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Characterisation of resistant starch from *Vigna unguiculata* for the development of an oral-colon delivery agent

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Summary There is a significant concern associated with the delivery of chemotherapeutics to the colon due to limited drug absorption in the gastrointestinal tract. Starch has received great interest as a delivery agent owing to its cost efficacy and high availability. In this study, native resistant starch and acid-modified resistant starch from *Vigna unguiculata* were used to produce microcapsules containing camptothecin as an oral-colon-specific drug delivery agent. Functional characteristics such as solubility, swelling power, amylose content, emulsifying, foaming together with water and oil absorption capacity of native resistant starch and acid-modified resistant starch were established. The prepared microcapsules were characterised and subjected to simulated digestion with the obtained residue evaluated for cytotoxicity using the MTT assay. It was observed that acid-modified resistant starch had the best functional properties (water absorption capacity: 2.76 g g^{-1}, oil absorption capacity: 3.13 g g^{-1}, amylose: 35%, foaming capacity: 17.73%, and emulsifying activity: 33.33%) as compared to its native resistant starch (water absorption capacity: 2.03 g g^{-1}, oil absorption capacity: 2.81 g g^{-1}, amylose: 32%, foaming capacity: 4% and emulsifying activity: 23.56%). The microcapsules had a particle size of 0.25–0.35 μm, moisture content between 1.12% and 3.41% and a high encapsulation efficiency of 96.40 and 98.96% for native resistant starch and acid-modified resistant starch capsules respectively. Results for MTT assay showed cancerous cells to be inhibited by microcapsules with noncancerous cells less affected.

Keywords Delivery system, microencapsulation, starch.

Introduction

Regulating the location or rate of bioactive compounds that are released from oral dosage forms has been prioritised to improve therapeutic potential (Sastry *et al.*, 2000). One region which would gain from this application of controlled drug release is the colon with extensive research focused on drug delivery systems to target the colon. This may possibly enhance the therapy of diseases affecting the colon such as colon cancer, while avoiding systemic adverse effects (Das *et al.*, 2010).

Researchers have shown interest in colon-specific drug delivery systems because of benefits such as a close to neutral pH, extended transit duration, and low activity of enzymes (Haupt & Rubinstein, 2002). According to Kumar *et al.* (2011), transport of bioactive compounds can provide benefits for the colon, including a decreased dosage, adverse effects of anti-inflammatory medication, as well as the prevention of undesired digestion and better bioavailability of beneficial drugs that can be absorbed by the system. When active chemicals are discharged through the gastrointestinal tract (GIT), they need to be efficiently suppressed by an optimal colon-specific drug delivery system. However, there may be significant difficulties due to pH fluctuations throughout the GIT, digestive enzymes, and the extended transport time (Hamman *et al.*, 2005). These challenges may be addressed by new advances in polymer composites, including the use of bacteria-degradable, ionic strength, pressure-sensitive, and time-dependent coating polymers, which offer prospects to accelerate the advancement of colon delivery carriers (Maroni *et al.*, 2012). In the pharmaceutical field, microencapsulation provides substantial benefits in targeted delivery of bioactive compounds for oral administration which promotes health and therapeutic enzymes. It also protects these delicate compounds by concealing them within diverse coating materials, allowing for stability over time and drug loss.

Among some of the materials vulnerable to colonic biodegradation, there is particular interest in polysaccharides which have risen in popularity in recent years

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due to their nontoxicity, safety, biodegradability and high bioavailability (Sinha & Kumria, 2003).

Polysaccharides have hydrophilic and gel-forming qualities and are biocompatible with mammalian tissue resulting in industrial interest through pharmaceutical formulations as tailored drug delivery systems (Tudu & Samanta, 2022). Starch, being the second most prevalent polysaccharide, has received much interest because of its minimal price, variety of sources, and high availability. Resistant starch (RS) resists digestion in the small intestine; however, colonic bacteria can easily digest RS therefore, having a significant function in digestive physiology (Fuentes-Zaragoza *et al.*, 2010).

According to Ríos-Covián *et al.* (2016), this provides extra benefits of the RS as a carrier material since anaerobic bacteria can ferment RS and create short-chain fatty acids (SCFA). *Vigna unguiculata*, also commonly known as cowpea, is a leguminous commonly grown in Africa. It has been utilised in a variety of traditional recipes such as snacks or soups and may be beneficial in the production of gelling agents, thickening agents, and textural enhancers. According to Ratnaningsih *et al.* (2016), it may also be used in pharmaceutical employment due to its resistance to acid and enzymatic hydrolysis. As evidenced by previous literature, cowpea had a RS content before modification between 3.55% and 9.62% (Moongngarm, 2013; Eashwarage *et al.*, 2017) as compared to other legumes like mung bean, soybean and white bean which ranged between 0.10% and 11.1% (Liljeberg Elmståhl, 2002; Moongngarm, 2013). After modifying the RS by different methods like the autoclaving-cooling or cross-linking method, the RS content in cowpea increased to 41.26% (Ratnaningsih *et al.*, 2020). Furthermore, RS is said to function as a substrate for the proliferation of probiotic microbes, which are necessary for colonic health (Ab'lah & Wong, 2020). By its drug release and colon health features, the utilisation of starch and RS are strong contenders for a drug delivery system. Therefore, the aim of this study was to create an oral-colon-specific drug delivery system produced with native and modified RS from *Vigna unguiculata* as a wall material for microencapsulation.

Materials and methods

Sample preparation

Preparation of native resistant starch

The isolation of starch was conducted according to the method of Rengadu *et al.* (2020a) with modifications. Cowpea was left to soak overnight and was thereafter dehulled and dried in an oven at 40 °C. The dried cowpea was milled, sieved with a 180 μm screen mesh and stored in a zip-lock bag. Flour samples were defatted using hexane (1:3 m v^{-1}) at 120 rpm for 24 h with excess hexane allowed to evaporate overnight. The defatted sample was then added to 0.025 M Na_2SO_3 (1:10), homogenised and left at room temperature for 1 h. Samples were then centrifuged at 5000 × ***g*** for 15 min before being washed twice with ethanol and stored at −80 °C until further analysis.

Preparation of acid-modified resistant starch

Starch modification was carried out according to the method of Goñi *et al.* (1996) with minor modifications. A starch slurry (25% w w^{-1}) was autoclaved at 121 °C for 30 min and suspended in 45 mL citric acid (0.1 M) overnight at room temperature. After the acid hydrolysation, the pH of the suspension was adjusted to 6 with 0.5 M NaOH and the hydrolysis residue isolated by centrifugation, neutralised by washing with water, and oven-dried at 60 °C for 24 h.

Isolation of resistant starch

Resistant starch was modified using methods of Zhang *et al.* (2014) with minor modifications. Native and modified starch samples (15 g) were added to 300 mL of citric acid buffer (pH 6.0) and hydrolysed by adding α-amylase (1.5 U mL^{-1}), which was incubated at 37 °C for 1 h in an orbital incubator shaker at 85 rpm. A citric acid solution was used to change the sample pH to 4.5 and thereafter incubated with amyloglucosidase (260 U mL^{-1}) at 60 °C for 1 h in an orbital shaker at 85 rpm. After hydrolysation, samples were centrifuged at 4000 × ***g*** for 10 min, the residue washed 3× with ionised water and a series of ethanol (75%, 85% and 95%). The resulting samples were then freeze dried and stored at −80 °C until further analysis.

Preparation of wall material and microencapsulation using freeze drying

Wall material preparation was conducted according to the methods of Guo *et al.* (2020) with minor modifications. The method of Laureanti *et al.* (2023) was followed with minor modifications, and briefly, samples (5 g) were mixed in distilled water (100 mL) using a high-speed homogeniser. The mixture was left overnight to settle and the camptothecin (10 mg) dissolved in 10 mL of methanol and topped to 100 mL which was added to the sample mixture. The sample mixture was subjected to rotary evaporation to remove the methanol and the resultant core-wall materials freeze dried and stored till analysis.

Simulated gastrointestinal digestion model

The simulated digestive system was conducted using methods of Mtolo *et al.* (2017). Samples (0.2 g) were combined with synthetic saliva (6 mL) made up of KCl (89.6 g L^{-1}), KSCN (20 g L^{-1}), NaH_2PO_4 (88.8 g L^{-1}), Na_2SO_4 (57 g L^{-1}), NaCl (175.3 g L^{-1}),

$NaHCO_3$ (84.7 g L^{-1}), urea (25 g L^{-1}), and 290 mg of α-amylase. Sodium hydroxide (0.1 M) was used to alter the pH to 6.8. Pepsin (0.5 g) was dissolved in 25 mL of 0.1 M HCl and added to the mixture. The solution was then altered to a pH of 2 with 6 M HCL and incubated for 2 h in a 37 °C shaking incubator at 120 rpm. After increasing the pH to 6.5 with 0.5 M $NaHCO_3$, 5 mL of (1:1; v v^{-1}) pancreatin (8 mg mL^{-1}) and bile salts (50 mg mL^{-1}) were diluted in 20 mL of distilled water and added to the mixture, which was then incubated for 2 h in a 37 °C shaking incubator at 120 rpm.

Physicochemical properties of resistant starch

Swelling power (SP) and solubility (S)

This was conducted according to methods of Klein *et al.* (2013) with starch samples (0.5 g) and distilled water (50 mL) added into a 50 mL centrifuge tube and heated at 80 °C for 30 min in a water bath. Sample was then cooled to 20 °C and centrifuged. The supernatant was poured onto a petri dish and dried in a convection oven at 60 °C overnight to obtain supernatant constant weight.

The sediment weight of starch and supernatant constant weight was measured, and solubility and swelling power were calculated using the formulae [dry sample weight (W0), supernatant constant weight (W1) and sediment weight (W2)]

$$\text{Solubility (S)} = \frac{W1}{W0} \times 100 \quad (1)$$

$$\text{Swelling power (SP)} = \frac{W2}{W0\left(1-\frac{S}{100}\right)} \quad (2)$$

Amylose content

Amylose content was conducted following the method of Hamid *et al.* (2015). Starch samples (20 mg) were added to 10 mL of 0.5 M KOH, transferred to a 100 mL volumetric flask and diluted to the mark with distilled water. An aliquot of each test starch solution (10 mL) was pipetted into a 50 mL volumetric flask and 5 mL of 0.1 M HCl added followed by the 0.5 mL of iodine reagent. The volume was diluted to 50 mL and the absorbance measured at 625 nm. The measurement of the amylose was determined from a standard curve developed using distilled water as a blank with eq. 3 used to calculate amylose content:

$$\text{Amylose}(\%) = \left[\text{Amylose concentration} \times \frac{100}{\text{Sample weight}}\right] \times 100\% \quad (3)$$

Foaming capacity (FC) and stability (FS)

Foaming capacity and stability were conducted following methods described by Naiker *et al.* (2020) with minor modifications. Starch samples (0.25 g) and distilled water (2.5 mL) were homogenised for 1 min with suspension volumes noted before and after homogenisation to calculate the foaming capacity (FC) using the following equation:

$$\text{FC}(\%) = \left[\text{Vol. after homogenization} - \frac{\text{Vol. before homogenization}}{\text{Vol. after homogenization}}\right] \times 100 \quad (4)$$

After 1 h of incubation time at room temperature the final volume of the foam was recorded to calculate foaming stability (FS):

$$\text{FS}\ (\%) = \frac{\text{Final vol. after incubation}}{\text{Initial vol. after homogenization}} \times 100 \quad (5)$$

Water and oil holding capacity

Water and oil holding capacities were conducted according to the method of Stone *et al.* (2015). Samples (0.25 g) (M1) were mixed in water/oil (2.5 g) in a 50 mL centrifuge tube, vortexed for 10 s every 5 min for a total of 30 min and centrifuged at 1000 × ***g*** for 15 min. The supernatant was then decanted, and the remaining pellet weighed (M2) with the water/oil holding capacity (g/g) calculated using the following equation:

$$\text{WHC/OHC} = \frac{M2-M1}{M1} \quad (6)$$

Emulsifying activity (EA) and emulsifying stability (ES)

Emulsifying activity (EA) and ES were determined according to the method by Naiker *et al.* (2019) with minor modifications.

Samples (0.25 g) were mixed with distilled water (2.5 mL), sunflower oil (5 mL) added and the solution homogenised. The sample was then centrifuged at 1100 × ***g*** for 5 min with the height of the emulsified layer and the total height recorded. The EA was determined using the following equation:

$$\text{EA}\ (\%) = \frac{\text{Height of emulsified layer in tube}}{\text{Height of total contents in tube}} \times 100 \quad (7)$$

Following incubation (80 °C for 30 min) and centrifugation (1100 × ***g*** for 5 min), the ES was calculated using the following equation:

$$\text{ES}\ (\%) = \frac{\text{Height of emulsified layer after heating}}{\text{Height of emulsified layer before heating}} \times 100 \quad (8)$$

Colorimetric analysis
Colour analysis of the resistant starch was measured using a colorimeter (ColorFlex EZ, Hunter Lab-SCFEZ 0840) according to Rengadu *et al.* (2020b). The instrument was standardised with Hunter lab colour standards, and L^* (lightness), a^* (redness and greenness), and b^* (yellowness and blueness) values were measured.

Characterisation of microcapsules

Encapsulation efficiency (EE)
Encapsulation efficiency was conducted using methods by Baracat *et al.* (2012) with minor modifications. Microcapsules (50 mg) were dispersed in ethanol (4 mL) and stirred for 5 min, followed by centrifugation at 4000 × ***g*** for 10 min. The supernatant was filtered through a 0.45 μm nylon syringe filter and the filtrate analysed spectrophotometrically at 243 nm to determine bioactive content. EE was calculated using the following equation:

$$\text{EE}\ (\%) = \left[\text{initial drug added} - \frac{\text{free drug}}{\text{initial drug added}}\right] \times 100 \quad (9)$$

Moisture content
The moisture content was determined using an MA-50 RADWAG moisture analyser. Each sample (0.5 g) was precisely placed in an aluminium pan and heated to 120 °C, after obtaining a stable weight, the moisture content (%) of each sample was recorded.

Particle size distribution
The particle size distribution of the microcapsules was analysed using a laser light scattering particle analyser (Litesizer 500, Anton Paar., Graz, Austria).

Anticancer activity

MTT assay
Breast cancer (MCF), colon cancer (CaCo-2-2) and a non-cancerous (C2C12) cell line were obtained and grown at 37 °C using 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) which contained 10% foetal bovine serum (FBS), penicillin (10 000 U mL^{-1}) and streptomycin sulphate (10 000 U mL^{-1}). For cell viability colon cancer cells (1×10^2 cells mL^{-1}) and 50 μL of DMEM were seeded in a 96-well flat bottom plate and incubated at 37 °C (5% CO_2) for 24 h.

Cells were treated with the samples (NS, NSCT, Am, AMCT, CT) and incubated for 24 h with camptothecin (C9911 Sigma Aldrich) powder which served as the positive control and cells without camptothecin serving as negative control. Sample absorbance was read at 570 nm and viability calculated using the following formula:

$$\text{Cell viability}\ (\%) = \frac{\text{absorbance of treated cells}}{\text{absorbance of untreated cells}} \times 10 \quad (10)$$

Statistical analysis

Results were analysed by one-way ANOVA ($P < 0.05$) (GraphPad Prism 5, San Diego, CA, USA). All analysis was conducted in triplicate with data represented as mean ± standard deviation.

Results and discussion

Physicochemical properties of resistant starch

Swelling power and solubility
Swelling power (SP) and solubility indices (SI) are generally directly proportional to temperature (Naiker *et al.*, 2019). At a temperature of 80 °C, NRS had a SP of 10.96 g g^{-1} and SI of 8% as compared to AMRS with a SP of 17.74 g g^{-1} and SI of 12% (Table 1). Results show that high water penetration may be attained at high temperatures for cowpea flour cultivars, with similar findings found in a study by Adebooye & Singh (2008) for the SP of two varieties of cowpea flour (8.0–15.4 g g^{-1}). According to Adebooye & Singh (2008), solubility of cowpea flour and starch is limited with cowpea starch SI approximately 50%. Results obtained for the SI in this study were much lower; however, similar findings were reported for corn starch which had a SI between 12% and 20% (Sandhu *et al.*, 2004) and rice starch of below 10% (Singh *et al.*, 2000). In this study, NRS was acid-modified using citric acid resulting in a pregelatinised starch. In the nutraceutical industry, pregelatinised starch and starch are generally employed as binders to increase tablet durability and integrity.

To improve tablet flow, pregelatinised starch is partly hydrolysed and dried with solubility a key criterion in attaining the correct concentration of drug in systemic circulation in order to elicit the intended pharmacological reaction (Vemula *et al.*, 2010).

The amylose content of NRS was found to be 32% as compared to acid-modified resistant starch (AMRS) of 35.33%with no significant difference observed. Similar amylose contents for cowpea were obtained in previous studies which ranged between 26.39% and 42.20% (Kaptso *et al.*, 2016; Kim *et al.*, 2018). Due to their high amylose concentration, rapid retrogradation, resistance to shear thinning, legume starches such as cowpea are becoming increasingly popular in food formulations (Kim *et al.*, 2018). A high amylose content forms higher strength gels therefore both NRS and AMRS can be used in the formation of gels with improved gelling properties. According to Gani *et al.*

Table 1 Colour and physicochemical test of native (NRS) and modified resistant starch (AMRS) from *Vigna unguicalata*

	NRS	AMRS
Physicochemical test		
Swelling power (g g^{-1})	10.96 ± 0.39[a]	17.74 ± 0.02[b]
Solubility (%)	8.00 ± 2.31[a]	12 ± 0.00[b]
Amylose content (%)	32.00 ± 3.12[a]	35.33 ± 0.75[a]
Foaming capacity (%)	4.00 ± 0.00[a]	17.73 ± 1.43[b]
Foaming stability (%)	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Water absorption capacity (g g^{-1})	2.03 ± 0.32[a]	2.76 ± 0.32[a]
Oil absorption capacity (g g^{-1})	2.81 ± 1.28[a]	3.13 ± 0.30[a]
Emulsifying activity (%)	23.56 ± 4.07[a]	33.33 ± 6.67[b]
Emulsifying stability (%)	9.31 ± 2.54[a]	17.22 ± 2.55[b]
Colour		
L	92.31 ± 0.01[a]	87.98 ± 0.00[b]
*a**	0.39 ± 0.01[a]	0.34 ± 0.01[a]
*b**	4.12 ± 0.11[a]	5.76 ± 0.02[a]
Whiteness	91.26 ± 0.01[a]	86.67 ± 0.01[b]

All data are expressed as mean ± standard deviation ($n = 3$). Values with different superscript letters are significantly different ($P < 0.05$).

(2020), the activity of amyloglucosidase and α-amylase enzymes on the α-(1–6) linkages of amylopectin molecules during RS extraction may be responsible for the increase of AMRS with a higher amylose content generally associated with a higher RS content. In the nutraceutical industry, the addition of RS enhances the coating's resistance to enzymatic digestion with RS successfully employed as a food grade enteric coating to protect encapsulated core material from stomach disintegration and release in the desired area (Dimantov *et al.*, 2004).

Water and oil absorption capacity

The capacity of starch to absorb water or oil is referred to as its water (WAC) and oil absorption (OAC) capacity (Table 1). WAC of starches may be impacted by granule structure variation and the participation of a greater proportion of hydroxyl groups in the formation of hydrogen and covalent bonds between starch chains than it is with water (Sandhu *et al.*, 2004). The hydrophilic regions in starch chains permit for hydrogen bonding interactions with water. According to Wani *et al.* (2016), at room temperature the association of amylose and amylopectin chains with water is regarded weak; however, when the temperature increases, thermal energy appears to weaken the hydrogen bonds and enhance the engagement with water, resulting in the absorption of starch and water. The WAC for NRS was 2.03 g g^{-1} as compared to AMRS of 2.76 g g^{-1}. Results show that the modification of native RS increased WAC and OAC which may be attributed to its high amylose content, which results in an increased water absorption and loose compaction of amylose and amylopectin. The WAC of starch is highly influenced by the source, amylose/amylopectin composition, extraction technique, and thermal stability (Gani *et al.*, 2020). Similar results were reported in a study by Giami (1993) for WAC of cowpea flour which ranged between 2.0% and 2.98 g g^{-1}. According to Ratnaningsih *et al.* (2016), cowpea starches with high WAC have the potential to be employed as a thickening and textural enhancer agent in the formulation of food. Although the OAC of NRS and AMRS were 2.81 and 3.13 g g^{-1} respectively after modification there was no significant difference. Cowpea RS OAC may be attributed to the presence of lipid or hydrophobic groups that are present (Rengadu *et al.*, 2020a). High OAC properties allow for flours suited for use in meat formulations such as meat replacers and as well as shelf-life extension in the production of baked processed items such as doughnuts (Naiker *et al.*, 2019).

Emulsifying activity and stability

According to Wani *et al.* (2010), emulsion activity (EA) refers to the total quantity of oil emulsified by protein dispersion, whereas emulsion stability (ES) refers to the ability of the emulsion generated to remain unaltered. In this study, EA for NRS was 23.56% as compared to AMRS of 33.33% (Table 1), while ES of NRS was 9.31% as compared to17.22% for AMRS. Overall, the MRS exhibited better emulsifying properties as compared to its NRS. However, values were fairly low indicating poor emulsifying properties of cowpea RS. Poor emulsifying activities were reported by Rengadu *et al.* (2020a) which ranged between 4.44% and 7.50% for EA and 0.00–22.41% for ES.

Other studies using cowpea starch had an EA between 34.06% and 35.05% and an ES of 73.81–96.04% (Naiker *et al.*, 2019) with results from the current study significantly different. Protein specificity might explain disparities in emulsion characteristics due to interactions between adsorbed protein molecules, such as the formation of disulphide, hydrogen, and hydrophobic bonds.

Colour analysis of resistant starch

Colour analysis was done on the RS where '*L*' determines the lightness, '*a*' determines the redness to greenness and '*b*' determines the yellowness to blueness. It was observed that NRS had an '*L*' and whiteness value of 92.31 and 91.26 respectively which was significantly different as compared to AMRS of 87.98 and 86.67 (Table 1) indicating that NRS was much whiter in appearance as compared to AMRS. According to Ratnaningsih *et al.* (2020), the Maillard reaction, which proceeded at high temperatures during autoclaving for AMRS, might have contributed to the shift in colour values, notably in lightness and degree

Table 2 Particle size, moisture and colour analysis of *Vigna unguicalata* microcapsules

	NS	NSCT	AM	AMCT
Particle size (μm)	0.32 ± 0.05[a]	0.35 ± 0.12[a]	0.25 ± 0.02[b]	0.29 ± 0.02[b]
Moisture (%)	3.41 ± 0.01[a]	3.01 ± 0.01[b]	1.60 ± 0.01[b]	1.12 ± 0.01[b]
Colour				
L	90.46 ± 0.01[a]	90.46 ± 0.01[a]	85.20 ± 0.00[b]	84.39 ± 0.00[b]
*a**	0.44 ± 0.01[a]	0.5 ± 0.01[a]	1.00 ± 0.02[b]	0.80 ± 0.01[b]
*b**	4.26 ± 0.01[a]	4.47 ± 0.01[a]	7.31 ± 0.02[b]	6.24 ± 0.00[b]
Whiteness	89.55 ± 0.01[a]	89.45 ± 0.00[a]	83.46 ± 0.00[b]	83.17 ± 0.00[b]

All data are expressed as mean ± standard deviation ($n = 3$). Values with different superscript letters are significantly different ($P < 0.05$).
AM, acid modified resistant starch; AMCT, acid modified resistant starch + camptothecin; NS, native resistant starch; NSCT, native resistant starch + camptothecin.

of whiteness. In rice starch, similar results were found for dual autoclaved-retrograded starch (Ashwar *et al.*, 2016) and oat starch (Shah *et al.*, 2016). Colour analysis is significant for food applications because colour differences might affect the final colour of food items with variation impacted by different types of colour components, particle size, and several contact sites (Naiker *et al.*, 2019). The values for '*a*' and '*b*' showed no significant difference between the NRS (0.39 and 4.12) and AMRS (0.34 and 5.76).

Characterisation of microcapsules

Encapsulation efficiency

Encapsulation efficiency is a significant measure used to assess the ability of various wall materials to encapsulate core components. EE also represents the amount of 'bioactive compound' enclosed in the freeze-dried microcapsule's wall material matrix, which is critical for stable and long-term preservation (Charles *et al.*, 2021).

It can be observed that the acid-modified resistant starch microcapsule containing camptothecin (AMCT) had the best EE of 98.96% as compared to the NRS microcapsules containing camptothecin (NSCT) of 96.40% indicating that the modification of the resistant starch improved its EE. Microencapsulation can be used to protect sensitive substances from the surrounding environment, conceal the organoleptic characteristics of the substance such as taste, and smell, obtain controlled drug release of the drug substance, for proper handling of toxic materials, obtain targeted release of the drug and avoid negative effects such as gastric irritation (Jyothi *et al.*, 2010).

Moisture content and colorimetric analysis

Colour analysis was performed on both the microcapsules and the RS used as the wall material. It was observed that NS and NSCT had the same lightness value of 90.46 and a whiteness value of 89.55 and 89.45 respectively as compared to AM and AMCT with a lightness value of 85.20 and 84.39 and a whiteness value of 83.46 and 83.17, respectively (Table 2). In contrast between the resistant starch on its own and the microcapsules, it was observed that NRS and AMRS were whiter and lighter than the produced microcapsules which may be attributed to the steps involved during the encapsulation process. Pieniazek & Messina (2017) stated that the heating process during the preparation of the RS causes a Maillard reaction, leading to mild browning, and dehydration, which impacts the drying and rehydration kinetics. It was observed that NS and NSCT had a higher moisture content of 3.41 and 3.01% respectively as compared to AM and AMCT with a moisture content of 1.60 and 1.12%. According to Rengadu *et al.* (2020b), the increased time in freeze drying and proper storage conditions contribute to a low moisture content. Low moisture contents were also observed for freeze-dried microcapsules using arrowroot starch which ranged between 0.24% and 3.35% (Charles *et al.*, 2021).

Particle size distribution

It was observed that NS and NSCT had a particle size of 0.32 and 0.35 μm respectively which was significantly different to AM and AMCT with a particle size of 0.25 and 0.29 μm (Table 2). Factors such as time, the speed of homogenisation and concentrations of reagents can affect the size of particles (Nwabor *et al.*, 2020). An essential aspect of microcapsules is sustained release, with the continuous release of microcapsules in air dependant on the core material penetration and migration into the shell, as well as diffusion on the shell surface. As a result, the microcapsule particle size has a substantial impact on the sustained release characteristics (Zhao *et al.*, 2019). Smaller-sized microcapsules such as the ones produced in the current study have the potential to exhibit a faster sustained release rate. It was observed that NS and NSCT had two distinct peaks which indicates a bimodal shape with all particle sizes <1 μm; therefore, this is especially relevant in the case of powders since the smaller particles may infiltrate the

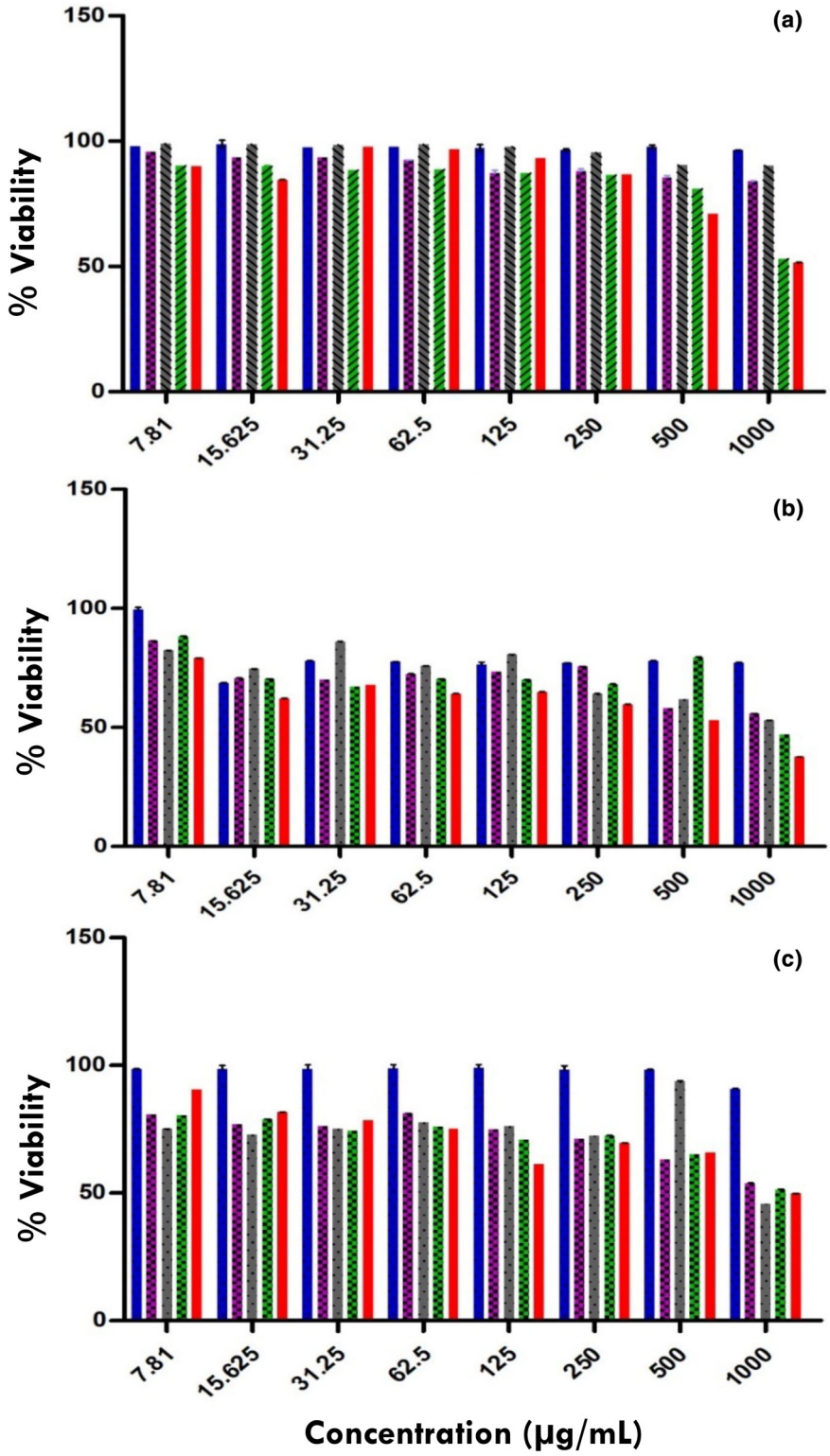

Figure 1 Viability of MCF (a), CaCo-2 (b) and C2C12 (c) cells after treatment with starch microcapsules. All data is expressed as mean ± standard deviation ($n = 3$).

Table 3 IC50 values of cancerous (MCF-7 and Caco-2) and non-cancerous (C2C12) cell lines treated with the *Vigna unguiculata* microcapsules

Cell line	NS (μg mL^{-1})	NSCT (μg mL^{-1})	AM (μg mL^{-1})	AMCT (μg mL^{-1})	CT (μg mL^{-1})
MCF-7	6.133 ± 0.54^{a}	4.038 ± 0.48^{a}	135.9 ± 0.08^{b}	80.04 ± 0.01^{c}	38.81 ± 0.01^{d}
Caco-2	87.47 ± 0.18^{a}	8.194 ± 0.40^{b}	184.9 ± 0.16^{c}	167.3 ± 0.09^{d}	169.6 ± 0.24^{d}
C2C12	381.2 ± 0.47^{a}	62.23 ± 0.10^{b}	133.7 ± 0.21^{c}	50.92 ± 0.09^{d}	49.97 ± 0.09^{d}

All data are expressed as mean ± standard deviation ($n = 3$). Values with different superscript letters are significantly different ($P < 0.05$).
AM, acid modified resistant starch; AMCT, acid modified resistant starch + camptothecin; CT, camptothecin; NS, native resistant starch; NSCT, native resistant starch + camptothecin.

crevices between the larger ones, needing less storage space (Carmona *et al*., 2013).

A simulated digestive system was conducted to mimic the digestion process in humans and evaluate the efficacy of the encapsulation process. Each microcapsule was subjected to digestion conditions for 4 h with the obtained residue that survived the digestion process and then treated on cancerous and non-cancerous cell lines. The MTT test was used to assess the cytotoxicity of all produced microcapsules including camptothecin which was used as a control. To be an effective anticancer drug, the microcapsules should be toxic to MCF, and CaCo-2 cell lines (cancerous cells) while being somewhat less toxic to C2C12 cells (non-cancerous cells). For the MCF and CaCo-2 cell lines at a concentration of 1000 μg mL^{-1} (Fig. 1a, b), AMCT had the lowest cell viability on these cancerous cell lines which indicates that the produced microcapsule has anticancer activity and can successfully inhibit cancerous cells.

It can also be observed in Fig. 1 that there is a trend showing AMCT to exhibit a lower cell viability on CaCo-2 cells as compared to MCF cells. This indicates that AMCT can successfully carry the anticancer drug to the afflicted region of the colon to inhibit colon cancer cells. The C2C12 cells exhibited a disproportionate pattern in which the greater the concentration of sample, the lower the toxicity on C2C12 cell viability (Fig. 1c). This shows that both NSCT and AMCT microcapsules are relatively less toxic to normal cells as it was observed that the IC50 values as shown in Table 3 were all above the IC50 value of the control CT. Overall, both NSCT and AMCT microcapsules were shown to exhibit anticancer activity as compared to NS and AM which did not contain camptothecin, this may be attributed to the stability of camptothecin in two forms, which is the active lactone and inactive hydrolysed carboxylate. The results from the IC50 values indicate that NSCT had the lowest values for toxicity to MCF and CaCo-2 and the highest value for C2C12 healthy cells, making it a potential anticancer agent. The AMCT microcapsules were also shown to have the lowest cell viability and IC50 values, thus making it a potential anticancer agent as well.

Conclusion

Native resistant starch from cowpea, after modification, resulted in improved functional properties as well as an increased yield of RS. The low moisture content and high EE of the microcapsules make RS an ideal wall material for a drug delivery agent. It is evident by the cell viability and low IC50 values that NSCT and AMCT microcapsules were more effective in the inhibition of cancerous cells as compared to NS and AM. Non-cancerous cells had a lower toxicity by NSCT and AMCT as compared to CT. Therefore, it can be concluded that AMCT had the best functional properties, but NSCT showed to have a better result for cytotoxicity with both NSCT and AMCT showing potential for use as a drug delivery agent.

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Author contributions

Priyanta Sewnarain: Investigation; formal analysis; methodology; writing – original draft. **Depika Dwarka:** Writing – review and editing; methodology; project administration; supervision. **John J. Mellem:** Conceptualization; funding acquisition; writing – review and editing; methodology; project administration; supervision; resources.

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Ethics approval was not required for this research.

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Author elects to not share data.

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