Supporting information

One-step assembly of zein/caseinate/alginate nanoparticles for encapsulation and improved bioaccessibility of propolis

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Methods

Propolis UV-vis spectroscopy and ABTS radical scavenging capacity

Propolis was observed to be quickly solubilized at pH 12.0 and the feasibility to prepare propolis loaded nanoparticles using pH-driven method was evaluated by monitoring propolis UV-vis spectroscopy and Trolox equivalent antioxidant capacity at different pH conditions. Propolis was pre-dissolved at 0.5 mg mL\textsuperscript{-1} in ethanol, and 1 mL of the propolis stock solution was mixed with 49 mL of double distilled water. The absorbance spectra of diluted propolis solutions at pH 4.0 before alkalization and after being acidified back to pH 4.0 were recorded with a spectrophotometer (Shimadzu UV-
The solutions without propolis were used as blanks. The antioxidant activity of propolis at different pH by the ABTS method was determined according to previous study\(^1\). The ABTS radical cation solution (7.4 mM ABTS\(^{••}\)) was prepared by mixing ABTS stock solution with 2.6 mM potassium persulfate at room temperature in dark for reacting 16 h. The resulting solution was diluted using phosphate buffer (0.1M pH 7.0) until the absorbance read 0.7 at 734 nm in a spectrophotometer (Shimadzu UV-2550). Subsequently, 1.0 mL of this ABTS\(^{••}\) working solution was mixed with propolis solution at different pH and incubated for 7 min. The absorbance was read at at 734 nm. The percentage of ABTS radical scavenging capacity was determined using Equation (3):

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\text{ABTS}^{•+} \text{ scavenging percentage (\%)} = \left( \frac{\text{Absorbance}_{\text{ABTS}^{•+}} - \text{Absorbance}_{\text{propolis solution}}}{\text{Absorbance}_{\text{ABTS}^{•+}}} \right) \times 100
\]

**Nanoparticle tracking analysis**

According to previous study\(^2\), NTA measurements were performed using a NanoSight NS300 (Malvern Instruments Ltd, Malvern, UK) at 25 °C. The NanoSight NS300 was equipped with a 638 nm laser sample chamber and a Viton fluoroelastomer O-ring. The samples were diluted 1000-fold in citrate buffer before being injected into the viewing chamber with syringes. The data was analyzed using NTA version 3.1 Build 3.1.45 (NanoSight Ltd, Malvern, UK).

**Influence of temperature**

According to previous report\(^3\), the temperature stability of the nanoparticles was examined using dynamic light scattering measurements (Nano-ZS90, Malvern...
Instruments Ltd., Worcestershire, UK). The samples were incubated at each temperature for 40 min prior to measurement.

**Results and discussion**

*Effect of the pH- cycle on propolis stability and ABTS radical scavenging capacity*

Previous studies have indicated that nutrients can be degraded under alkaline conditions\(^4\text{-}^5\). At pH 12.0, propolis became water soluble and deep red in color. This observation prompted us to evaluate the reversibility of the structural changes to propolis components by visible light spectroscopy and TEAC analysis during the processes of pH-induced one-step assembly method. The UV–vis absorption spectra of propolis before alkaline dissolution and after being acidified back to pH 4.0 were identical (Figure. S1A), suggesting no change in the structure of propolis with the pH conditions studied\(^6\text{-}^7\). This result was expected because previous studies have proved that some nutrients such as rutin\(^8\), curcumin\(^5\), eugenol\(^9\) and clove bud oil\(^7\) can resist alkaline degradation due to short-term alkaline treatment. The composition of propolis is complex with over 300 compounds being reported in different species, such as phenolic acids, flavonoids, aromatic aldehydes, fatty acids and sugars\(^10\). This obviously poses a challenge to evaluate single component changes during the pH cycle. Therefore, the influence of the pH cycle on the biological activity of propolis was measured by ABTS radical scavenging capacity analysis (Figure. S1B). With pH increasing from 4.0 to 12.0, the scavenging activities of propolis significantly \((P < 0.05)\) decreased from 84.58% to 78.81%. When pH was subsequently acidified to pH 4.0, the scavenging activities of propolis significantly \((P < 0.05)\) increased from 78.81% to 81.43%,
indicating protonation of the propolis phenolic components. The scavenging activities of propolis after being acidified back to pH 4.0 decreased by 3.15% compared to pristine propolis indicating the decomposition of some flavonoids into small phenolic acids at alkaline pH. Overall, propolis retained most biological activity and the degradation of propolis during the pH cycle is expected to be relatively small.

Figure. S1: Comparison of (A) UV-vis absorption spectra of propolis before alkalization at pH 4.0 and after being acidified back to pH 4.0. (B): Trolox equivalent antioxidant capacity of propolis at pH 4.0 (4 original), adjusting to pH 12.0 (12 up), after subsequent pH adjustment to pH 7.0 (7 down), and after subsequent pH adjustment
to pH 4.0 (4 down).

Nanoparticle tracking analysis

As shown in Fig. S2, compared to the samples fabricated by method 1 and method 2, more nanoparticles with smaller size were observed for M 3 sample. The particle concentration of M 3 sample was higher than M 1 and M 2 samples. The NTA data further supported the count rate data in DLS (Table 1).

Figure. S2: Size distribution as three-dimensional plots (size, number and light intensity) of three samples prepared with three different encapsulation approaches.
Temperature stability of zein/NaCas/Alginate nanoparticles

As shown in Fig. S3, the particle size was not affected with the decreasing of temperature from 25 °C to 5 °C. This finding was different from previous report. Fuenzalida et al\textsuperscript{3} report that cooling from 37 to 4 °C, results in increased particle size of the Alg-Lyz nanocomplexes. They assumed that hydrogen bond was the preponderant force, as the formation of the NCXs seems favored at a lower temperature. While, different finding in our work may due to minor role of hydrogen bond in our nanoparticles. Obviously, heating caused the unfolding and aggregating of protein, resulting in an increase in the size of nanoparticles. What’s more, heating induced structure rearrangement may also have resulted in the smaller PDI values of the heated nanoparticles. Previous studies\textsuperscript{8,11} also observed similar phenomenon.

Figure S3 Effect of temperature on the Z-average diameter and PDI of zein/NaCas/alginate nanoparticles (method 3 sample).
Figure S4: The zeta potential of the zein and casein at different pHs

References


