

To investigate effect of solvent, curcumin degradation was evaluated in the presence of an aqueous (water) as well as a non-aqueous (absolute ethanol) solvent (Figure. 5). As shown in Figure. 5, ethanol does not degrade curcumin whereas water remarkably decomposes it. Moreover, light accelerate the curcumin degradation rate especially in the aqueous medium.

To test of protective effects of BSA, casein, and β-lactoglobulin on curcumin degradation, the kinetic of water-mediated curcumin degradation was investigated in the presence of proteins. In phosphate buffer solution, the degradation is accompanied by a substantial decrease in the fluorescence emission intensity. By plotting of the maximum emission intensity of spectra against time, the kinetic profiles of curcumin decomposition were obtained (Figure. 6). BSA and casein improves the curcumin stability while β-lactoglobulin increases the curcumin degradation rate, significantly.

There is this possibility that employed proteins protect curcumin against light-mediated degradation. The kinetic profiles of curcumin light-mediated degradation were created by plotting of the absorbance at 420 nm versus time. As can be seen in Figure. 7, BSA and casein decrease the curcumin degradation rate whereas β-lactoglobulin unstable the curcumin.

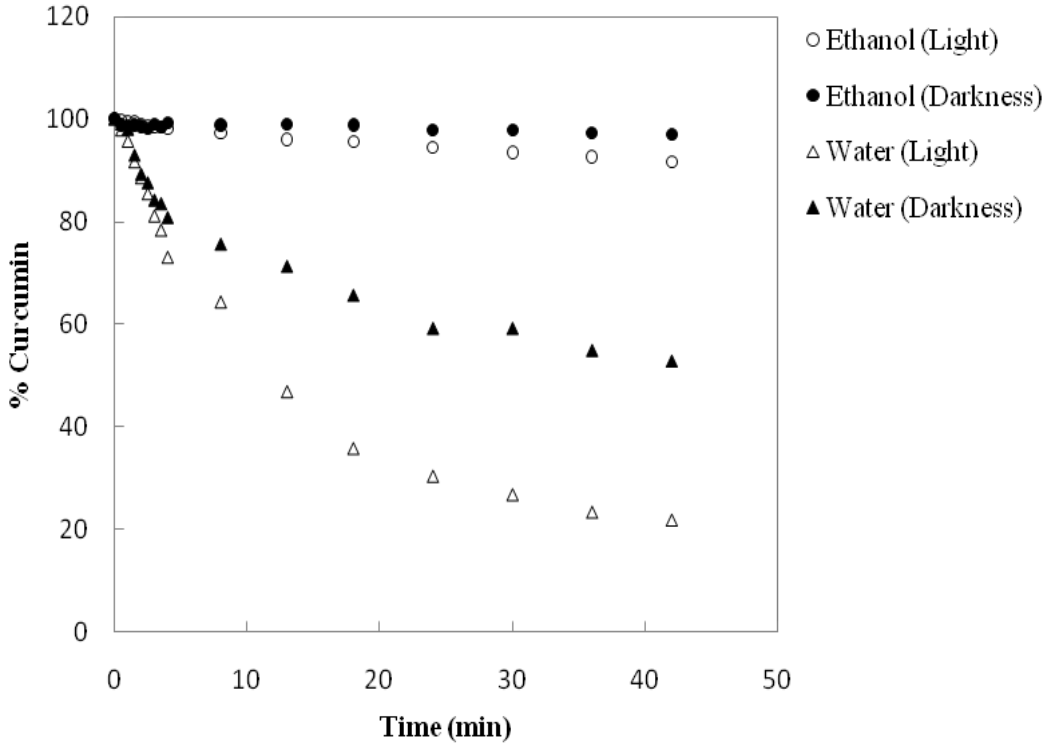

Figure 5. Effect of water activity and light on the curcumin stability, at room temperature.

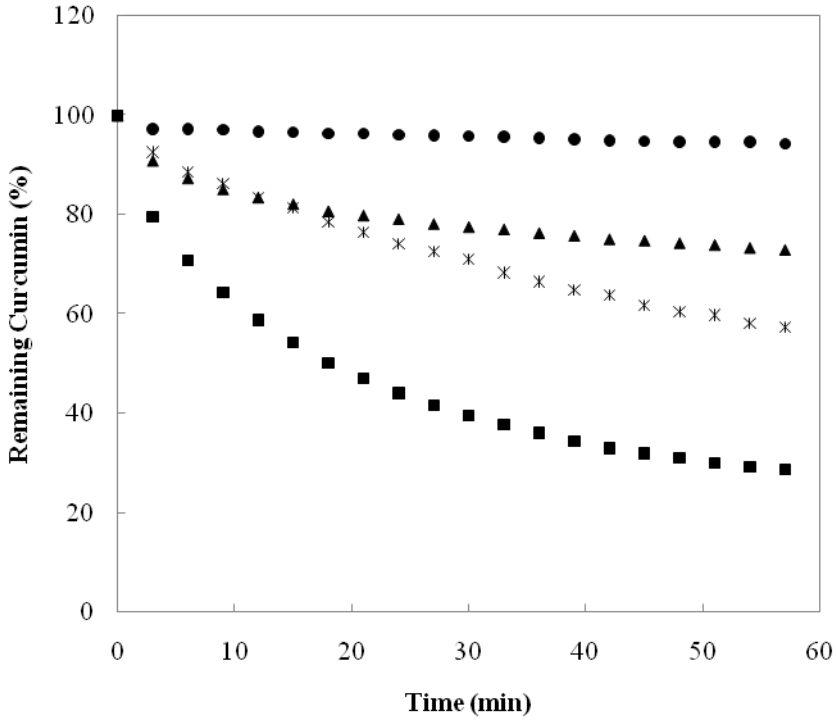

Figure. 6. Curcumin stability in the presence of BSA (filled circle), β-lactoglobulin (filled square), casein (filled triangle) and in buffer alone (pH 7, asterisk), at 37 °C.

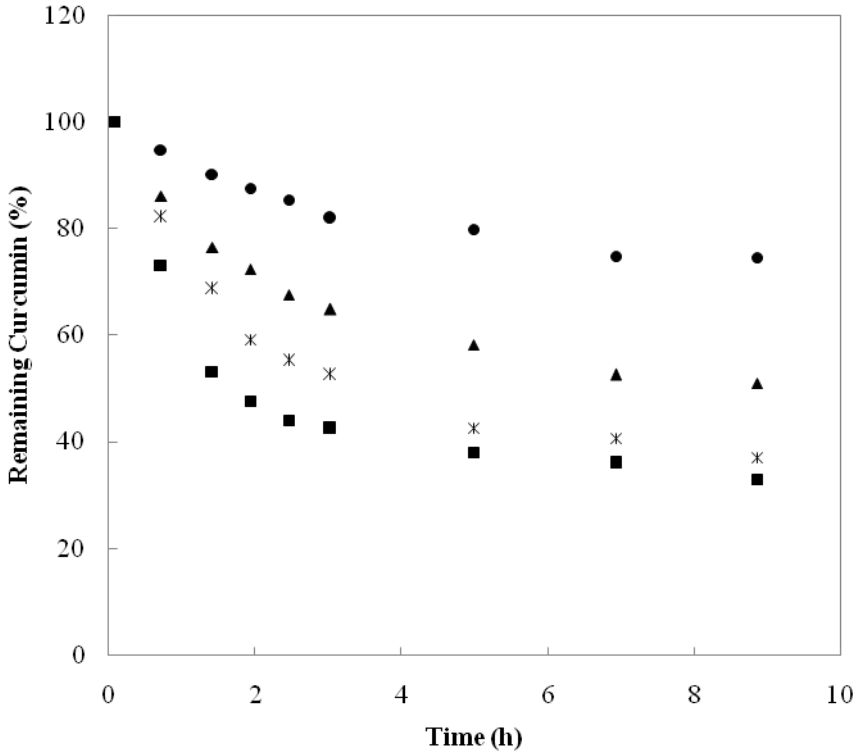

Figure 7. Effect of three proteins; BSA (filled circle), β-lactoglobulin (filled square), casein (filled triangle) on the curcumin stability against direct light, at 37 °C, Buffer alone (pH 7, asterisk).

DISCUSSION

Curcumin, with considerable pharmaceutical activity, is a hydrophobic polyphenolic compound with low water solubility and stability [1, 7]. Hence, curcumin requires a convenient carrier system to deliver to different parts of the body. Recently, various systems such as encapsulation in nanoparticles, cyclodextrins, micelles, and proteins are being expanded for drug delivery investigations [3, 10]. In this study, the stability characteristics of curcumin in the presence of various proteins have been studied. Previous studies have shown that alkaline hydrolysis is the main process in the degradation of curcumin in buffer solution [3]. Curcumin is partially deprotonated initially, which is followed by fragmentation into trans-6-(4´-hydroxy-3´-methoxyphenyl)-2,4-dioxo-5-hexanal [8]. This product is then further decomposed into smaller molecules such as vanillin, feruloyl methane, and ferulic acid [3, 8]; these molecules contribute negligibly to the absorption of 420 nm light.

As indicated in Fig. 5, curcumin remains intact in the presence of ethanol while it decomposes significantly in the polar medium. In other word, both light and water activity accelerate curcumin degradation rate. Additionally, curcumin degradation rate has been evaluated in the presence of two polarity reducing compounds, ethylene glycol (EG) and polyethylene glycol 6000 (PEG). The results showed that these two compounds has similar protective effects so that they reduced curcumin degradation rate by almost 10-fold (86% and 98%, respectively, data not shown). This is probably due to the weak and dynamic hydrophobic interactions between the exposed hydrophobic regions of polarity reducing agents and curcumin.

Investigation of the degradation of curcumin in the presence of BSA, casein and βLG is of interest as these food-grade proteins are believed to act as natural transporting molecules. Figure. 6 shows the intact curcumin decays to approximately 30% of the initial value in 60 min. In contrast, the curcumin water-mediated degradation is negligible in the presence of BSA and casein. Similar to kinetic of water-mediated degradation, albumin and casein also show capability to protect curcumin against light-mediated degradation while β-lactoglobulin promotes curcumin decomposition by unknown mechanism (Figuer. 7).

There are several reports on the binding of curcumin to the serum albumin and casein [2, 3, 20]. It seems to be stabilization of curcumin in the presence of BSA and casein, as indicated in this study, is due to interaction between proteins and curcumin. This supposition is confirmed by pervious study which indicates the plasma proteins (human serum albumin and fibrinogen) interact with curcumin and stabilize it against hydrolytic degradation [3]. Overall, Our results along with previous reports clearly highlight the intrinsic ability of BSA and casein to stabilize curcumin.

CONCLUSION

In brief, it can be suggested that "protein-curcumin" complexes may be effective tools for curcumin delivery *in vivo*. This laboratory suggest that mixing of curcumin with milk or its consumption as albumin containing capsules may lead to more stability, bioavailability and therapeutic efficiency, *in vivo*.

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