

Comparison of Whole and Skim Milk as Loading Medium for Curcumin

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Abstract:

Curcumin is known as a natural component of turmeric, which provides numerous health benefits due to its anti-inflammatory, antioxidant and antiseptic properties. However, the bioavailability of curcumin is limited partially due to its low solubility in water (61 µg/mL). While there are various delivery systems that have been examined to improve the loading and stability of curcumin, this study examines the usage of milk as a delivery system, specifically the usage of whole and skim milk. The hypothesis is that the lipids and proteins in the milk would help improve the solubility of curcumin. Inherent fluorescence of curcumin was used to measure its concentration in situ. Initially 0.0025 g of curcumin was dissolved in 5mL of ethanol. Then, milk was heated and stirred until a temperature of 80°C was reached and then cooled. 0.02% sodium azide was added to prevent bacterial growth in the milk. 5mM of 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) was added in half of the samples to be used as a treatment. AAPH is an oxidizer that accelerates oxidative degradation of the curcumin in solution. Samples were stored at 8° C and 22° C. Curcumin concentration was measured over a two-week period. The refrigerated samples showed a 41.69% decrease in skim milk when AAPH was used and 10.05% decrease when no AAPH was present. The room temperature samples of skim milk with AAPH showed a 58.33% decrease, whereas the skim milk without AAPH showed a 11.8% decrease. The refrigerated whole milk samples showed a 39.6% reduction with AAPH and a 6.25% increase without the presence of AAPH. While the room temperatures

samples showed a 48.37% reduction with AAPH and 11.12% reduction without AAPH. Both whole and skim milk showed similar stability readings. This experiment depicts that milk may be used as a suitable loading medium for curcumin.

Introduction:

Curcumin is a natural chemical found within the turmeric plant that is known for its numerous health benefits, such as its anti-inflammatory, anti-microbial and antioxidant properties (Perrone et al., 2015). However, the bioavailability of curcumin is limited partially due to its low solubility in water (61 µg/mL) (Zhang et al., 2011). Various delivery systems have been studied to improve the loading and stability of curcumin. Some experiments studied using natural matrixes to suspend the curcumin. One study questioned the usage of various cellulose as a matrix to improve the bioavailability yet with varying results (Li, Konecke, Wegiel, Taylor, & Edgar, 2013). Another study used rubusoside, a natural sweetener, which improved the solubility 38 times (Zhang et al., 2011). The use of cyclodextrin, a compound used to stabilize and solubilize lipophilic compounds had a 10^4 increase in solubility (Tønnesen, Másson, & Loftsson, 2002). The use of proteins to increase bioavailability and stability in curcumin has been examined in both milk and soy. Soy protein isolate was used to isolate curcumin with approximately an 80% success rate (Tapal & Tiku, 2012). There have been other experiments previously that considered some alternatives using milk as a system. These however, focused on non-fat milk when looking at the relationship between the milk protein casein and curcumin. One study investigated the binding capability of curcumin when heat was applied, heating of the milk caused the denaturation of the whey protein thus allowing for a higher binding capacity of curcumin ((Rahimi Yazdi & Corredig, 2012). Another study involving milk, investigated the

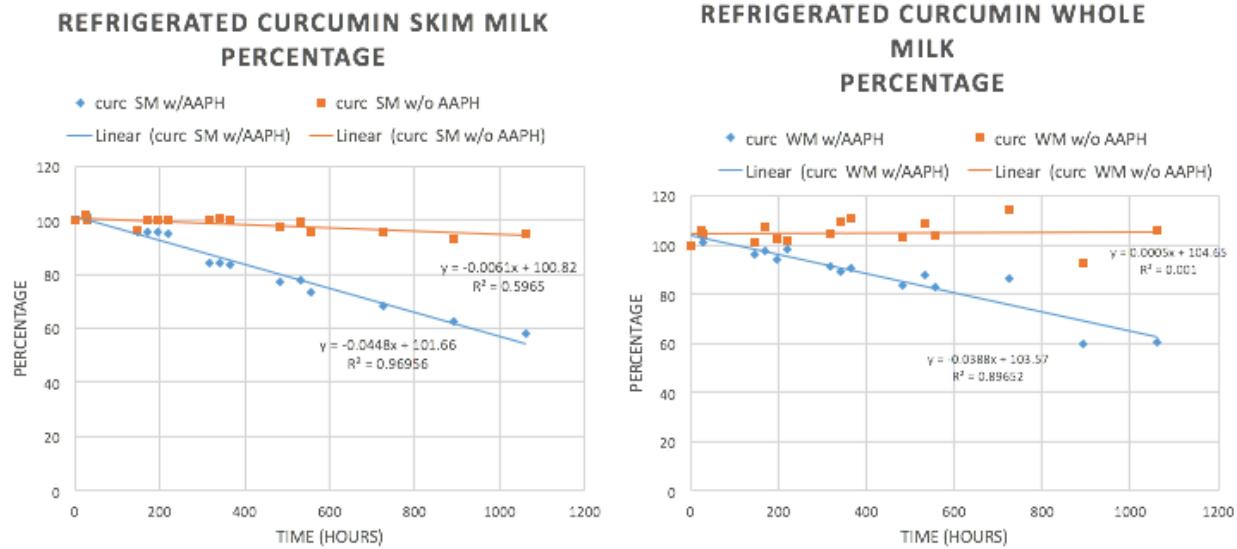
usage of high pressure processing to improve the binding of curcumin to the milk protein casein (Yazdi et al., 2013). While these experiments have shown a multitude of mediums to be good carriers of curcumin, few have looked at the relationship between milk with fat and the binding stability in such a solution. In the preliminary stages of this experiment other polar solutions such as acetone were used to determine an efficient solution to disperse curcumin. Ethanol proved to be the most effective solvent for the curcumin. A fluorescence spectrum was run to determine the wavelength range of the curcumin and ethanol solution.

Materials and Methods:

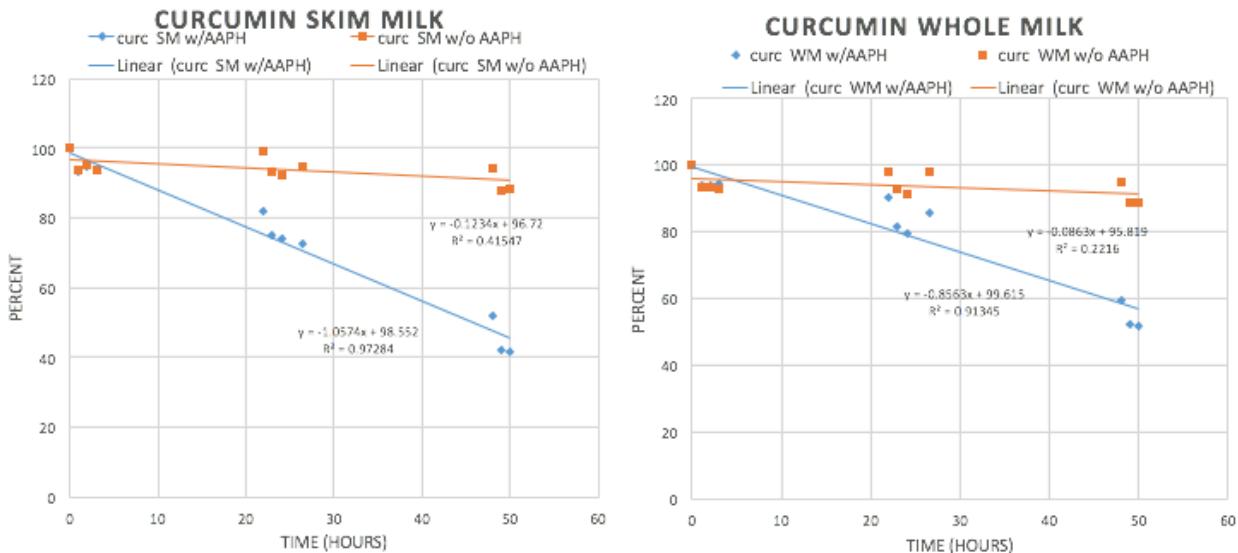
Skim milk and whole milk were purchased from the local on-campus convenience store. 98% Curcumin was purchased from Acros Organics, the Sodium Azide was purchased from fisher Biotech, ethanol and the 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) were purchased from (Sigma-Aldrich Inc., St Louis, MO). Four tests were run for each milk sample; a control of milk and ethanol without curcumin with AAPH, a control without AAPH, treatment with curcumin without AAPH and finally a treatment with curcumin and with AAPH. In all experiments, the milk sample was heated and stirred on a hotplate to 80°C. While the sample was heating, 0.0025 g of curcumin was weighted and placed in 5 mL of ethanol. Once the target temperature was reached, the curcumin solution was added, stirred for 2 minutes, and then cooled in a water bath. 0.02% w/v of sodium azide was then added and stirred for 2 minutes. When AAPH was used, a 20 mM sample of AAPH was placed in the solution under a fume hood and stirred for 2 minutes. The milk was then labeled and stored in 50 mL centrifuge tubes at both ambient and 8 °C temperatures. A fluorescence curve was created using various dilutions of the solutions. 200 µL of each sample was measured 4 days a week in a black microplate. A

Spectromax M5e Multimode Microplate Readers was used to measure the fluorescence of the samples between 420 nm and 520 nm.

Results and Discussion:



(Figure 1: Refrigerated skim and whole milk curcumin samples over a 44 day period)



(Figure 2: Room Temperature skim and whole milk curcumin samples over a 50 hour period)

The results from the experiment are shown in the following figures. Figures 1 depicts the percent change in the absorbance from the experiment change of curcumin in refrigerated temperature at 4°C. Figure 2 shows the percentage change of curcumin at a room temperature of 22 °C. The skim milk samples refrigerated with AAPH had a 41.69% decrease in absorbance over the 44 days whereas the whole milk with AAPH had a comparative 39.6% decrease in absorbance. The skim milk without AAPH showed a 10.05% decrease while the whole milk sample without AAPH had a 2.25% increase. In the room temperature sample, which took place over a 50-hour period, showed a 58.33 % decrease with AAPH, followed by whole milk with a 48.37% decrease in absorbance. The skim milk samples at this temperature had a 11.8% decrease while the whole milk without AAPH had a 11.12% decrease. Throughout the experiment, samples with AAPH showed relatively stable readings and no noticeable changes can be observed.

Conclusion:

This experiment shows that milk may be used as a suitable loading medium for curcumin, due to the milk's retention of curcumin over long periods of time. The conclusion of this experiment varied from the hypothesis. Since curcumin is considered a hydrophobic substance, the hypothesis was thought that it would bind more efficiently with milk with a higher lipid content. This was not the case as the results showed milk is a suitable medium and the lipid content does not significantly change the stability.

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