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Encapsulation of *N*-acetylcysteine (NAC) using protein-polysaccharide combinations through spray drying and air drying

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ABSTRACT

Although *N*-acetylcysteine (NAC) has shown significant medical and antioxidant activities, its strong sour taste and sulfur odor limits its use in food. Here we spray dried and air dried a mixture of proteins and polysaccharides that served as matrix materials to entangle NAC and delay its release in water. Proteins including egg white, gelatin, whey protein isolate, and casein, were combined with polysaccharides such as hydroxypropyl methylcellulose, and carboxymethyl cellulose and electrostatic interactions with NAC were formed. The resulting solutions were then air or spray dried. The powders were analyzed by SEM, FTIR, LC-MS, NMR, and conductometry. The resulting powders exhibited slower release in water, particularly when polysaccharides were incorporated with proteins as matrix materials. SEM results showed the spray-dried samples displayed spherical and concaved structures with particle sizes ranging from 2 to 10 μm. While in the air-dried samples, NAC crystals were observed on the surface. Conductometry revealed that gelatin, with different bloom strengths exhibited different patterns in their NAC release profiles upon spray drying. In comparison to the spray-dried samples, samples obtained from air drying showed a faster NAC release in water.

1. Introduction

N-acetylcysteine (NAC) is a water-soluble amino acid and has shown positive effects on health conditions such as inflammation, lipid accumulation, insulin resistance, and oxidative stress (Dludla et al., 2019; Millea, 2009). However, NAC exhibits a very unpleasant sulfur taste and smell, which makes it unacceptable for direct consumption or incorporation into food products (V. Bhilare et al., 2016).

Encapsulating small bioactive molecules into a polymeric matrix has gained substantial interest as a method for protecting products, extending shelf-life, and controlling the release of nutraceutical and pharmaceutical supplements (Pérez-Masiá et al., 2015). Previous researchers have encapsulated NAC using nonfood-grade materials such as PEGylated nano-niosomes (Firozian et al., 2020) and PLGA nanoparticles (Karimi Zarchi et al., 2015) or by using an organic solvent in the fabrication process, such as chloroform (Ourique et al., 2014). However, encapsulating NAC for controlled release in a food-safe manner with food-safe ingredients has not been widely reported (Enayati et al., 2022).

Proteins are good candidate materials to encapsulate NAC. Previously, we proved that NAC, when hydrolyzed, could be tightly bound to the hydrophobic pocket of ovalbumin, the main protein in egg white, forming a complex via hydrogen bonding (J. Wang et al., 2020). Polysaccharides are added to the proteins used as the matrix materials to encapsulate water-soluble bioactive compounds like NAC through electrostatic interactions between proteins and polysaccharides to improve drying properties (Rodríguez Patino & Pilosof, 2011) and increase the oxidative stability of the encapsulated ingredients (Le Tan et al., 2017; Li et al., 2017; Rusli et al., 2006). The physicochemical properties of protein-polysaccharide matrix materials can be impacted by pH, ionic strength, and processing conditions (Ye, 2008). Therefore, materials for the matrix composition need to protect the core materials from physicochemical degradation and evaporation, while achieving an acceptable controlled release profile (Pérez-Masiá et al., 2015; Saifullah et al., 2019; Woo et al., 2007).

Spray drying is the most extensively applied technology used for the

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microencapsulation of food ingredients (Nedovic et al., 2011). The advantages of spray drying include cost, speed, and equipment. Numerous studies have used gelatin as the protein source to encapsulate food ingredients by spray drying since gelatin has strong emulsifying and stabilizing properties and forms three-dimensional networks (Gharsallaoui et al., 2007). Egg whites, casein, and whey protein isolate have also been used in microencapsulation due to their functional properties (Gharsallaoui et al., 2007; Jarunglumlert & Nakagawa, 2013; Khanji et al., 2018; Nogueira et al., 2020; Shishir et al., 2018; Tavares & Zapata Noreña, 2019).

Air drying, allowing solvent to evaporate at room temperature and ambient pressure, is another widely used drying technology that is used to encapsulate bioactive compounds. Air drying is inexpensive and requires no special equipment. One potential drawback of air drying is oxidation, the prolonged drying time under ambient conditions has been shown to increase the likelihood that both the matrix materials and the encapsulated organic molecules are air oxidized (Menin et al., 2018). Regarding encapsulation, ionic gelation between a protein and a polysaccharide, or between two different polysaccharides is commonly used, and the developed products have different microstructures, particle sizes, and encapsulation efficiencies compared to samples obtained by other drying methods (Liu et al., 2022; López de Dicastillo et al., 2023; Timilsena et al., 2019).

The goal of our work was to find a method to slow down the release of NAC in water long enough for it to be ingested without interacting with the tastebuds so that it can be consumed without the perception of an unpleasant flavor. To this end, combinations of proteins and polysaccharides were used as encapsulating matrix materials to incorporate NAC via spray drying and air drying. Choosing the most appropriate wall materials is another factor that determines the properties of the resulting materials. EW is an efficient wall material due to its high emulsifying property and it has been shown to form complexes with a wide range of phytochemicals (Samborska et al., 2021). Gelatins are the most commonly used spray drying animal-based protein sources in food ingredients due to its high complexation property, good morphological features, and desirable encapsulation efficiency when it is combined with other wall materials (Cortés-Morales et al., 2021). Here, we used EW or gelatin in combination with different polysaccharides as wall materials which formed networks to trap and protect NAC with a goal to slow down NAC release in water. Subsequently, the NAC release profiles of the resulting solids were evaluated. The morphology of the samples was evaluated using SEM, while LC-MS, FTIR and NMR were used to assess NAC incorporation. Conductometry was used to evaluate the release profiles of NAC for each combination of protein, polysaccharide, and encapsulation technique.

2. Materials and methods

2.1. Materials

N-acetylcysteine (NAC) was purchased from Ark Pharmacies Co (Austin, TX, USA). Unflavored gelatin (Knox, Phoenix, AZ, USA) was purchased from the local market (Ithaca, NY, USA). Gelatins with two bloom strengths, 90 and 250, were purchased from Gelita (Sioux, IA, USA). Bulk liquid egg white (EW) was purchased from the local market (Ithaca, NY, USA). Whey protein isolate (WPI), casein, maltodextrin (MD), Mannose (Mann) and sodium hydroxide were obtained from Sigma-Aldrich (St. Louis, MO, USA). Methylcellulose (MC) was obtained from Tic Gums (Belcamp, MD, USA). Carboxymethyl cellulose sodium salt (CMC) and hydroxypropyl methylcellulose (HPMC) were purchased from Alfa Aesar (Ward Hill, MA, USA). MilliQ water (Millipore, Molsheim, France) was utilized throughout the experiments.

2.2. General preparation of NAC-protein-polysaccharide solutions

The solutions for spray drying and air drying were prepared by mixing protein (with or without polysaccharide) solutions with a NAC solution in water with a magnetic stirrer for 2 h or until complete dissolution/hydration was reached. As an example, Sample E-2 (Table 1) was fabricated by first dissolving 6.0 g of EW and 2.0 g of CMC in 100.0 mL of MilliQ water in one beaker and 12.0 g NAC in 50 mL water in another beaker. After the proteins and polysaccharides were dissolved, the solutions in the two beakers were poured together and stirred for an additional 2 h. The different formulations of NAC, protein, and polysaccharides are presented in Table 1, and details regarding formulations can be found in Tables S1–S3.

2.3. Encapsulation of NAC by spray drying and air drying

The spray drying process (Fig. 1) was carried out using a LabPlant SD-Basic, spray dryer (NorthYorkshire, U.K.). Solutions were fed into the drying chamber through a peristaltic pump with the inlet air temperature of 175 ± 5 °C and outlet air temperature of 75 ± 5 °C with an airflow of 600 L h^{-1}. The feeding rate was 16.7 mL min^{-1}, with an atomization pressure of 20 psi.

To air dry the samples, an aliquot (50 mL) of the prepared NAC-protein-polysaccharide solution was poured onto a food-grade polypropylene plastic plate (26 cm in diameter) on the lab bench and air-dried at room temperature for at least 72 h. Afterward, the dried films were ground using a mortar and pestle into a fine powder, sealed in ziplock bags, and stored in a dry environment for future analysis (Fig. 1).

2.4. Scanning electron microscopy (SEM)

The morphology of the spray-dried and air-dried samples was analyzed by SEM using a Zeiss Gemini 500 Field Emission Microscope (LEO Electron Microscopy, Thornwood, NY, USA). Samples were sputter-coated with an ultra-thin gold layer, and the images were collected.

2.5. Conductometry measurement

The NAC release profiles of the fabricated samples in water were quantified using a Metrohm 856 conductometer module (Metrohm, Riverview, FL, USA) with a 5-ring probe electrode (0.7 constant, range 5 μS cm^{-1} - 20 mS cm^{-1}). Before measuring, the conductometer was equilibrated for 60 s by submerging the electrode in 80 mL MilliQ water in a beaker with constant stirring (300 rpm). Then, the NAC release experiments were carried out by adding 133.3 mg of the spray-dried sample or 1333.3 mg of the air-dried sample into MilliQ water (80 mL), to achieve a NAC concentration of 1.0 g L^{-1} for spray-dried samples and 10.0 g L^{-1} for air-dried samples. The control was prepared by adding 80 mg or 800 mg pure NAC into 80 mL MilliQ water for spray-dried and air-dried samples, respectively. The calibration curve of pure NAC dissolved in water is available in the Supplementary Information (Fig. S1). The release percentage was calculated by dividing the released NAC (mg) in solution at 180 s by total amount of NAC released after 24 h of shaking in water as quantified by the LC-MS data (Eq. (1)).

$$\frac{(\text{mg of NAC from conductometry at 3 min})}{(\text{mg NAC released after 24 h per LC}-\text{MS})\times(\text{Sample Size})}\times 100 = \%\ \text{NAC released in 3 min.} \quad (1)$$

2.6. Liquid chromatography mass spectrometry (LC-MS)

The NAC release in 24 h in water of the fabricated samples was quantified using an Agilent 1100 series LC equipped with a mass spectrometer (MS) (Agilent 1100 Series, Santa Clara, CA, USA) equipped with a Luna Omega (Phenomenex, Torrance, CA, USA) Polar C18 100 Å, LC column, 100 × 4.6 mm, 3 μm, at reverse-phase chromatography. Liquid chromatography eluents, A and B, under gradient elution were DI-water (formic acid 0.1 mg mL^{-1}) and acetonitrile, respectively. The

Table 1
Sample composition with sample numbers. Precise formulations can be found in Tables S1–S3.

Egg White		Gelatin (Knox)		Gelatin Bloom 90		Gelatin Bloom 250	
Spray-dried Samples							
E-1	NAC/EW	**G-1**	NAC/Gelatin	**G90-1**	NAC/Gelatin-90/HMW HPMC	**G250-1**	NAC/Gelatin-250/MC
E-2	NAC/EW/CMC	**G-2**	NAC/Gelatin/CMC	**G90-2**	NAC/Gelatin-90/CMC	**G250-2**	NAC/Gelatin-250/MC/MD
E-3	NAC/EW/Casein	**G-3**	NAC/Gelatin/Casein	**G90-3**	NAC/Gelatin-90/MC/MD	**G250-3**	NAC/Gelatin250/MC/Mann
E-4	NAC/EW/HPMC	**G-4**	NAC/Gelatin/HPMC	**G90-4**	NAC/Gelatin-90/VHMW HPMC	**G250-4**	NAC/Gelatin-250/CMC
E-5	NAC/EW/WPI	**G-5**	NAC/Gelatin/WPI	**G90-5**	NAC/Gelatin-90/VHMW HPMC/MD	**G250-5**	NAC/Gelatin-250/HMW HPMC
						G250-6	NAC/Gelatin-250/VHMW HPMC
						G250-7	NAC/Gelatin-250/VHMW HPMC/MC/MD
Air-dried **Samples**							
				A90-1	NAC/Gelatin-90/VHMW HPMC	**A250-1**	NAC/Gelatin-250/VHMW HPMC
				A90-2	NAC/Gelatin-90/HMW CMC	**A250-2**	NAC/Gelatin-250/HMW CMC
				A90-3	NAC/Gelatin-90/MC	**A250-3**	NAC/Gelatin-250/MC

Egg White (EW), *N*-acetylcysteine (NAC), Gelatin Bloom Strength 90 (G-90), Gelatin Bloom Strength 250 (G-250), Whey Protein Isolate (WPI), Carboxymethyl cellulose (CMC), High Molecular Weight hydroxypropyl methylcellulose (HMW HMPC), Very high Molecular Weight hydroxypropyl methylcellulose (VHMW HMPC), High Molecular Weight Carboxymethyl Cellulose (HWM CMC), Methylcellulose (MC), maltodextrin (MD), Mannose (Mann).

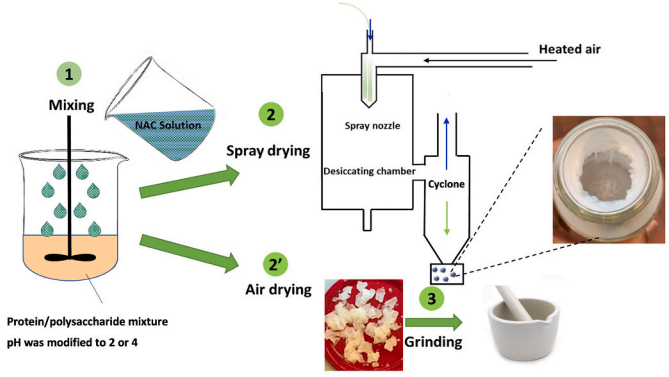

Fig. 1. Fabrication of NAC encapsulated samples through spray-drying and air-drying.

solvent flow started from 90% of eluent A and 10% of eluent B for 1 min. Then, they changed to 50% A and B over 5 min. These conditions were maintained for an additional 3 min, and then they went back to 90% A and 10% B in 3 min (total run time was 12 min). MS (Finnigan LTQ mass spectrometer) with an electrospray ionization interface (ESI) in positive mode was used for mass data acquisition. The optimized parameters for the ionization source included sheath gas flow rate at 20 arbitrary units, the capillary voltage at 41.0 V, spray voltage set at 4.00 kV, tube lens set at 125.0 V, and capillary temperature at 350 °C.

For the calibration curve, a stock solution was made by adding 10 mg of NAC into 10 mL MQ-water including 0.1 mL/dL of formic acid. The standard solutions of 10, 25, 50, 100, 200, 300, 400, and 500 mg L^{-1} NAC were prepared from the stock solution via serial dilution, and a calibration curve was created ($R^2 = 0.99$) (Fig. S2). Samples were prepared by grinding, and then 7.5 mg of the spray-dried or air-dried mixtures were dispersed in 15.0 mL MilliQ-water (containing formic acid 0.1 mg mL^{-1}) to afford a 500 mg L^{-1} solution of each mixture (spray dried or air dried). These samples were placed on an orbital shaker for 24 h and then filtered using 0.22 μm PVDF syringe filters for LC-MS analysis. Samples were not homogenized nor extracted further and because of this, the retentate includes NAC that is still tightly held or trapped by the matrix materials. Further agitation, or more extreme extraction conditions, such as homogenization, Soxhlet extraction, or ultrasonication, would be required in order to release 100% of the NAC from the matrix materials (Tan et al., 2018).

2.7. Fourier transform infrared (FTIR) spectroscopy

Attenuated total reflectance Fourier transform infrared spectra (ATR-FTIR) of pure NAC, spray-dried and air-dried samples were acquired using a Shimadzu IRAffinity-1S spectrophotometer (Columbia, Maryland, USA) measured at room temperature. The resolution was set at 2 cm^{-1} with 64 scans, and the frequency range was set at 400-4000 cm^{-1}.

2.8. Nuclear magnetic resonance (NMR) spectroscopy

Nuclear magnetic resonance (NMR) Spectroscopy was performed using a Bruker AV500 NMR spectrometer (Billerica, MA, USA) at 23 °C. To obtain ^1H and ^{13}C NMR spectra, samples were prepared in DMSO-d_6 with occasional shaking for 24 h then filtered through Nylon filters (0.22 μm).

3. Results and discussion

3.1. Particle size and morphology by SEM

SEM images of the powders obtained by spray and air drying revealed that the encapsulation technique affected the morphology of the particles (Fig. 2 and S3). The spray-dried samples using EW and CMC or HPMC as matrix materials showed concave spherical particles with particle sizes ranging from 2 μm to 10 μm. These structures were attributed to the moisture loss and shrinkage of the particles (Papoutsis et al., 2018).

The addition of polysaccharides into gelatin as matrix materials, such as CMC and HPMC, resulted in smooth surfaces in the spray-dried samples, representing the enhanced elasticity and film-forming property in those samples (Fig. 2) (Rajabi et al., 2015; Sun et al., 2018). Similar results were also found in the literature, in which the spray-dried particles developed by the complex of gelatin and gum Arabic showed smooth and intact spheres (Rajabi et al., 2015). Powders obtained by air drying showed a flattened structure with NAC crystals observed on the surface highlight by white arrows (Fig. 2), suggesting that the NAC was not completely incorporated into the system and that air drying, because it is a slower process, allowed some of the NAC to move through the matrix and recrystallize.

3.2. Structural characterization of the air-dried and spray-dried samples

3.2.1. FTIR analysis

The ATR-FTIR spectrum of pure NAC showed typical absorption bands at 3370, 2550, 1715, 1530, and 535 cm^{-1}, which are assigned to the N–H stretching vibration, free S–H stretching, carbonyl group, N–H bending, and carboxyl group stretching, respectively (Fig. S4) (Du et al., 2019; Hamedinasab et al., 2020; Pavia et al., 2014). Upon interaction with EW (Fig. S4a) and gelatin (Fig. S4b), the intensity of the S–H

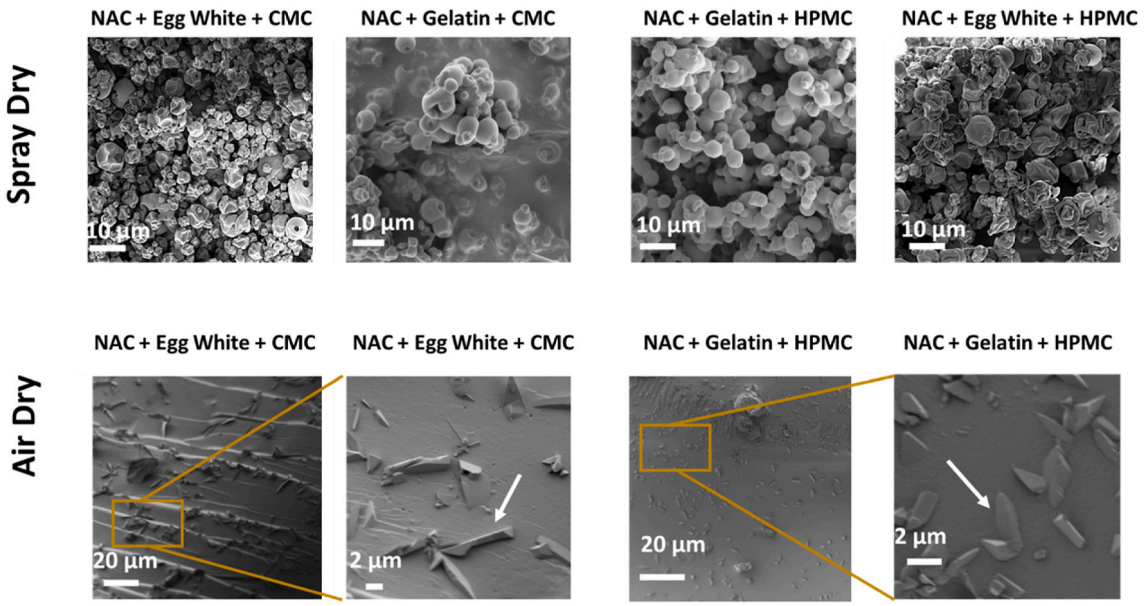

Fig. 2. Representative SEM images of (A) spray-dried and (B) air-dried NAC samples with different matrix materials. The gold boxes indicate the area that was enlarged by imaging at 2 µm in the next image. The white arrows represent NAC crystals present on the microcapsules external surface.

stretching and *N*–H stretching vibration bands decreased dramatically, which is attributed to the intra- and intermolecular disulfide bond formation between NAC with EW, and within EW, or the hydrogen bond formation between NAC with EW or gelatin (Aldini et al., 2018; Hoque et al., 2010; J. Wang et al., 2020).

Other bands, such as *N*–H bending (1530 cm^{-1}), and carbonyl group (535 cm^{-1}) stretching, were present in all samples. A similar trend was observed in the spray-dried samples using gelatin (G90 and G250) and polysaccharides as matrix materials (Fig. S5), where the characteristic bands of NAC disappeared upon interacting with the protein and polysaccharides. However, in air-dried samples, the prominent bands of NAC were still found in FTIR spectra (Fig. S6). This represents that NAC was not fully incorporated chemically into the system due to the low-temperature processing of the air-dried samples (Fig. S6).

3.2.2. NMR analysis

We performed NMR analysis of some representative spray-dried samples prepared using EW or gelatin/polysaccharide mixture as encapsulating matrix materials to confirm the presence of NAC in our products (Figs. S8 and S9). All samples showed the characteristic peaks for NAC after grinding, dispersal, and storage in DMSO-d_6 for 24 h (samples were filtered before NMR measurements). However, due to the involvement of hydrogen bonding in interactions between proteins and polysaccharides, the hydrogens from –SH, –NH, and –COOH were not observed. The corresponding ^{13}C NMR spectra of these samples exhibited all of the expected carbon peaks of NAC (Enayati et al., 2022).

3.3. LC-MS analysis of NAC release

Spray-dried and air-dried samples were stirred in water for 24 h, then the NAC released into the water was quantified using LC-MS (Fig. S7, Table S1). All of the NAC is incorporated into the wall matrix; we might consider this 100% incorporation; however, the LC-MS shows less than quantitative recovery because some of the NAC was retained in the matrix materials and removed via filtration during sample preparation for LC-MS analysis. When EW was used as the protein source, only 28% of the NAC was recovered after 24 h (E-1). While for EW combined with other proteins, the recovery was higher. For Casein 43% (E-2) of the starting NAC was recovered while for WPI the recovery was only 38% (E-3) (Table S1). We attribute the changes in NAC recovery between proteins to the cysteine groups which increase the interactions between EW and NAC, and thus we recover less of the NAC from the EW matrix after 24 h of shaking in water (Hoober et al., 1999; Hoque et al., 2010). To test this, we used gelatin as another protein source and found that on average, gelatin retained slightly less NAC in a 24 h period. NAC releases ranging from 39 to 52% were observed for all Knox gelatin samples (G-1 to G5) (Table S1) and gelatins with different bloom strengths (90 or 250) also showed lower NAC retention after 24 h (Table S2). Because gelatin lacks cysteine groups, this observation supported the conclusion that the interactions between NAC and the cysteine groups of EW were a primary reason for the increased retention of NAC in those samples (Priftis & Tirrell, 2012).

3.4. NAC release profile in water

Conductometry has been widely used for measuring the release profiles of hydrophilic compounds (Contreras et al., 2010). Recently, conductometry has been reported as an acceptable method to measure the release profile of encapsulated NAC in water (Madarshahian et al., 2022).

To be sure that the NAC would not release in the oral cavity in quantities large enough to impart an off flavor, we sought a NAC release profile in water of 4 min and at less than 25% of our formulated concentration. These values are below reported oral cavity residency times and detectable flavor concentrations for NAC (Iqbal et al., 2021; Madarshahian et al., 2022; Martínez-Las Heras et al., 2017). We used protein alone, protein-protein combinations, or protein-polysaccharide complexes as matrix materials to investigate their effect on NAC release in solutions after spray-drying from solutions at pH 2 and pH 4. The initial pH, pH 2, was chosen because this is the pH of NAC in water, however at pH 4 the solutions were less viscous, and the resulting spray dried samples were more homogeneous. In all cases, we observed a lower than quantitative NAC release based on the 24 h LC-MS data indicating that after 3 min some of the NAC remained in the matrix (Table S1, Fig. 3a and b). Partial replacement of EW with polysaccharides (CMC, HPMC) or another protein, such as WPI, were the weakest matrix materials, releasing over 75% of the NAC within 3 min (**E-2, E-4, E-5,** Table S1). The sample composed of EW and carboxymethyl cellulose (**E-3**) showed the best NAC retention within 3 min at 54% (Table S1). EW, whether it is combined with other proteins or

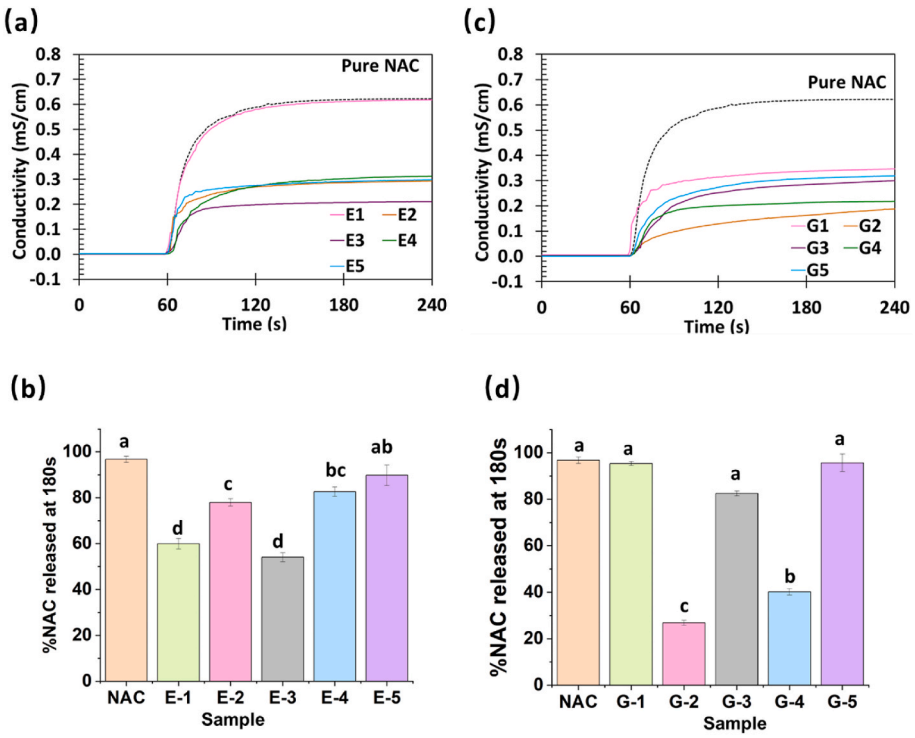

Fig. 3. NAC release profile measured by conductometry of spray-dried samples using (a–b) EW/polysaccharide and (c–d) Knox gelatin/polysaccharide. Formulations and reaction conditions for these samples are presented in Table S1. Different letters on bar graphs indicate significant differences. ($p < 0.05$).

polysaccharides as matrix materials, had a smaller than expected effect on slowing NAC release (Table S1). When gelatin (Knox) was combined with CMC (G-2), or HPMC (G-4), the NAC release was 27% and 40% at 3 min, respectively (Fig. 3c and d).

We attribute the differences between EW and gelatin to the different kinds of aggregates that are formed. These aggregates had different shapes and their properties are driven by the structural attributes of the different proteins. For example, heating EW changes its secondary structure increasing its surface hydrophobicity and creating a more stretchable protein structure. These changes allow EW to connect more strongly to the polysaccharide helices through electrostatic interactions (He et al., 2021). Gelatin and polysaccharides, however, undergo coil-to-helix transitions when heated and upon cooling their networks intertwine forming a biopolymer-rich network domain connected by helices (C.-S. Wang et al., 2018). The combination of gelatin with other proteins, such as casein (G-3, NAC release 82%) or WPI (G-5, NAC release 95%), produced NAC release profiles which were similar to Knox gelatin alone (G-1, NAC release 95%).

Because the samples with matrix materials composed of gelatin showed the slowest NAC release, we focused on gelatin with bloom strength 90 and bloom strength 250 in combination with different polysaccharides. The mixture of NAC with these matrix materials produced mixtures at pH 2 that were very viscous and ultimately unsuitable for spray drying. However, when the pH was increased to 4, the viscosity was greatly reduced. We attribute this difference in viscosity to the nature of the protein structure, in particular the gelatin. At lower pH (compared to isoelectric point), gelatin by itself has higher viscosity due to the presence of a less compact structure with larger hydrodynamic radius and longer drainage time, thus at pH 2 the solutions are more viscous. At pH 4, the viscosity was lower due to the formation of fewer triple helices, and gelatin's innate lower viscosity as it approaches its isoelectric point (Masuelli & Sansone, 2012).

Gelatin bloom index, or bloom strength as measured by gel strength and stiffness, is an essential physicochemical parameter that determines mechanical, rheological, and thermal properties (Chou et al., 2016). The physicochemical properties of gelatin with different bloom strengths, such as gel stiffness, can be very different because of the amino acid content, and secondary structure (Chou et al., 2016). As a result, changes are observed in the way these proteins interact with NAC which changes the NAC release profile. Therefore, the present study used gelatin with two bloom strengths, 90 (G90) and 250 (G250), combined with other polysaccharides, as a NAC-encapsulating matrix material and used conductometry to study their release profiles (Table S2, Fig. 4 a and b). The control sample containing spray-dried NAC solution with no matrix materials released 97% of the NAC in the first 3 min (Table S2). Among all samples with G90, the slowest NAC release was found when the matrix material consisted of G90 combined with MC or VHMW HPMC were added MD (G90-3 and G90-5, 39%), followed by the HMW HPMC (G90-1, 41%). We attribute these observations to the increased electrostatic interactions between the positively charged gelatin and the negatively charged polysaccharides (Table S4). At pH 4, NAC solutions have a negative zeta potential while gelatin-90 and gelatin-250 have positive zeta potentials (Table S4). These charge differences between NAC and gelatin-90 and gelatin-250 contribute increased electrostatic interactions and thus may contribute to these samples showing the slowest NAC release (Table S2).

The combination of G90 and CMC (G90-2) at pH 4 had the fastest release rate 85% of the NAC was released in 3 min. This phenomenon is in accordance with previously reported studies which used a gelatin and CMC coacervate as encapsulating agents (Priftis et al., 2012). Despite the electrostatic interactions between gelatin and CMC polymers, dissolution of the coacervates occurred when the mixing pH was under 4.25. Therefore, under pH 4, the complex dissociated and caused fast NAC release.

In the spray-dried samples using G250 and polysaccharides as matrix materials, the slowest NAC release, (17% in 3 min), was found in G250-1 where G250 and MC were used as matrix materials (Table S2, Fig. 4 c and d). The addition of MD or mannose did not further slow the release of the NAC (release was 31% for G250-2 and 46% for G250-3 Table S2). The observed difference in the NAC release profiles between gelatin-90

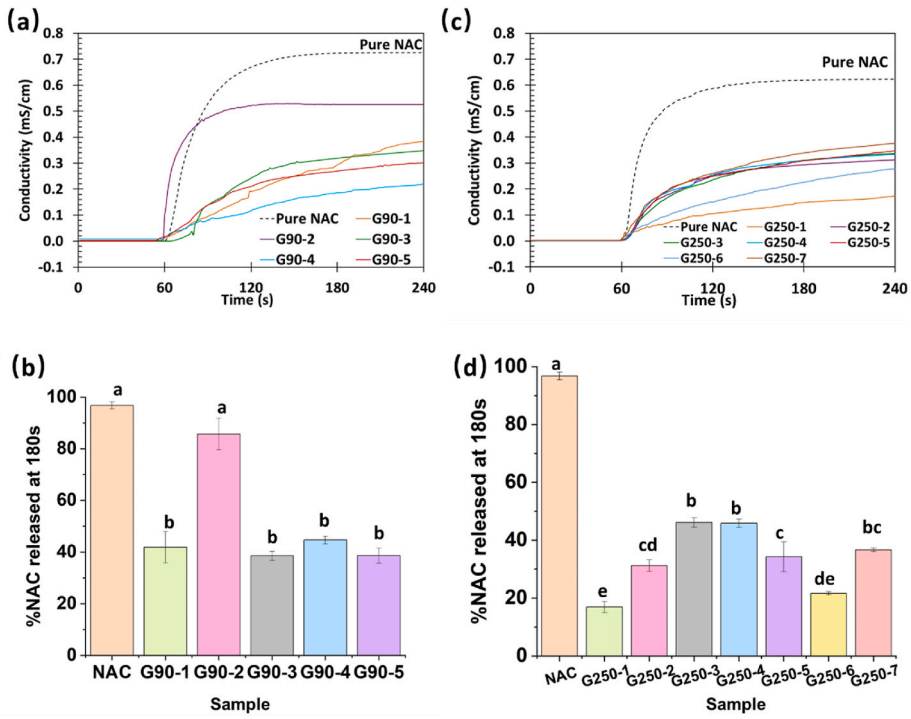

Fig. 4. NAC release profile in water measured by conductometry of spray-dried samples using (a–b) gelatin (bloom strength of 90)/polysaccharide and (c–d) (bloom strength of 250)/polysaccharide as matrix material. Formulations and reaction conditions for these samples are presented in Table S2. Different letters on bar graphs indicate significant differences. ($p < 0.05$).

and gelatin-250 can be attributed to the structural differences between the two types of gelatins. A study of the release of tea polyphenol using gelatin with varying bloom strengths as encapsulating agents found that gelatin with higher bloom strength had a correspondingly higher content of α-helices than β-sheets compared to the gelatin with lower bloom strengths (Kuai et al., 2020). As a result, the high content of α-helices resulted in more triple helix structures which have been shown to promote gel stabilization, reduce the degree of particle swelling, and improve gel mechanical properties thus retaining encapsulated materials at a higher rate. In addition to that, gelatin with higher bloom strength exhibits a significant reduction in the water absorption compared to gelatin with low bloom strength, this also caused affect the

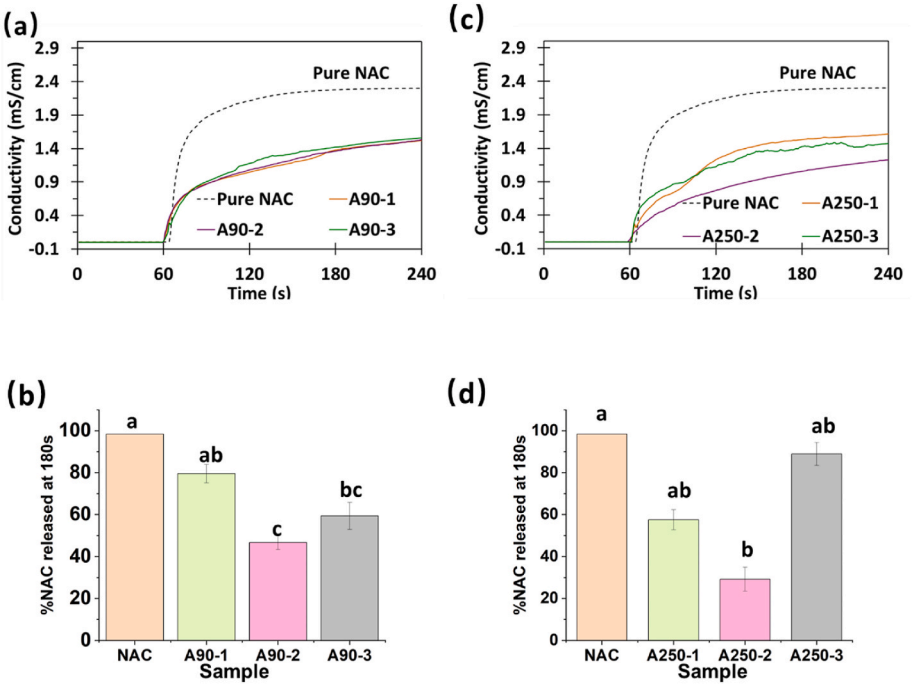

Fig. 5. NAC release profile in water measured by conductometry of air-dried samples using (a–b) gelatin (bloom strength of 90)/polysaccharide and (c–d) (bloom strength of 250)/polysaccharide as matrix material. Formulations and reaction conditions for these samples are presented in Table S3. Different letters on bar graphs indicate significant differences. ($p < 0.05$).

NAC release in water (Kempka et al., 2014).

3.5. NAC release from air-dried samples

Air drying using G90 and G250 combined with three polysaccharides, MC, HPMC, and HMW CMC were chosen as encapsulating agents because of their performance as matrix materials in spray drying. In the air-dried samples, pH 2 was used in the sample fabrication to take advantage of the higher gel viscosity and consequently the slow the movement of NAC toward the surface during the drying process. Under this condition, NAC is expected to be tightly locked into the polymer network. Overall, the air-dried samples showed substantially faster NAC release within 3 min compared to the spray-dried samples, the slowest release was found in the combination of G90 with HMW CMC (A90-2, 47%) (Table S3, Fig. 5a and b). For G250, again the slowest release was found when CMC was added as a matrix material (A250-2, 29%) (Table S3, Fig. 5 c and d), this is possibly due to the carboxymethyl groups of CMC interacting with lysine/hydroxylysine side groups of gelatin, as a result, the gel structure and strength were dramatically improved (Duhoranimana et al., 2017). The overall results are consistent with FTIR data, in which the characteristic –SH band of NAC was also present in all the air-dried samples indicating weaker interactions between the matrix materials and NAC. This was also confirmed by the SEM data where NAC crystals were observed on the surface of the films rather than incorporated into the matrix materials (Fig. 2). This was not entirely unexpected as the rate of air drying is slower and would allow for recrystallization of the NAC, preferably at the surface.

4. Conclusion

We investigated the encapsulation of NAC by protein/polysaccharide complexes through spray drying and air drying for controlled release. Egg white, casein and gelatin mixed with polysaccharides, MD, HPMC, CMC, and MC, were combined and used as matrix materials to slow the release of NAC in water. The structure, morphology, NAC loading, and release, were analyzed by FTIR, SEM, conductometry, LC-MS, and NMR. SEM results showed that the spray-dried samples displayed spherical and concaved particles ranging from 2 to 10 μm, whereas the air-dried samples exhibited a crystal-shaped amorphous structure. The conductometry results showed that gelatin with different bloom strengths had discriminative interaction mechanisms with polysaccharides. Using 90 bloom gelatin combined with either MC and MD (G90-3, NAC release 39%) or VHMW HPMC (G90-4, NAC release 39%), and 250 bloom strength gelatins combined with MC alone (G250-1, 17%) as matrix materials showed the slowest NAC release profiles in water. Compared to the control sample, the FTIR spectra showed that the intensity of characteristic bands of NAC decreased in all spray-dried samples, but in the air-dried samples, the state of those peaks remained unchanged. NMR analysis showed the hydrogens from –SH, –NH, and –COOH could not be observed, suggesting the NAC was hydrogen bonding with the matrix materials thus accounting for the slower release rates for all spray-dried samples. This research showed that spray drying using protein and polysaccharides as matrix materials could be a promising method to achieve delayed release of NAC.

Credit author statement

J.W.: Conceptualization, Methodology, Investigation, Data curation, Writing - original draft, Writing - review & editing. **M. E.:** Conceptualization, Investigation, Data curation, Writing - review & editing. **S. M.:** Data curation, Writing - review & editing. **G. U.:** Resources, Writing - review & editing. **B.Y.:** Resources, Writing - review & editing. **A. A.:** Conceptualization, Supervision, Project administration, Funding acquisition, Resources, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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