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Thermal and oxidative stability of curcumin encapsulated in yeast microcarriers

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ABSTRACT

This study evaluated the effects of the intracellular constituents of yeast microcarriers on the thermal and oxidative stability of encapsulated curcumin. Intact yeast cells and plasmolyzed yeast, *i.e.* yeast cell wall particles (YCWPs), of *Saccharomyces cerevisiae* were compared to Pickering emulsions in this study. Peroxyl radicals were generated with 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) and thermal pasteurization was carried out at 70 °C and 90 °C. Analysis of variance (ANOVA) and kinetic modeling were also employed. YWCPs provided significantly higher thermal stability to curcumin (91.8 ± 1.0% and 99.7 ± 3.1% at 70 °C and 90 °C respectively) compared to intact cells and Pickering emulsions; these results in YCWPs were attributed to the lack of native subcellular structures which are prone to denaturation and subsequently release curcumin. Native yeast, however, provided significantly higher oxidative stability to encapsulated curcumin. This oxidative stability in intact cells was ascribed to endogenous, cytoplasmic antioxidants and confirmed with ferric ion reducing antioxidant power (FRAP) assays.

1. Introduction

Oxidation of bioactives, both during food processing and storage, is one of the key factors that limits the incorporation of bioactives into food and other functional products (Del Caro et al., 2004; Patras et al., 2010). In order to mitigate losses of these labile ingredients due to oxidation and thermal enhanced oxidation and other degradation reactions, formulations often include the use of metal ion chelates such as ethylenediamine tetraacetic acid (EDTA) which sequester redox-active metal ions and sacrificial antioxidants such as butylated hydroxytoluene (BHT) and certain vitamins (Choe & Min, 2009; Let et al., 2007; Nielsen et al., 2004). Although these methods demonstrate some efficacy in retarding oxidation, their incorporation may be waning due to strict regulatory requirements and negative consumer perception attributed to the use of synthetic compounds (Miková, 2001; André et al., 2010).

In addition to chemical methods, encapsulation of these labile ingredients into various encapsulation systems has been employed to mitigate losses due to oxidation. Commonly used encapsulation systems include emulsions and particles (Luo et al., 2011; Pan et al., 2013). In many cases, the encapsulation systems both the dispersed and continuous phase of the encapsulation systems are modified using physical and chemical approaches to prevent undesirable oxidative reactions. Physical modifications of the lipid dispersed phase often entail changes to the lipid core, *e.g.* nanostructured lipid carriers (NLC) and solid lipid nanoparticles (SLN) (Üner, 2005a, 2005b). Despite significant potential promise of this approach, recent studies have shown that exclusion of encapsulated compounds from the solid-lipid core can enhance exposure of encapsulated compounds to oxidants such as free radicals and metal ions (Pan et al., 2016). The other physical modifications have focused on engineering the interface of encapsulation systems using both biopolymer coatings or nanoparticles such as Pickering emulsions (Tikekar et al., 2013; Wang et al., 2014; Yang et al., 2011; Zheng et al., 2013). In many cases only marginal improvement in stability of encapsulated compounds have been reported (Davis et al., 2015; Pan et al., 2016; Tikekar et al., 2013). Furthermore, translation of these concepts to commercial products is hindered by additional cost and labor required for these modifications. In contrast to physical modifications, industrial applications have mostly focused on chemical additives, such as those mentioned earlier, to improve oxidative stability. These chemical additives not only increase cost of food formulations but also lowers consumer acceptability (Peschel et al., 2006).

In contrast to common encapsulation systems, yeast are naturally complex, containing both a multilayered cell wall and various internal compartments and antioxidant mechanisms (Lipke & Ovalle, 1998, Bachhawat et al., 2009). Due to this innate complexity, their prevalence

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in food systems and ease to culture at industrial scale, yeast have been employed as carriers for a variety of bioactive compounds, including biopolymers such as proteins and DNA (Ciamponi et al., 2012; Soto & Ostroff, 2008; Young et al., 2017). Despite yeast's potential as carriers, no research has been conducted to evaluate if these cell structures and endogenous antioxidant systems within the cytoplasm have a prophylactic effect against thermally- or oxidative-induced degradation of encapsulated bioactives.

The proposed hypothesis for this study was that the intracellular constituents of yeast microcarriers moderate the degradation of bioactives during both thermal processing and accelerated oxidation. Native yeast cells and chemically and thermally plasmolyzed cells, *i.e.* yeast cell wall particles (YCWPs), were selected as model microbial carriers and compared to a Pickering emulsion stabilized by Ludox HS-30 colloidal silica. Pickering emulsion was selected as the interfacial composition is semi-impermeable like cell walls of yeast cells. Furthermore, unlike surfactants and emulsifiers, nanoparticles in the continuous phase (not adsorbed at the interface) of the Pickering emulsion do not bind encapsulated molecules.

In summary, the results of this study will illustrate the potential of cell based and cell wall-based carriers to improve oxidative and thermal stability of encapsulated bioactives. Comparison between yeast and yeast cell wall particles will illustrate the significance of intracellular composition in influencing oxidative and thermal stability of bioactives. Furthermore, comparison among the selected formulations will guide development of formulations that can enhance stability of bioactives compounds.

2. Materials and methods

2.1. Materials

Curcumin derived from *Curcuma longa* (Turmeric) [≥65%, HPLC], Ludox HS-30, an anionic colloidal silica, methanol, acetone, sodium acetate, sodium chloride, sodium phosphate, mono- and di-basic, 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), iron(III) chloride ($FeCl_3$) were purchased from Sigma-Aldrich (St. Louis, MO). Absolute ethanol was obtained from Koptec (King of Prussia, PA). Sodium hydroxide, hydrochloric acid and were procured from Fisher Scientific (Pittsburgh, PA). Isopropyl alcohol was purchased from Spectrum Chemical (New Brunswick, NJ). Fleischmann's Active Dry yeast, *Saccharomyces cerevisiae*, (Memphis, TN) and organic canola oil were obtained from a local grocery store. Ultrapure water (18 MΩ cm) was obtained using the in-lab Milli-Q RG water purification system from EMD Millipore (Billerica, MA).

2.2. Preparation of yeast

For preparation of yeast, 1 g of active dry yeast was washed, twice, with an excess of 10% (v/v) ethanol solution and once with an excess of ultrapure water. The cells were pelleted down at 2100 × *g* for 5 min between each washing and once more to remove excess water.

2.3. Preparation of yeast cell wall particles (YCWPs)

Yeast cell wall particles (YCWPs) were prepared using the method adapted from Young et al. (2017). Briefly, 20 g yeast were suspended in 200 mL of 1 M NaOH. The suspension was heated to 80 °C and stirred for 1 h. The suspension was allowed to cool for 10–15 min while maintaining stirring. The suspension was centrifuged at 2500 × *g* for 10 min to pellet down the cells. Once the supernatant is decanted, the cells were resuspended in 200 mL of 1 mM HCl, the pH was adjusted to 4.2 and the suspension was heated to 55 °C for 1 h. The yeast cell wall particles were centrifuged, the supernatant decanted and the washed twice with an excess of water, four times with isopropanol and twice with acetone before drying under vacuum. YCWPs were rehydrated with 100 mM phosphate buffer at pH = 6.5 (PB) prior to encapsulation.

2.4. Encapsulation into yeast microcarriers via vacuum infusion

Yeast microcarriers were encapsulated under saturated conditions using the methods proposed by Young et al. (2017). Briefly, 1 g of native yeast or reconstituted YCWPs were dispersed in 5 mL of an aqueous solution of 35% ethanol (v/v). To the yeast microcarrier suspension, 440 μL of 2.5 mg/mL curcumin in absolute ethanol were added to give a final ratio of curcumin to yeast of 1.1 mg to 1 g yeast. Yeast cells prepared with curcumin were sealed in 6″ × 8″ boilable vacuum bags (Prime Source) at 50, 75 or 99% vacuum (Ultravac 250, Koch Equipment LLC) with a hold time of 5 s. The sealed samples were covered with aluminum foil and allowed to incubate at room temperature (25 °C) for 10 min. Samples were decanted into 50 mL centrifuge tubes, centrifuged at 2 100 × *g* for 5 min and washed 5x with ultrapure water.

2.5. Encapsulation of curcumin into emulsions

Curcumin was added to canola oil at the level of 1 mg/g and the mixture was stirred and heated to 80 °C for 10 min followed by centrifugation at 16 100 × *g* for 10 min to precipitate the un-dissolved curcumin. The canola oil with dissolved curcumin could then be used to prepare the emulsion. First a coarse emulsion was made with 0.75g canola oil with dissolved curcumin and 5 mL of the 12% w/v stock of Ludox HS-30 solution, prepared in PB, using a high-speed disperser (Ultra-Turrax model T25, IKA Works, Wilmington, NC) set at 9 500 rpm (67 rcf) for 2 min with an IKA S 18 N 19 G dispersing probe. A fine emulsion was then produced by sonicating at 50% amplitude (Ultrasonic processor Q55, QSonica, Newtown, CT) for 30 s. A control emulsion, *i.e.* without curcumin, was created in a similar manner.

2.6. Shear disruption of yeast microcarriers

Yeast cell lysis was achieved by weighing 150 mg of yeast microcarriers, either freshly washed native cells or YCWPs, into 2.0 mL microcentrifuge tubes to which 1 mL of ultrapure water was added. Cells were disrupted with 0.5 mm glass beads and 0.5 mm zirconia/silica beads with a tissue homogenizer (FastPrep-24™ 5G Instrument, MP Biomedicals) set to 6 m/s. Each sample was homogenized 6x for 30 s intervals, followed by submersion in an ice bath for 30 s after each pulse. The cell homogenate was centrifuged at 16 100 × *g* and 4 °C for 30 min and the supernatant was collected for further analysis. Complete cell lysis was confirmed via light microscopy at 40x objective.

2.7. Ferric ion reducing antioxidant power (FRAP)/Trolox equivalent antioxidant capacity (TEAC) assay

A FRAP assay was conducted on the supernatant from the cell homogenate in order to determine the antioxidant capacity of yeast microcarriers. The assay was conducted as detailed by Benzie and Strain (1996). The following reagents were employed in this assay: 10 mM TPTZ in 40 mM HCl, 20 mM $FeCl_3$ and 300 mM sodium acetate buffer at pH = 3.6. Briefly, a working solution of FRAP reagent was prepared by mixing 2.5 mL of TPTZ solution, 2.5 mL of $FeCl_3$ solution and 25 mL of sodium acetate buffer. An aliquot of 900 μL of working FRAP solution was mixed with 90 μL of ultrapure water and 50 μL of the supernatant of the cell homogenate. After vortexing, the absorbance of the resulting solution was immediately measured at 593 nm and normalized to a blank, composed of 900 μL of working FRAP solution and 140 μL of ultrapure water. Antioxidant capacity was determined via a standard curve prepared with the use of a reference standard, Trolox.

2.8. Thermal treatment of microcarriers

First, yeast microcarriers were dispersed into 100 mM phosphate

buffer at pH = 6.5 with 0.9% NaCl (w/v) (PBS) at a ratio of 50 mg yeast microcarrier to 5 mL of PBS. Ludox HS-30 emulsions were diluted to 5% carrier oil (w/v) with PBS. The final concentrations of curcumin in the Ludox-HS30 stabilized emulsions were 15 μg/mL. Then, 2 mL aliquots of each samples were pipetted into 5.75 in (length) Pasteur pipettes (Fisher Scientific International, Inc., Hampton, NH) which were flame sealed, retaining only the top 4 in. The emulsions and yeast microcarriers were treated at 70 °C for 30 min and at 90 °C for 1 min. The tubes were monitored with a thermocouple (Type K, OM-DAQPRO-5300, Omega Engineering, Inc., Stamford, CT) and allowed to equilibrate to the appropriate temperature before treatment time began. Immediately after treatment, the samples were submerged into an ice bath to cool to room temperature.

2.9. Accelerated oxidation with AAPH

2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), a peroxyl radical initiator, was used to simulate accelerated oxidation of encapsulated curcumin in yeast microcarriers and Ludox HS-30 emulsions. Briefly, yeast microcarriers were dispersed in 20 mM AAPH in PBS while stock emulsions, 15% carrier oil (w/v), were diluted 1:2 with 60 mM AAPH in PBS to give a final concentration of 5% carrier oil (w/v) and 20 mM AAPH. Samples were covered from ambient light; shaken with a rotary incubator at 150 rpm and room temperature (20 °C); and sampled at regular intervals. The total treatment interval with AAPH was 24 h.

2.10. Extraction and percent recovery of bioactive

Curcumin was extracted with 1 mL methanol from 0.01 g of yeast cells (on a wet basis). The yeast-methanol suspensions were then vortexed and bath sonicated for 10 min (Branson 2510, Branson Ultrasonics). For emulsion samples, a liquid-liquid extraction method was utilized to determine the amount of curcumin retained in the carrier oil. First, curcumin was extracted by disrupting 200 μL of emulsion with 1 mL methanol and vortexed. All samples, yeast microcarrier and emulsion, were then centrifuged at 16 100 × *g* for 10 min. 500 μL of the supernatant was pipetted into a UV cuvette and the absorbance (λ_{max} = 425 nm for curcumin) was measured using a UV–vis spectrophotometer (GENESYS 10S Series, Thermo Scientific). A blank was prepared by extracting the control samples with methanol. The percent recovery was determined as follows:

$$\%\ Recovery = \frac{Absorbance_{Treated}}{Absorbance_{Before}} * 100\%$$

where $Absorbance_{Before}$ is the absorbance measurement of the extracted curcumin at 425 nm prior to thermal or oxidative treatment and $Absorbance_{Treated}$ is the absorbance measurement at 425 nm of the recovered curcumin extracted after treatment to heat or oxidation.

2.11. Confocal imaging

To image yeast and YCWP, a Leica TCS SP8 STED (Leica Microsystems Inc.) with a pulsed laser tuned to 495 nm was used in confocal mode. A HyD time-gated, hybrid detector was used to collect signal from 509 to 551 nm range, corresponding to the fluorescence of curcumin. A second HyD detector was used at 410–490 nm range to detect for the contrast dye, DAPI. A PMT Trans detector was used with the same excitation to produce the white light image. All images were taken with a 63x oil objective.

2.12. Curve fitting

The percent recovery (% recovery) data from treatment with AAPH was fitted to the following kinetic model using Matlab R2017a (MathWorks, Torrance, CA):

$$y(t) = C_0 e^{-(t/\tau)^a}$$

where y(t) is the fractional recovery of curcumin at the specified time point, C_0 is the initial fractional recovery, *i.e.* 100%, t is the time in hours, τ is the delay time or time required for exponential decay in hours, and a is the shape parameter. Matlab Curve Fitting Tool was used to fit the data with a non-linear regression model with "Trust Region" algorithm.

2.13. Statistical analysis

Statistical analyses were performed using RStudio. Analysis of variance (ANOVA) was used to determine if experimental values were significantly different. In addition, *post hoc* Tukey's analyses were conducted to determine any significant difference between groups. Significant values were reported with a 95% confidence interval, *i.e.* $p < 0.05$. For each repetition, three samples were extracted and analyzed via UV–vis spectrophotometry. All samples for extractions were conducted in triplicate at a minimum.

3. Results and discussion

3.1. Thermal degradation of curcumin

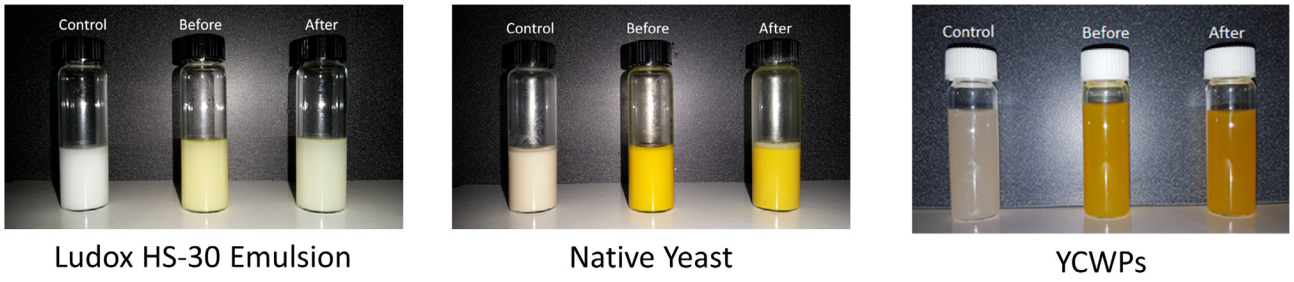
Yeast microcarriers and Pickering emulsions samples were subjected to thermal pasteurization to evaluate the effect of the carrier on the stability of encapsulated curcumin. Ludox HS-30 emulsions were selected based on the results in prior work. Ludox HS-30 emulsions have a semi-permeable relatively thick interface which resembles the structure and permeability of the cell wall of yeast. In addition, Ludox HS-30 emulsions were physicochemically stable at elevated temperature, do not form micelles in solution and have no purported antioxidant properties (Prestidge & Simovic, 2006). Table 1 shows the percent recovery of curcumin for Pickering emulsions, native yeast and YCWPs at two pasteurization conditions. The percent recoveries at 70 °C for 30 min were 66.2%, 75.8% and 91.8% for Pickering emulsions, native yeast and YCWPs respectively. Similarly, the percent recoveries at 90 °C for 1 min were 89.7%, 78.6% and 99.7% for Pickering emulsions, native yeast and YCWPs respectively. The yeast microcarriers, however, showed no significant difference among samples of the same type at longer timescales of processing.

The two-factor ANOVA conducted on the data set suggested that there was a significant difference associated with single variables which were pasteurization method and type of carrier. However, the interaction of these variables, *i.e.* pasteurization method:type of carrier, was not significantly different, suggesting that the method and carrier type together contributed less to the stability of curcumin during processing than the individual factors. Furthermore, there was no significant difference ($p > 0.05$) in the percent recoveries in native yeast for the two processing conditions while stability of the bioactive in both Pickering emulsions and YCWPs were significantly different between the selected set of processing parameters. The results seem to suggest that YCWPs, even devoid of most of its intracellular content, have a better protective ability against thermal mediated degradation than native yeast.

Fig. 1, which shows a confocal image of curcumin encapsulated in yeast before and after thermal processing, did not demonstrate an obvious change in the homogeneous distribution of curcumin; however, the images do show a notable decrease in the fluorescence intensity to 56% normalized fluorescence intensity percent. Fig. 2, which shows the confocal images for YCWPs before and after processing, did show specific intra-particle localization of curcumin in these particles based on the fluorescence signal. However, neither the distribution nor the fluorescence of the signal changed visibly in the YCWPs. In this case, the normalized fluorescence intensity percent decreased to only 87%.

Table 1
Comparative percent recovery of curcumin in emulsions, native yeast and yeast cell wall particles (YCWPs) microcarriers after processing at 70 °C for 30 min and 90 °C for 1 min.

Sample	4% Ludox HS-30 Emulsion	Yeast Encapsulated Curcumin	YWCPs
% Recovery (70 °C, 30 min)	66.2 ± 3.6	75.8 ± 0.9	91.8 ± 1.0
% Recovery (90 °C, 1 min)	89.7 ± 1.0	78.6 ± 3.5	99.7 ± 3.1



As noted in Young et al. (2017), the residual constituents within the YCWPs seem to be DNA as they bind strongly to 4′,6-diamidino-2-phenylindole or DAPI, a DNA intercalating dye. Curcumin has been shown to bind to DNA and other biopolymers (Nafisi et al., 2009; Pucci et al., 2012; Yazdi & Corredig, 2012). Furthermore, the release of curcumin from these structures was likely moderated by denaturation of these curcumin-biopolymer complexes. In the case of YCWPs, the residual fraction of proteins/DNA in the YCWPs was already denatured during preparation of the carriers. Moreover, the association of curcumin with the denatured biopolymers was likely not perturbed by heating, protecting the labile compound from heat and hydrolysis (Tapal & Tiku, 2012; Yazdi & Corredig, 2012). However, in native yeast cells, the biopolymers were denatured through heating. This process may have altered the association of curcumin with biopolymers in native yeast cells and may have contributed to the enhanced hydrolysis.

3.2. Peroxyl radical permeation into yeast microcarriers and Pickering emulsion

The ability of the cell structure, *i.e.* cell wall and intracellular components, to limit the degradation of curcumin during accelerated oxidation was measured using the peroxyl radical initiator, AAPH. AAPH has a half-life of about 175 h in aqueous solution (Li et al., 2011), which insured a constant concentration of peroxyl radicals throughout the timescale of the experiment. The permeation into the core of the carriers and subsequent degradation of curcumin by the peroxyl radical

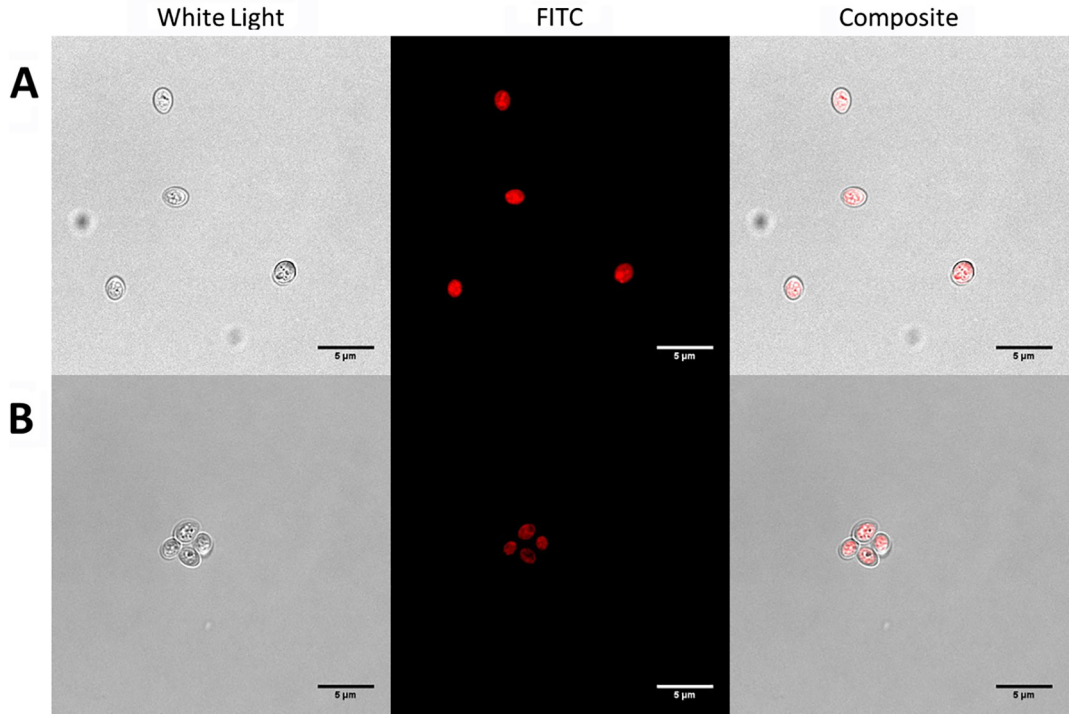


Fig. 1. Confocal fluorescence images of native yeast cells treated with thermal processing. Native yeast cells with encapsulated curcumin were subject to pasteurization at 70 °C for 30 min. A) No treatment and B) heat pasteurization at 70 °C for 30 min. Magnification: 63x with digital zoom.

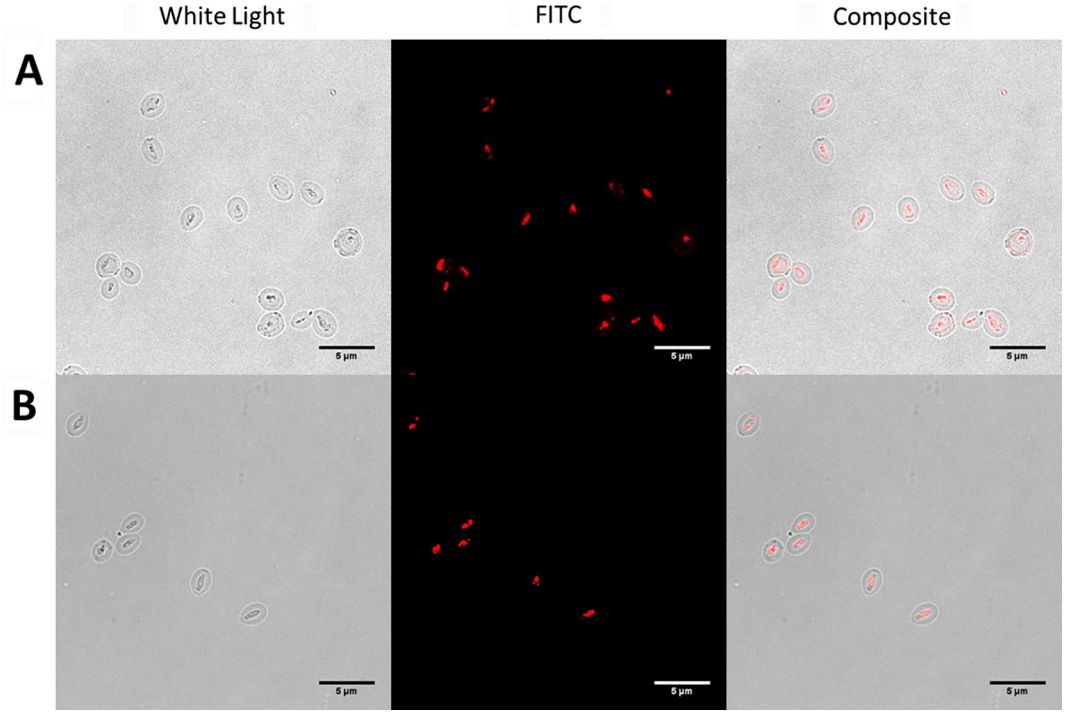


Fig. 2. Confocal fluorescence images of yeast cell wall particles (YCWPs) treated with thermal processing. YCWPs with encapsulated curcumin were subject to pasteurization at 70 °C for 30 min. A) No treatment and B) heat pasteurization at 70 °C for 30 min. Magnification: 63x with digital zoom.

was measured by observing changes in the absorbance. Curcumin is a peroxyl radical sensitive compound, and as such, is readily oxidized in lipid carriers (Ansari et al., 2005, Ak & Gülçin, 2008). Fig. 3 shows the change in the percent of recovered curcumin in A) native yeast cells; B) YCWPs; and C) Pickering emulsions after exposure to 20 mM AAPH in PBS over the course of 24 h. Fig. 3A shows the degradation of curcumin encapsulated in colloidal silica-stabilized emulsion. As mention before, Ludox HS-30 emulsions were selected as a reference emulsion-based encapsulation system for comparison with yeast microcarriers. After 3 h, there was a significant reduction ($p < 0.05$) in the recovery of curcumin as compared to the control. After 24 h of exposure to AAPH, only 39.9% of the curcumin was recovered from Pickering emulsions. It is important to note that previous studies have demonstrated that oxidation induced by AAPH on an accelerated time scale is correlated with the storage stability of these formulations (Pan et al., 2013; Tikekar et al., 2013).

Fig. 3B shows there was no significant change ($p > 0.05$) in the concentration of curcumin in native yeast cells as compared to the control samples suspended in PBS during the first three hours. However, after 24 h, the concentration of recovered curcumin from both the

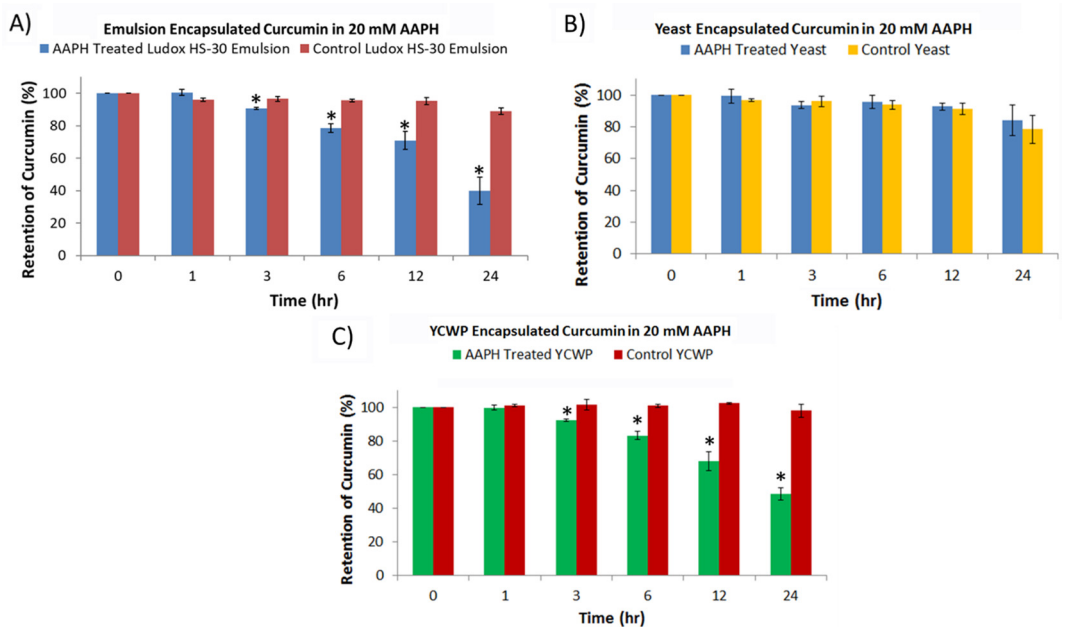


Fig. 3. Oxidative stability of curcumin encapsulated into (A) Ludox HS-30 (Pickering) emulsion; (B) yeast cell wall particles (YCWPs); and (C) native yeast. Both yeast microcarriers and emulsions were subjected to 20 mM AAPH, a water soluble peroxyl radical generator, in phosphate buffered saline.

Table 2
Nonlinear regression fit parameters of fractional retention of curcumin in various carriers exposed to AAPH as a function of time.

Sample	τ	a	R^2
Ludox HS-30 Emulsion	26.93	1.104	0.98
YCWPs	32.06	1.032	0.99
Native Yeast Cells	237.60	0.788	0.89

controls and the samples treated with AAPH decreased to about 80%, which was significantly different from the initial values at t = 0 h. This degradation was likely due to autolysis of subcellular constituents to which curcumin is bound, which led to its release and subsequent hydrolysis in the aqueous cytoplasm.

Fig. 3C, which reports the rate of curcumin degradation in YCWPs, was quite different from the trend observed in native yeast cells. In YCWPs, there was a significant difference ($p < 0.05$) between the control and treated samples after only 3 h, as the % retention of curcumin decreased to less than 90% in the treated samples compared to 99% in the controls. This trend was further observed throughout the remaining time points with a final curcumin concentration of 48% at 24 h. The difference in the rate of permeation of peroxyl radicals into native yeast and YCWPs was likely negligible as reactive oxygen species (ROS) are readily able to diffuse through the cell wall due to their small size. It is more likely that these observed differences in curcumin degradation rates between native cells and YCWPs is due to the intercellular constituents.

3.3. Antioxidant activity of yeast microcarriers and kinetic modeling

The antioxidant activity of yeast microcarriers was measured using the FRAP assay, which correlates the reduction of ferric-tripryidyltriazine complex to its ferrous form in the presence of a redox active compound (Benzie & Strain, 1996). A water-soluble vitamin E analogue, Trolox, was used as a reference standard to determine the antioxidant capacity of yeast microcarriers. Based on measurements, the Trolox equivalent values for YCWPs and native yeast are 0.20 μM and 7.48 μM respectively. A low Trolox equivalent value should be expected from YCWPs as the caustic conditions and high heat would oxidize and degrade any innate antioxidant systems within the cytoplasm. However, native cells, which may have up to 10 mM glutathione (Penninckx, 2002), an essential intracellular oxidative stress regulator which is constitutively expressed, should have a much higher Trolox equivalent value to correspond to the trends observed in Fig. 3B and Table 2. Espindola Ade, Gomes, panek, and Eleutherio (2003) suggested that desiccation of yeast cells decreases the ratio of GSH:GSSG, *i.e.* the ratio of the reduced form of glutathione to the oxidized form, which may partially account for the diminished Trolox equivalent value. However, this assay did not account for enzymatic mechanism for moderating oxidative stress in yeast such as catalase, which can degrade free peroxyl radicals and may contribute to a greater extent to the reduced degradation of curcumin in native yeast.

To quantify the relative differences in kinetic rates amongst yeast microcarriers and Pickering emulsion samples, the experimental data was fitted to the equation in Section 2.12; the results of this analysis are shown in Table 2. Comparison of the kinetic parameters suggested that both Pickering emulsions and YCWPs follow first-order kinetics, which is denoted by *a* values approximately equal to one (Priyadarsini, 1997). Colloidal silica has not been reported in the literature to have antioxidant capacity which was demonstrated by a relatively short τ and adherence to first-order kinetics. However, the rate kinetics observed in native yeast was less than one which may suggest some inhibitory effect diminishing the reaction of curcumin with the peroxyl radicals. Further comparisons showed that the exponential delay times, *i.e.* τ, for Ludox HS-30 and YCWPs were around 30 hr, while the value for native yeast cells was nearly 8x longer than the other encapsulating carriers in this study. This further supports the hypothesis intracellular constituents contributed to the retardation of peroxyl-mediated oxidation.

4. Conclusion

This study demonstrated an integrated approach to evaluate the thermal and oxidative stability of bioactives encapsulated within yeast microcarriers. The results presented in this study demonstrated that plasmolyzed yeast microcarriers, *i.e.* yeast cell wall particles, provided a better barrier against thermal degradation compared to native yeast microcarriers and Pickering emulsions. This observation was evidenced by the resistance of residual biopolymer against further denaturation which prevented hydrolysis of bound curcumin. In contrast, native yeast cells provided a better oxidative barrier against peroxyl radicals due to the presence of endogenous antioxidant systems which are absence in YCWPs and Pickering emulsions. Critical analysis of this study may help to establish formulations which employ yeast microcarriers to protect labile bioactives during thermal processing and storage.

Conflict of interest

The authors of this study declare that they have no conflicts of interest pertaining to the funding sources or to the materials presented in this study.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.foodchem.2018.08.121.

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