Research Article

Preparation and *In Vitro* Release of Drug-Loaded Microparticles for Oral Delivery Using Wholegrain Sorghum Kafirin Protein

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Kafirin microparticles have been proposed as an oral nutraceutical and drug delivery system. This study investigates microparticles formed with kafirin extracted from white and raw versus cooked red sorghum grains as an oral delivery system. Targeted delivery to the colon would be beneficial for medication such as prednisolone, which is used in the management of inflammatory bowel disease. Therefore, prednisolone was loaded into microparticles of kafirin from the different sources using phase separation. Differences were observed in the protein content, *in vitro* protein digestibility, and protein electrophoretic profile of the various sources of sorghum grains, kafirin extracts, and kafirin microparticles. For all of the formulations, the majority of the loaded prednisolone was not released in *in vitro* conditions simulating the upper gastrointestinal tract, indicating that most of the encapsulated drug could reach the target area of the lower gastrointestinal tract. This suggests that these kafirin microparticles may have potential as a colon-targeted nutraceutical and drug delivery system.

1. Introduction

Kafirin is a storage protein extracted from sorghum grain and shares many similarities with zein, the storage protein extracted from maize (e.g., an analogous pattern of peptide subunits) [1]. Zein microparticles loaded with the corticosteroid drugs prednisolone [2] and hydrocortisone [3] have been investigated for use as orally administered delivery systems. However, kafirin is known to be more hydrophobic and of lower digestibility than zein [1, 4] and as such may have greater potential as an oral drug delivery system that

can delay or target the release of medications to the lower gastrointestinal tract (GIT). Kafirin microparticles loaded with condensed tannins and catechins have previously been investigated as an oral delivery system [5].

Problems such as dose-limiting side effects restrict current pharmacological management options for colonic conditions such as inflammatory bowel disease (IBD). This is often due to the inability to effectively and efficiently deliver pharmacological therapy to the affected areas of the lower GIT. Corticosteroids such as prednisolone are used in the management of IBD and are drugs that would benefit from

a system that could delay or target delivery to the lower GIT [2].

Sorghum grain can be classified into white, red, black, and brown types based on the appearance of the grain and the extractability of the polyphenolic compounds [6]. These polyphenolic compounds are secondary plant metabolites that provide protection for the plant from insect, animal, and disease attack [6]. The two main groups of polyphenolics in sorghum grain are phenolic acids and flavonoids [6], and they are thought to contribute to the low protein digestibility of sorghum grain by forming complexes with the kafirin protein, inhibiting digestive enzyme activity [4]. Polyphenolic compounds, particularly those responsible for pigmentation (anthocyanins), are reportedly coextracted with kafirin from sorghum grain when ethanol is used as the extractant [7]. Any differences in polyphenolic compounds coextracted with kafirin may influence the digestibility and drug release properties of kafirin microparticles.

The lower digestibility of sorghum grain protein in humans compared to that of other cultivated cereal grain proteins is thought to be due to the unique structure of kafirin protein bodies and the hydrophobic nature of the kafirin [4]. There are four classes of kafirin: α (α_1 and α_2), β , γ , and δ [4, 8], and disulphide bond crosslinks are reportedly formed between β - and γ -kafirin, further reducing kafirin's susceptibility to hydrolysis by human digestive enzymes [9]. Variations in kafirin digestibility between sorghum cultivars can also arise due to differences the profile of kafirin subunits present [4]. Determining varietal differences in kafirin digestibility will be useful for identifying optimal sources of kafirin for targeting and controlling the release of bioactive agents to different regions of the GIT. Aside from the differences between the sorghum cultivars, processing methods are also known to affect kafirin digestibility [4]. It is well documented that cooking kafirin in water can further reduce its digestibility through the formation of protein crosslinks [9]. This significant impairment in digestibility through "wet cooking" appears to be unique to kafirin, as this effect is absent in other grains [9, 10]. Hence, it is possible that microparticles made using kafirin extracted from cooked sorghum grain may be less digestible than those made from kafirin extracted from the raw grain and therefore more effective for targeting the release of encapsulated drugs or nutraceuticals to the lower GIT.

The aim of this work was to compare prednisolone loading into microparticles formulated using kafirin extracted from a red grain (var. MR Buster) and a white grain (var. Liberty) sorghum cultivar. The red sorghum grain was also "wet-cooked" prior to kafirin extraction for comparison with the extract from the raw grain. The morphology, *in vitro* protein digestibility, and polyphenolic content of the microparticles, along with their *in vitro* drug release behaviour in simulated gastric and small intestinal conditions, were characterised.

2. Materials and Methods

2.1. Raw Materials. Kafirin was extracted from two different sorghum hybrid cultivars, one with red grains (MR Buster)

and one with white grains (Liberty), sourced from the Department of Agriculture, Fisheries and Forestry, Brisbane, Australia. The white grain cultivar is likely to have "no detectable tannins or anthocyanins and very low total extractable phenol levels" [6], and the red grain cultivar is likely to "have no tannins but have a red pericarp with significant levels of extractable phenols" [6]. The wholegrain sorghum was milled using a hammer mill until 100% of the flour passed through an 800 μ m sieve. To investigate the effects of wet cooking, red sorghum flour was cooked in boiling water (1:10 w/v) for 30 min immediately before kafirin was extracted.

2.2. Kafirin Extraction. Kafirin was extracted from the wholegrain sorghum flour using a method adapted from a glacial acetic acid extraction protocol [11], and all reagents were of analytical and HPLC grades. In brief, the flour in batches of approximately 200 g was soaked in 1% sodium metabisulphite (1:10 w/v; Ajax Finechem, Taren Point, Australia) while being mixed using a magnetic stirrer at room temperature for 16 h. The flour was recovered using centrifugation at 11,662 $\times$ g, at 4°C for 30 mins (RC5C, Sorvall DuPont, Wilmington, DE, USA) and washed three times by mixing with a minimal amount of water and repeating the centrifugation. Kafirin was then extracted from the washed flour residue with glacial acetic acid (1:5 w/v; Scharlau, Gillman, Australia) for 2 h with vigorous stirring, after which the supernatant extract was recovered by centrifugation as described above. Glacial acetic acid was removed from the extract by dialysis (Spectra/Por 3 Dialysis Membrane, MWCO 3,500 Da; Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA) against MilliQ water (1:20 v/v extract to water ratio) at 10°C with two water changes over 24 h. Kafirin was precipitated by adjusting the extract to pH 5 using 18 M sodium hydroxide, recovered by centrifugation as described above, and then washed three times by mixing with a minimal amount of water and centrifuging before being freeze-dried. The freeze-dried kafirin extract was stored at 4°C in airtight containers until use in microparticle formulation studies.

2.3. Moisture and Protein Content. Moisture content of the sorghum flours was determined in triplicate, using the AOAC oven drying method [12], while that of the kafirins and microparticles was determined by the Karl Fischer titration method [13]. Protein content of the flours and kafirins were determined in triplicate by Dumas combustion (AACC Standard Method 46-30) [14], and a nitrogen to protein conversion factor of 5.7 was used.

2.4. Total Polyphenolic Content. Polyphenolic compounds were extracted in duplicate from flours, extracted kafirins, and empty and drug-loaded microparticles using a method described by Awika et al. [15]. The polyphenolic content of the samples was expressed as gallic acid equivalents (mg of GAE/g dry basis), and calibration curves in the range of 0–250 μ g/mL were used to quantify the amount of gallic acid. As prednisolone interferes with this polyphenolic assay [16], the values for the prednisolone loaded microparticles

were corrected for absorbance given by a blank containing prednisolone equivalent to the amount present in the microparticle samples.

2.5. *In Vitro* Protein Digestibility. Protein digestibility of the flours, extracted kafirins, and empty and drug-loaded microparticles was measured as described by Lau et al. [3], by incubating the samples with pepsin at pH 2 and then quantifying the nitrogen content of the undigested fraction.

2.6. *Microparticle Formulation.* Microparticles were formulated in 50 mL centrifuge tubes (Becton Dickinson, Franklin Lakes, NJ, USA) using a phase separation method as per Lau et al. [16]. In brief, 350 mg prednisolone (99%, Sigma Aldrich, St. Louis, MO, USA) was dissolved in 12 mL of 70% (v/v) ethanol (Merck, Darmstadt, Germany) and then heated in a 70°C water bath for 10 min. Kafirins (400 mg) was added to the solution, agitated using a vortex mixer (30 Hz; Zx³, Velp Scientifica, Usmate, Italy) for 1 min, and then returned to the water bath for 1 min. The vortexing and incubation process was repeated another four times over a total of 10 min. Then 8 mL of 0.1 M sodium chloride solution was used to induce phase separation, and vortex mixed for 2 min, which led to the formation of the microparticles. The microparticle solutions were left overnight in a -80°C freezer until solid and then freeze-dried (Alpha 2-4 LD freeze dryer, Martin Christ GmbH, Osterode am Harz, Germany) overnight until all the solvent was removed and a dried sample remained. A drug control formulated using only prednisolone and a protein control consisting of only empty kafirin microparticles were used. Each formulation of microparticles was made in triplicate. Reagents were of analytical and HPLC grades.

2.7. *Prednisolone Loading into Microparticles and Encapsulation Efficiency.* Freeze-dried microparticles (triplicate 10 mg samples from each replicate formulation) were washed by vortex mixing for 20 seconds with 1 mL of ethyl acetate (Ajax Finechem, Taren Point, Australia) to remove the nonencapsulated prednisolone [16]. The nonencapsulated prednisolone was recovered and quantified using HPLC as per Lau et al. [2]. The kafirin microparticles were not dissolved to analyse the amount of prednisolone encapsulated, since Lau et al. [2] demonstrated that the amount of prednisolone washed out during the ethyl acetate washes can be used to accurately quantify the amount of encapsulated prednisolone.

The amount of prednisolone encapsulated in the microparticles was calculated by subtracting the amount of nonencapsulated prednisolone from the amount of prednisolone added initially. The amount of prednisolone encapsulated was then used to determine prednisolone loading (i.e., the amount of prednisolone contained within the microparticles) and loading efficiency (i.e., the proportion of prednisolone initially added that became encapsulated) [2].

2.8. *Scanning Electron Microscopy.* The kafirin microparticles were mounted onto carbon tabs and coated with platinum to a thickness of approximately 10 nm using a Baltec MED 020

sputter coater. The samples were viewed at 5 kV and a working distance of 8 mm using a field emission scanning electron microscope JEOL JSM 6300F (JEOL, Tokyo, Japan).

2.9. *In Vitro* Drug Release in Conditions Simulating the Stomach and Small Intestine. The United States Pharmacopeia Dissolution Apparatus 2 fitted with a small volume vessel (100 mL) was used [17]. The medium used to simulate the different conditions of the stomach and small intestine was made according to the British Pharmacopoeia using increasing pH, from pH 1.5 at 0-1 h for gastric conditions, followed by small intestinal conditions at pH 4.5 from 1 to 4 h, and then pH 7.2 from 4 to 7 h [18]. Drug release was also measured in the presence of enzymes, where pepsin (0.32 g, 0.7 FIP-U/mg; AppliChem, Darmstadt, Germany) was added to the medium at 0 h, and pancreatin (1 g, 23664 FIP-U/g; AppliChem, Darmstadt, Germany) was added at 1 h. Drug release from approximately 30 mg of microparticles containing 5 mg of prednisolone was compared with that of a commercially available 5 mg prednisolone tablet (Panafcortelone, Aspen Pharmacare, St Leonards, Australia). Samples were retrieved at regular time intervals and analysed by HPLC [2].

2.10. *Nonreducing and Reducing Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis.* Samples were prepared for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing and nonreducing conditions using a protocol as per Lau et al. [16].

2.11. *Statistical Analysis.* One-way ANOVA was used to compare (a) the mean prednisolone loading and loading efficiency of each kafirin source, (b) the percentage of prednisolone released after one hour of the dissolution study from the different kafirin microparticles in the presence and absence of enzymes, and (c) the compositional parameters of the sorghum flours, kafirins, and microparticles. A Bonferroni *post hoc* test was used in GraphPad Prism 5.04 (GraphPad Software, San Diego, CA, USA) for comparisons, with the level of significance set as $p < 0.05$.

3. Results

3.1. *Protein and Polyphenolic Content.* The raw wholegrain flours contained 10% protein (mean g/100 g db $\pm$ SEM: white grain, 9.2 ± 0.0 ; red grain 10.8 ± 0.0), and this was increased to 68% ($p < 0.05$) by the extraction process (mean g/100 g db $\pm$ SEM: from white grain, 68.7 ± 0.3 ; from raw red grain, 68.4 ± 0.9). In addition, the protein level of kafirin extract from the cooked red sorghum (78.3 ± 0.0) was higher ($p < 0.05$) than that from the raw red sorghum.

As expected based on grain colour, the red sorghum flour, and the kafirins extracted from it, had higher ($p < 0.05$) total polyphenolic levels than the white grain flour and its extracted kafirin (Table 1). The total polyphenolic content of the extracted kafirins was higher ($p < 0.05$) than that of the flour from which it was extracted, highlighting the copurification of both kafirin and polyphenols under the conditions used in the present study.

TABLE 1: *In vitro* protein digestibility and total polyphenolic content of wholegrain flours, isolated kafirins, and empty and drug-loaded kafirin microparticles from sorghum cultivars with white grains (Liberty) and red grains (MR Buster).

Sample	<i>In vitro</i> protein digestibility (%)	Total polyphenolic content (mg GAE/g dry basis)
Sorghum flour		
Raw white	11.8 ± 1.3 ^a	0.72 ± 0.02 ^a
Raw red	13.9 ± 1.5 ^a	3.36 ± 0.07 ^c
Kafirin		
From raw white sorghum	30.7 ± 0.5 ^b	2.92 ± 0.14 ^c
From raw red sorghum	62.6 ± 0.3 ^c	13.65 ± 0.61 ^f
From cooked red sorghum	40.7 ± 1.7 ^{cd}	10.19 ± 0.13 ^e
Empty kafirin microparticles		
From raw white sorghum	ND	0.58 ± 0.07 ^{ab}
From raw red sorghum	37.6 ± 2.7 ^{bc}	2.13 ± 0.02 ^{bc}
From cooked red sorghum	32.4 ± 2.6 ^{bc}	9.87 ± 0.02 ^e
Drug-loaded microparticles		
From raw white sorghum	ND	0.09 ± 0.07 ^a
From raw red sorghum	60.8 ± 0.8 ^c	4.70 ± 0.10 ^d
From cooked red sorghum	48.2 ± 3.3 ^d	9.60 ± 0.03 ^e

Data are means of at least duplicate analyses ± SEM. Values within a column with different superscript letters are significantly different ($p < 0.05$). GAE: gallic acid equivalents; ND: not determined.

TABLE 2: Drug loading, loading efficiency, and prednisolone release (mean ± SEM, $n = 3$) of prednisolone-loaded kafirin microparticles prepared using kafirin extracted from wholegrain flour of sorghum cultivars with white grains (Liberty) and red grains (MR Buster).

Kafirin source	Kafirin from raw white grain flour	Kafirin from raw red grain flour	Kafirin from cooked red grain flour
Prednisolone loading into kafirin microparticles			
Prednisolone loading (%)	18.78 ± 0.31 ^a	12.95 ± 2.03 ^{ab}	7.73 ± 0.85 ^b
Loading efficiency (%)	42.76 ± 0.70 ^a	29.48 ± 4.63 ^{ab}	17.60 ± 1.93 ^b
Prednisolone release from kafirin microparticles in simulated gastric and small intestinal conditions			
Prednisolone release in the presence of enzymes (%)	15.04 ± 0.61 ^a	15.17 ± 2.33 ^a	15.41 ± 1.60 ^a
Prednisolone release in the absence of enzymes (%)	15.18 ± 0.69 ^a	16.68 ± 5.11 ^a	27.18 ± 4.41 ^a

Values within a row (two rows for prednisolone release) with different superscript letters are significantly different ($p < 0.05$).

On formation of microparticles (either empty or drug-loaded), a significant reduction in the levels of polyphenolics ($p < 0.05$) was seen for the white sorghum and raw red sorghum derived samples (Table 1). For the microparticles derived from cooked red sorghum, however, no decrease ($p > 0.05$) occurred in the levels of polyphenolics, possibly due to irreversible protein-polyphenolic binding as a result of the cooking process, which in turn might have potential to impact microparticle digestibility and drug release.

3.2. *In Vitro* Protein Digestibility. Isolation of kafirin from the sorghum flour resulted in a significant increase in protein digestibility ($p < 0.05$) compared to that of the raw flours, with the kafirin from raw white sorghum being less digestible ($p < 0.05$) than the kafirin from the red sorghum (Table 1). In addition the kafirin from the cooked red sorghum flour was less digestible ($p < 0.05$) than that from the raw red sorghum flour.

The empty microparticles from raw red sorghum kafirin had lower protein digestibility ($p < 0.05$) than the kafirin

they were formulated from. However, incorporation of prednisolone into the microparticles resulted in higher protein digestibility ($p < 0.05$) than their empty microparticle counterparts.

3.3. *Microparticle Formulation.* Although drug loading and loading efficiency of kafirin microparticles was greatest for those prepared using white sorghum flour, statistically it was only higher than the cooked, but not the raw red sorghum ($p > 0.05$) (Table 2). Using the kafirin extracted from the raw white sorghum grain, it can be calculated that approximately 20 mg of microparticles would be required to deliver a clinically relevant dose of prednisolone (5 mg). This amount of microparticles is realistic and practical for oral delivery (i.e., is a reasonable amount of material to swallow).

Multiple scanning electron micrographs were taken of the microparticles, and two representative micrographs for each formulation are presented. These micrographs confirmed the presence of round particles that were varied in size, with an irregular and crinkled surface structure (Figure 1).

FIGURE 1: Scanning electron micrograph of empty and prednisolone-loaded microparticles made using kafirin extracted from raw white grain, raw red grain, and cooked red grain. (A, B) empty and (C, D) prednisolone-loaded microparticles of kafirin from raw white grain; (E, F) empty and (G, H) prednisolone-loaded microparticles of kafirin from raw red grain; and (I, J) empty and (K, L) prednisolone-loaded microparticles of kafirin from cooked red grain. Scanning electron micrographs (A), (C), (E), (G), (I), and (K) were captured at 20,000x while (B), (D), (F), (H), (J), and (L) were captured at 50,000x magnification.

A comparison of the empty (Figures 1(B), 1(F), and 1(J)) and prednisolone-loaded microparticles (Figures 1(D), 1(H), and 1(L)) at high magnification revealed that the prednisolone-loaded microparticles generally were more crenated and also appeared to have pores or pits on the surface.

3.4. Prednisolone Release in Simulated Conditions of the Gastrointestinal Tract. In conditions simulating the stomach (0–1 h; pH $1.5 \pm$ pepsin) and small intestine (1–4 h with pH 4.5 followed by 4–7 h with pH $7.2 \pm$ pancreatin), only 15–27% of prednisolone was released from the microparticles (Table 2, Figure 2). This is in contrast to the 100% release from the commercial tablet. In both the commercial tablet and the microparticles, the maximum amount of prednisolone was released by 30 min (Figure 2). The amount of prednisolone released from the microparticles made using the three sources of kafirin was not significantly different, and the presence of pepsin and pancreatin did not impact drug release (Table 2).

3.5. SDS-PAGE. The SDS-PAGE under nonreducing conditions (Figure 3(a)), of all kafirin extract samples (lanes 2, 5, and 8), empty microparticles (lanes 3, 6, and 9), and drug-loaded microparticles (lanes 4, 7, and 10) derived from raw white sorghum grains, raw red sorghum grain, and cooked red sorghum grain, respectively, showed similar protein banding patterns. Predominant banding was observed that corresponded to the molecular weights of the subunits of

the major sorghum prolamins, α -kafirins (~ 22 – 29 kDa) and γ -kafirin (~ 27 kDa) with very faint banding corresponding to the molecular weight of β -kafirin monomers (~ 18 kDa) [5, 19, 20]. The other predominant banding corresponded to a molecular weight of ~ 45 kDa matching that reported for kafirin dimers, whilst the array on bands corresponding to molecular weights of >66 kDa is likely to represent kafirin trimers and higher-order polymers [19]. Under reducing conditions (Figure 3(b)), all samples again gave similar banding patterns; however the pattern was different to that under nonreducing conditions (Figure 3(a)). Under reducing conditions, by far the most predominant bands were those corresponding in molecular weight to the α -kafirins and γ -kafirin monomers, with a more distinct band corresponding to that of β -kafirin monomers than that seen in the nonreducing conditions. Under reducing conditions (Figure 3(b)), only faint banding corresponding to the kafirin dimers and trimers was observed, and bands corresponding to higher-order kafirin polymers were absent.

4. Discussion

Kafirin was successfully isolated from the sorghum flours, though further purification steps will be necessary to increase the protein concentration for commercial pharmaceutical applications. The higher protein digestibility in the isolated kafirin compared to the corresponding raw flours is presumably due to the disruption of the kafirin protein bodies found in raw sorghum grain, whereby cross-linked β - and γ -kafirin

FIGURE 2: *In vitro* drug release profile of a commercially available 5 mg prednisolone tablet compared to prednisolone release from the three different types of kafrin microparticles containing 5 mg prednisolone. Prednisolone release was measured in conditions simulating the stomach for 1 h, followed by the small intestine for 6 h, (a) without enzymes and (b) in the presence of the enzymes pepsin and pancreatin. Values are mean $\pm$ SEM ($n = 3$).

FIGURE 3: (a) Sodium dodecyl sulphate-polyacrylamide gel electrophoresis under nonreducing conditions and (b) under reducing conditions of kafrin from raw white sorghum grain (lane 2); empty kafrin microparticles from raw white sorghum (lane 3); drug-loaded microparticles from raw white sorghum (lane 4); kafrin from raw red sorghum (lane 5); empty kafrin microparticles from raw red sorghum (lane 6); drug-loaded microparticles from raw red sorghum (lane 7); kafrin from cooked red sorghum (lane 8); empty kafrin microparticles from cooked red sorghum (lane 9); and drug-loaded microparticles from cooked red sorghum (lane 10). Molecular weight markers are seen in lanes 1 and 11.

form a protective layer around the more inherently digestible α -kafrins [21].

The lower *in vitro* protein digestibility of extracted kafrin and drug-loaded kafrin microparticles observed from cooked red sorghum compared to raw red sorghum is in agreement with previous reports [1]. Moist cooking can reduce the digestibility of sorghum protein through

increasing kafrin cross-linking and rearrangement of the secondary structure to β -pleated sheets [1]. However, the SDS-PAGE under nonreducing conditions of the raw and cooked kafrins were similar in overall banding patterns (Figure 3), not supporting the formation of extensive new cross-links between kafrin subunits during the cooking process. In the present study differences in the *in vitro* protein digestibility

of the drug-loaded microparticles did not translate into differences in their corresponding *in vitro* drug release profiles. Interaction between polyphenolics and kafirin is one proposed mechanism for the low protein digestibility of sorghum grain, interactions that can be enhanced by cooking [4]. However, although the kafirin extracts from the hybrid red grain cultivar (MR Buster) had higher total polyphenolic contents than the white grain sorghum (Liberty), they also had higher protein digestibility. The red commercial hybrid cultivar used in the present study is classified as tannin-free, and of all the classes of polyphenolics in sorghum grain, it is condensed tannins that most potentially reduce protein digestibility, while nontannin polyphenolics may not appreciably reduce protein digestibility [4, 6].

Drug loading and loading efficiency were highest using kafirin extracted from the white grain sorghum. This may be in part due to red grain sorghum cultivar containing significantly higher levels of total polyphenolics than the white grain sorghum [22]. These polyphenolic compounds, particularly those responsible for pigmentation (anthocyanins), are coextracted with kafirin when using ethanol [7]. Glacial acetic acid was used in this study to extract kafirin from the wholegrain, and it also coextracted the polyphenol pigments with the kafirin; an orange-red pigment was observed throughout the extraction process and in the final kafirin extract of the red grain sorghum but not the white grain, and the total polyphenolic content of the kafirin extracts from the red grain was significantly higher than that from the white grain. The presence of more polyphenolic pigments in the red grain kafirin extracts may have interfered with prednisolone binding to the kafirin protein during the microparticle formation process, resulting in reduced drug loading into the microparticles formed using kafirin from the red grain compared to the white grain sorghum. Overall, drug loading into microparticles described herein appears to be comparable to the corresponding microparticles formulated using distiller's dried grain kafirin (prednisolone loading $12.35\% \pm 0.43$) [16]. Despite the differences in the kafirin sources and the extraction methods used, this observed similarity in drug loading may be because the microparticles were formulated using the same method. Scanning electron micrographs of the wholegrain kafirin microparticles and distiller's dried grain kafirin microparticles, both with and without drug, showed the presence of spherical microparticles that were varied in size [16].

The initial burst release of prednisolone in the simulated gastric conditions was similar to that reported in the literature [2, 5]. However, while the second phase of the release was reported to be slow and sustained from zein microparticles [2], the prednisolone release from kafirin microparticles appears to have stopped after the initial burst release, giving a plateau. No further release occurred when the pH changed to that simulating the small intestine, unlike kafirin microparticles containing condensed tannins or catechins [5]. The initial burst release is commonly attributed to the core material (e.g., prednisolone) that was adhered to the surface or close to the surface of the microparticles but had not been removed in the washing step [2, 5]. The slower sustained release after the initial burst has been explained by

the slower diffusion of the encapsulated material from the inner regions of the microparticles or the gradual breakdown of the coating material [2]. The lack of further prednisolone release after the initial burst suggests that the microparticle structure was not conducive for prednisolone diffusion and that the kafirin microparticle structure was not being broken down.

In the simulated gastric and small intestinal conditions, less prednisolone was released from kafirin microparticles (Figure 2) compared to zein microparticles [2]. This difference might be explained by the more digestible and less hydrophobic nature of zein compared to kafirin [1, 4]. A lack of prednisolone release from kafirin microparticles in the simulated gastric and small intestinal conditions is desirable as it maximises the dose of encapsulated drug that reaches the target area of the lower GIT. Therefore, the drug release profile from the kafirin microparticles observed in the present study is promising in terms of its potential as an orally administered drug delivery system that targets the lower GIT.

5. Conclusion

Prednisolone was successfully loaded into microparticles formulated using kafirin from different sorghum wholegrain sources. Based on formulations prepared in the present study, the amount of microparticles required to obtain a clinically relevant dose of prednisolone is realistic and feasible for an oral delivery system. The *in vitro* prednisolone release profile in simulated gastric and intestinal conditions suggests that kafirin may have potential as a delayed-release drug delivery system as the microparticles were able to retain the majority of the prednisolone load through the simulated gastric and small intestinal conditions. The *in vitro* protein digestibility of kafirins and drug-loaded kafirin microparticles from the varying sources differed, but this did not translate to differences in the *in vitro* drug release profiles of microparticles made from these kafirins. Further studies are now required to understand the kinetics of prednisolone release from kafirin microparticles under simulated colonic conditions.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

- [1] P. S. Belton, I. Delgadillo, N. G. Halford, and P. R. Shewry, "Kafirin structure and functionality," *Journal of Cereal Science*, vol. 44, no. 3, pp. 272–286, 2006.
- [2] E. T. L. Lau, S. K. Johnson, D. Mikkelsen, P. J. Halley, and K. J. Steadman, "Preparation and in vitro release of zein microparticles loaded with prednisolone for oral delivery," *Journal of Microencapsulation*, vol. 29, no. 7, pp. 706–712, 2012.
- [3] E. T. L. Lau, S. J. Giddings, S. G. Mohammed et al., "Encapsulation of hydrocortisone and mesalazine in zein microparticles," *Pharmaceutics*, vol. 5, no. 2, pp. 277–293, 2013.
- [4] K. G. Duodu, J. R. N. Taylor, P. S. Belton, and B. R. Hamaker, "Factors affecting sorghum protein digestibility," *Journal of Cereal Science*, vol. 38, no. 2, pp. 117–131, 2003.
- [5] T. Janet, J. R. N. Taylor, P. S. Belton, and M. Amanda, "Kafirin microparticle encapsulation of catechin and sorghum condensed tannins," *Journal of Agricultural and Food Chemistry*, vol. 57, no. 16, pp. 7523–7528, 2009.
- [6] J. M. Awika and L. W. Rooney, "Sorghum phytochemicals and their potential impact on human health," *Phytochemistry*, vol. 65, no. 9, pp. 1199–1221, 2004.
- [7] L. S. da Silva and J. R. N. Taylor, "Physical, mechanical, and barrier properties of kafirin films from red and white sorghum milling fractions," *Cereal Chemistry*, vol. 82, no. 1, pp. 9–14, 2005.
- [8] H. K. C. Laidlaw, E. S. Mace, S. B. Williams et al., "Allelic variation of the beta-, gamma- and delta-kafirin genes in diverse *Sorghum* genotypes," *Theoretical and Applied Genetics*, vol. 121, no. 7, pp. 1227–1237, 2010.
- [9] B. R. Hamaker, A. W. Kirleis, L. G. Butler, J. D. Axtell, and E. T. Mertz, "Improving the *in vitro* protein digestibility of sorghum with reducing agents," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 84, no. 3, pp. 626–628, 1987.
- [10] E. T. Mertz, M. M. Hassen, and C. Cairns Whittern, "Pepsin digestibility of proteins in sorghum and other major cereals," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 81, no. 1, pp. 1–2, 1984.
- [11] J. Taylor, J. R. N. Taylor, M. F. Dutton, and S. de Kock, "Glacial acetic acid—a novel food-compatible solvent for kafirin extraction," *Cereal Chemistry*, vol. 82, no. 5, pp. 485–487, 2005.
- [12] Association of Official Agricultural Chemists, *AOAC Official Methods of Analysis*, AOAC International, Gaithersburg, Md, USA, 2008.
- [13] American Society for Testing and Materials, "Standard test method for determination of water in liquid petroleum products by Karl Fischer reagent," ASTM D1744-92, American Society for Testing and Materials, West Conshohocken, Pa, USA, 1992.
- [14] American Association of Cereal Chemists, *Approved Methods of the American Association of Cereal Chemists*, American Association of Cereal Chemists, Saint Paul, Minn, USA, 10th edition, 2000.
- [15] J. M. Awika, L. W. Rooney, X. Wu, R. L. Prior, and L. Cisneros-Zevallos, "Screening methods to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products," *Journal of Agricultural and Food Chemistry*, vol. 51, no. 23, pp. 6657–6662, 2003.
- [16] E. T. L. Lau, S. K. Johnson, R. A. Stanley et al., "Formulation and characterization of drug-loaded microparticles using distiller's dried grain kafirin," *Cereal Chemistry*, 2015.
- [17] United States Pharmacopeial Convention, "The United States pharmacopeia and national formulary," Tech. Rep. USP 30-NSP 25, United States Pharmacopeial Convention, Rockville, Md, USA, 2007.
- [18] British Pharmacopoeia Commission, "Appendix XII B. Dissolution ANNEX: recommendations on dissolution testing," in *The British Pharmacopoeia 2012*, TSO, Norwich, UK, 2012.
- [19] I. N. A. El Nour, A. D. B. Peruffo, and A. Curioni, "Characterisation of sorghum kafirins in relation to their cross-linking behaviour," *Journal of Cereal Science*, vol. 28, no. 2, pp. 197–207, 1998.
- [20] Y. Wang, M. Tilley, S. Bean, X. S. Sun, and D. Wang, "Comparison of methods for extracting kafirin proteins from sorghum distillers dried grains with solubles," *Journal of Agricultural and Food Chemistry*, vol. 57, no. 18, pp. 8366–8372, 2009.
- [21] N. J. de Mesa-Stonestreet, S. Alavi, and S. R. Bean, "Sorghum proteins: the concentration, isolation, modification, and food applications of kafirins," *Journal of Food Science*, vol. 75, no. 5, pp. R90–R104, 2010.
- [22] M. H. Dicko, H. Gruppen, A. S. Traoré, W. J. H. VanBerkel, and A. G. J. Voragen, "Evaluation of the effect of germination on phenolic compounds and antioxidant activities in sorghum varieties," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 7, pp. 2581–2588, 2005.