Sorghum condensed tannins encapsulated in kafirin microparticles as a nutraceutical for inhibition of amylases during digestion to attenuate hyperglycaemia

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Short title

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ABSTRACT

Type 2 diabetes (T2D) is increasing rapidly in Africa. An appropriate therapeutic approach is to inhibit intestinal carbohydrate digesting enzymes using plant polyphenols. A crude preparation of sorghum condensed tannins (SCT) was highly effective (approx. 20,000 times) at inhibiting α-glucosidase compared to acarbose, while acarbose was a better α-amylase inhihitor (approx. 180 times). Kafirin microparticles (KEMS) were investigated as an oral delivery system for SCT. Using a simple aqueous alcohol coacervation method, the encapulation efficiency of SCT in the KEMS was approx. 48%. Quantitative data and electron microscopy revealed that KEMS encapsulating SCT were digested to only a limited extent during simulated gastrointestinal digestion with pepsin and trypsin-chymotrypsin. Hence, SCT encapsulated in KEMS retained their inhibitory activity against both amylases, throughout simulated gastrointestinal digestion, whereas unencapsulated SCT lost most of their inhibitory activity. Thus, KEMS encapsulating SCT have potential as a nutraceutical to attenuate hyperglycaemia and control T2D.

Keywords: Amylase inhibition, kafirin, microparticles, sorghum condensed tannins, type 2 diabetes

1. Introduction

Diabetes has recently emerged as one of the major non-communicable diseases in sub-Saharan Africa (Mbanya, Motala, Sobngwi, Assah & Enoru, 2010). The International Diabetes Federation (IDF) estimates that by 2035, 41.4 million African residents will suffer from diabetes (IDF, 2013). Type 2 Diabetes (T2D) is the most common form of the condition and is characterised by decreased insulin secretion and insulin resistance (Moller, 2001). A reason for this rise in T2D is that the diets of populations in sub-Saharan Africa area becoming progressively "Westernized" (Diabetes Leadership Forum: Africa, 2010). Such diets are rich in foods containing high glycaemic index carbohydrates, leading to post-prandial hyperglycaemia (high blood glucose) (Preuss, 2009). Hyperglycaemia is most commonly associated with diabetes and is responsible for chronic complications such as retinopathy and nephropathy (Mbanya et al., 2010; Moller, 2001). Controlling the rate of glucose absorption in the small intestine is an important physiological target to delay, prevent and control the development of T2D (Mbanya et al., 2010; Moller, 2001; Ross, Gulve & Wang, 2004).

A therapeutic approach for decreasing post-prandial hyperglycaemia is to prevent glucose absorption by inhibiting digestive amylase enzymes, specifically α-glucosidase and α-amylase (Shobana, Sreerama & Malleshi, 2009). However, drugs that are used for this purpose can have multiple undesirable side effects (Ross et al., 2004; Shobana et al., 2009). Drug therapies can also fail to significantly alter T2D development or prevent chronic complications (Moller, 2001). Further, in sub-Saharan Africa a specific problem is that the use of drug-based therapies is severely limited due to lack of funds, facilities and trained staff (Mbanya et al., 2010; Diabetes Leadership Forum: Africa, 2010). Thus, the development of

nutraceutical type treatments for T2D using components from locally grown plants would be highly beneficial.

Tannins extracted from a number of plant foods can inhibit can inhibit α-amylase and glucoamylase (Barrett et al, 2013). Sorghum is one of the major crops in Africa and there is significant production in 41 African countries (FAOSTAT, 2011). Extracts of polyphenols from sorghum grain have demonstrated *in vitro* and *in vivo* inhibitory activity against α-amylase and α-glucosidase (Kim, Hyun & Kim, 2011; Kim & Park, 2012). However, oral administration of such polyphenolic extracts has various limitations. Sorghum tannins (proanthocyanidins/procyanidins) bind proteins (Butler, Riedl, Lebryk, & Blytt 1984), which lead to the sensation of astringency and unpleasant bitter tastes (Kobue-Lekalake, Taylor, De Kock, 2007). Further, sorghum condensed tannins (SCT) bind to proteins in a non-specific way (Butler et al., 1984), which could lead to a reduction or complete loss in their enzyme inhibitory activity in the small intestine, where the major digestive amylases are secreted.

Thus, a suitable delivery system is needed to ensure that SCT reach the target site (small intestine) with enough biological activity to effectively inhibit carbohydrate digesting enzymes and thereby decrease hyperglycaemia. Zhao, Iyer, Flores, Donhowe & Kong (2013) demonstrated that by encapsulating tannic acid in calcium alginate microspheres, α-amylase could be substantially inhibited in the small intestine phase in a simulated gastrointestinal digestion. Kafirin, the sorghum prolamin storage protein, is notably hydrophobic and resistant to pepsin digestion (Belton, Delgadillo, Halford & Shewry, 2006). It has been found that microparticles made from kafirin (KEMS) can encapsulate and release SCT during simulated digestion (Taylor,., Taylor, , Belton & Minnaar, 2009a). The aim of this work was

to determine the potential of encapsulating SCT in KEMS as a simple delivery vehicle for SCT to the small intestine to inhibit digestive amylases.

2. Materials and Methods

2.1 Materials

Type III tannin sorghum, cultivar PAN 3860, was used to prepare SCT. For preparation of KEMS, total kafirin (82% protein (as is basis)) was extracted from condensed tannin-free, white pericarp, tan plant sorghum cultivar (Orbit) as described by Emmambux and Taylor (2003). Porcine α-amylase (EC 3.2.1.1) (>1000U/mg, where one unit will liberate 1.0 mg of maltose from starch in 3 minutes at pH 6.9 at 20 °C), yeast α-glucosidase (EC 3.2.1.20), (≥100 U/mg, where one unit will liberate 1.0 micromole of D-glucose from p-nitrophenyl a-D-glucoside per min at pH 6.8 at 37 °C) ,pepsin (EC 232.629.3) (800-2,500 U/mg, where 1 U will produce ΔA_{280}, of 0.001/min at pH 2.0 at 37 °C), trypsin (EC 232.650.8) (13,000-20,000 BAEE U/mg protein, where 1 U will produce ΔA_{253} of 0.001/min at pH 7.6 at 25 °C), chymotrypsin (EC 232.671.2) (83.9 U/mg, where 1 U will hydrolyse 1.0 µm of N-benzoyl-L-tyrosine ethyl ester/min at pH 7.8 at 25 °C), acarbose and ρ-nitrophenyl-α-D-glucopyranoside (PNPG) were obtained from Sigma-Aldrich (Johannesburg, South Africa). Amylase high range (HR) reagent (non-reducing-end blocked *p*-nitrophenyl maltoheptaoside) was obtained from Megazyme International (Wicklow, Ireland).

2.2 Methods

2.2.1 SCT preparation

Condensed tannins were extracted according to Price, Van Scoyoc and Butler (1978) with slight modifications. Sorghum bran was obtained by decorticating whole grain with a Tangential Abrasive Dehulling Device (TADD) (Venables Machine Works, Saskatoon,

Canada). SCT were extracted from the bran (75 g) with 750 ml methanol for 20 min with constant stirring at ambient temp. (22 $^{\circ}$C). The suspension was centrifuged at 4500 $\times$ g for 10 min. The supernatant was collected and the solvent evaporated off at ambient temp. in a fume hood. The dried SCT were defatted with hexane, ground to a fine powder with a pestle and mortar and stored at -20 °C until analysis. The assayed condensed tannin content of the dried extract was 312 mg/100 mg (as is basis) catechin equivalents. This shows that this crude extract was very high in tannins, notwithstanding the fact that catechin as a standard overestimates tannin content (Price et al., 1978).

2.2.2 KEMS preparation

Encapsulation of SCT in KEMS by coacervation using different solvents was investigated:

i) KEMS were prepared by coacervation from a solution of kafirin in glacial acetic acid, as previously described (Taylor et al., 2009a). Defatted kafirin (1.95 g, 82% protein) was dissolved in glacial acetic acid (5 g) with gentle stirring while the temperature was slowly raised to 30 °C to ensure full solvation. The solution was allowed to 'rest' for 16 h. SCT (500 mg) was dissolved in distilled water at 50 °C. The SCT solution was then added to the kafirin-acetic acid solution using a peristaltic pump (Watson-Marlow Bredel, Falmouth, UK) at a rate of 1.4 ml/min with stirring. Upon addition of water, KEMS formed. After washing with distilled water to remove unbound SCT, the KEMS were air dried, ground to a fine powder and stored at 10 °C until analysis.

ii) KEMS encapsulating SCT were formed from an aqueous ethanol solution, according to a coacervation procedure described by Liu, Sun, Wang, Zhang and Wang (2005) with some modifications. Kafirin (2.7 g) was separately dissolved in 70% (w/w) aqueous ethanol (15 ml) at 70 °C and SCT (500 mg) was also dissolved in 15 ml 70% (w/w) ethanol at 70 °C. The latter was added to the kafirin solution at a rate of 3.6 ml/min with constant stirring. To coacervate the KEMS, distilled water was then added to the kafirin-SCT solution at a rate of

3.6 ml/min with constant stirring to a total weight of 110 g. To prepare KEMS alone, kafirin was dissolved in aqueous ethanol and precipitated via coacervation with distilled water. The aqueous ethanol prepared KEMS were then treated as described for the acetic acid method.

2.2.3 Simulated gastrointestinal digestion

Twenty mg KEMS encapsulating SCT (18 mg for the control to make up for bound SCT) and 2 mg SCT alone were incubated with 3.7 mg pepsin in 1.75 ml sodium citrate buffer, pH 2 at 37°C. Digestion was assayed after 10 and 120 min incubation. After pepsin digestion, the samples were centrifuged to remove the supernatant. The pellet was suspended and further digested with a mixture of trypsin (3.9 mg)and chymotrypsin (4.6 mg) in 1.75 ml 0.05 M phosphate buffer, pH 6.9 at 37 °C. The samples were then treated as described by Taylor et al. (2009a).

2.3 Analyses

2.3.1 Tannin content

This was measured by the vanillin HCl assay (Price et al., 1978). Reagent blanks that corrected for colour of SCT were included. Catechin was used as a standard.

2.3.2 Protein content

Protein (N x 6.25) was determined by a Dumas standard combustion Method 46–30 (AACC, 2000).

2.3.3 SCT binding and encapsulation efficiency

Percentage SCT binding was estimated from the decrease in protein content of the KEMS. Encapsulation efficiency was calculated as the amount of SCT bound to KEMS, divided by the amount added initially and expressed as a percentage.

2.3.4 Alpha-amylase inhibition

An adaptation of the method of Tadera, Minami, Takamarsu and Matsuoka (2006) was used in conjunction with the Cerealpha α-amylase assay (Megazyme International, Wicklow, Ireland). Incubation was at 37 °C for 20 min. Alpha-amylase inhibitory activity of SCT and the supernatants of KEMS and KEMS encapsulating SCT were assayed after pepsin and after trypsin-chymotrypsin digestion. The whole pellet after digestion or the supernatant (50 µl) was reacted with either 200 µl for the pellet or 100 µl for the supernatant α-amylase (0.1 mg/ml) and 200 µl (pellet) or 100 µl (supernatant) 10 mM HR reagent. The supernatants from KEMS and KEMS encapsulating SCT reaction mixtures were used for inhibition determination as the high pH of the α-amylase stopping reagent, 1% tri-sodium phosphate (pH 11), caused the encapsulated SCT to separate from microparticles. The inhibitory drug, acarbose (Ross et al., 2004) was assayed as a standard.

2.3.5 Alpha-glucosidase inhibition

An adaptation of the method by Kim et al. (2011) was used. Alpha-glucosidase inhibitory activity of SCT and the supernatants of KEMS and KEMS encapsulating SCT were assayed after pepsin and after trypsin-chymotrypsin digestion as for α-amylase inhibition. Reaction mixtures consisting of 100 µl α-glucosidase (0 04 mg/ml) in 0.1 M phosphate buffer (pH 6.9) and SCT/KEMS supernatant/acarbose (100 µl) were pre-incubated at 37 °C. The substrate ρ-nitrophenyl-α-D-glucopyranoside (10 mM) (100 µl) in phosphate buffer (pH 6.9) was then added. Incubation was for 20 min and stopped using 1 M Na_2CO_3 (pH 11).

2.2.6 IC$_{50}$

IC$_{50}$ is defined as the concentration of inhibitor required to inhibit 50% of enzyme activity (Lacroix & Li-Chan, 2013). Alpha-glucosidase and α-amylase activities were assayed (as described) in the presence SCT alone and acarbose at various concentrations. Inhibition percentage was plotted against SCT and acarbose concentrations and IC$_{50}$ was determined by linear regression.

2.3.7 Microscopy

Suspensions of KEMS and SCT were prepared for scanning electron microscopy (SEM) and transmission electron microscopy (TEM) as described by (Taylor et al., 2009a). For SEM, samples were chemically dried using hexamethylenediamine (HDMA) and viewed using a Jeol JSM-840 SEM (Tokyo, Japan). TEM was performed using a Jeol JEM-2100F Field Emission Electron Microscope.
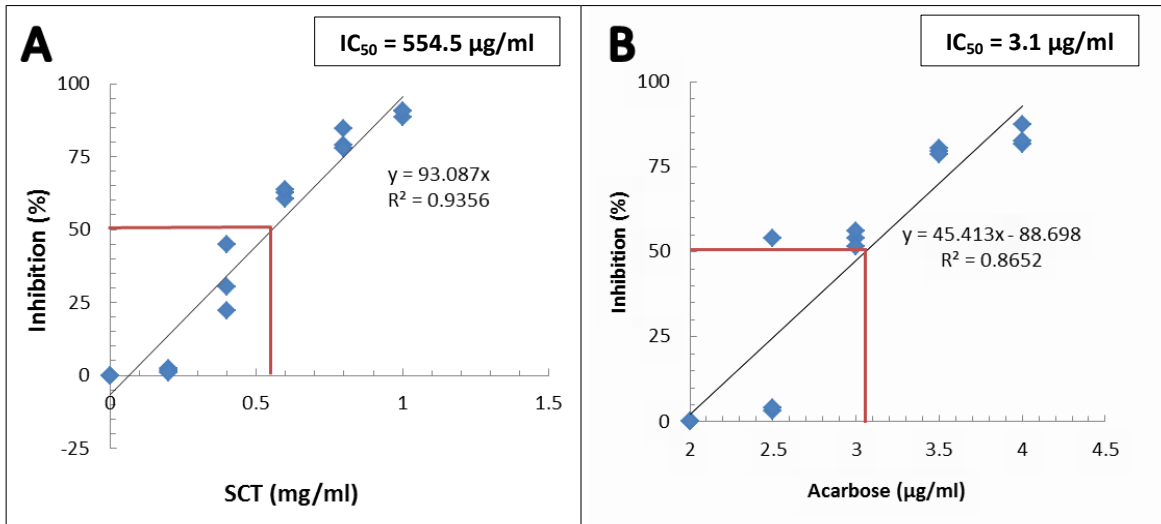
2.3.8 Statistical analysis

Data were analysed by one-way analysis of variance (ANOVA) and mean differences were assessed by Fisher's least significant difference test using Statistica software version 10 (StatSoft, Tulsa, OK).
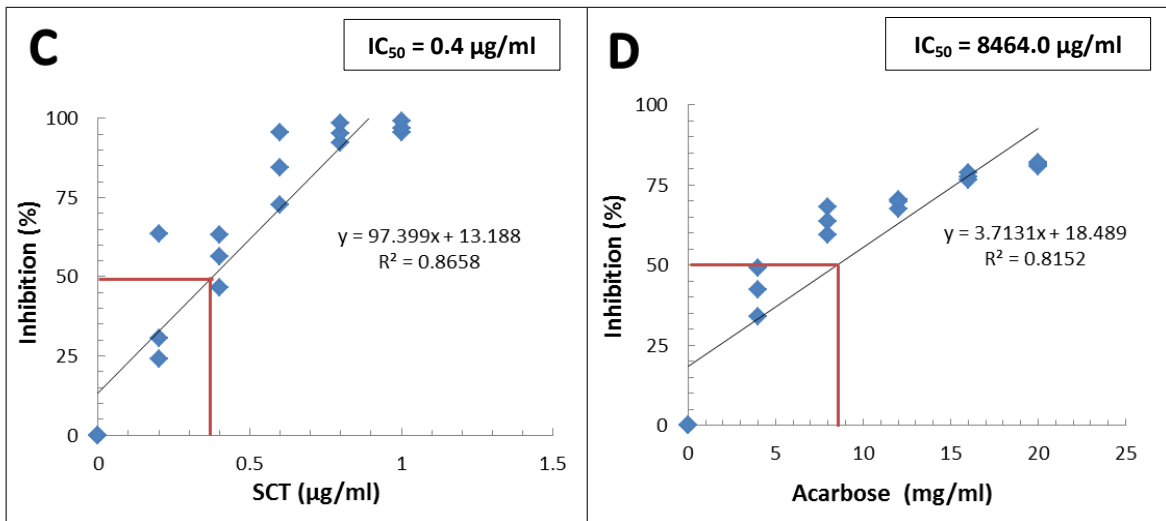
3. Results and Discussion

3.1 Inhibition of amylases by SCT

SCT were effective inhibitors of α-amylase (IC$_{50}$ = 554.4 µg/ml) and α-glucosidase (IC$_{50}$ = 0.4 µg/ml) with essentially linear dose responses (r = 0.97*)* and (r = 0.93), respectively

α-amylase inhibition

A IC$_{50}$ = 554.5 µg/ml

$y = 93.087x$
$R^2 = 0.9356$

Inhibition (%)
SCT (mg/ml)

B IC$_{50}$ = 3.1 µg/ml

$y = 45.413x - 88.698$
$R^2 = 0.8652$

Inhibition (%)
Acarbose (µg/ml)

α-glucosidase inhibition

C IC$_{50}$ = 0.4 µg/ml

$y = 97.399x + 13.188$
$R^2 = 0.8658$

Inhibition (%)
SCT (µg/ml)

D IC$_{50}$ = 8464.0 µg/ml

$y = 3.7131x + 18.489$
$R^2 = 0.8152$

Inhibition (%)
Acarbose (mg/ml)

Figure 1. Dose dependant inhibition of α-amylase and α-glucosidase by SCT and acarbose. Intercept indicates IC50. (A, C) inhibition by SCT and (B, D) inhibition by acarbose.

(Figure 1). Acarbose was a more powerful α-amylase inhibitor ($IC_{50} = 3.1$ µg/ml) than SCT, with approx. 180 times higher inhibitory activity. However, SCT had a far higher (approx. 20,000 times) α-glucosidase inhibitory effect than acarbose ($IC_{50} = 8464.0$ µg/ml).

SCTs in Type III sorghum consist of polymerised flavan-3-ol and flavan-3,4-diol units linked by C4-C8 interflavan bonds with (-)-epicatechin as extension units and catechin as terminal units (reviewed by Dykes & Rooney, 2006). A proanthocyanidin-rich extract from sorghum bran similar to that used in this study was found to contain approx. 23% monomers, 18% dimers, 15% trimers, 11% tetramers, 4% pentamers, 3% hexamers and 26% were unresolved polymeric proanthocyanidins (Wu, Huang, Qin, Meng, Zou, Zhu & Ren, 2011). Proanthocyanidin polymers show much stronger inhibitory activity against α-amylase than oligomers (reviewed by Xiao, Ni,Lai & Chen, 2013), whereas proanthocyanidin oligomers show greater inhibitory activity than polymers against α-glucosidase (Lee, Cho, Tanaka & Yokozawa, 2007). According to Spencer et al. (1988), proteins rich in proline bind more tannins than other proteins. Neither α-amylase nor α-glucosidase have particularly high proline content, approx. 4% and 5%, respectively (Brayer, Luo & Withers, 1995). Importantly, however, it has long been known that SCT bind to proteins in a non-specific way (Butler et al., 1984). Notwithstanding this, there is a direct relationship between sorghum tannin content and amylase inhibition (Adetunji, Khoza, de Kock & Taylor, 2013).

Kim et al.(2011) previously investigated the α-amylase and α-glucosidase inhibition of 70% ethanol phenolic extracts from tannin sorghum, while Hargrove, Greenspan, Hartle and Dowd (2011) studied the effects of 50% methanol extracts from Sumac (tannin) and black (non-tannin) sorghum varieties on α-amylase inhibition. The SCT (100% methanol extract from

sorghum) used in this study was a stronger α-glucosidase inhibitor than the sorghum phenolic extract of Kim et al. (2011) (IC_{50} = 0.4 µg/ml versus 1.1-1.4 µg/ml), using a similar inhibition assay. However, the sorghum phenolic extracts of Kim et al. (2011) (lowest IC_{50} = 2.9 µg/ml) and Hargrove et al. (2011) (1.4 µg/ml) were apparently far stronger α-amylase inhibitors than the SCT used in this study (554.5 µg/ml). The α-amylase inhibitory data are, however, not directly comparable. These authors used a non-specific α-amylase assay (starch substrate) as opposed to the specific α-amylase maltoheptaoside substrate (McCleary & Sheeran, 1987) used in this study.

The particularly low IC_{50} of the SCT against α-glucosidase indicates that they have the potential to control post-prandial hyperglycaemia associated with T2D. However, due to the challenges with the oral administration of SCT, as described, KEMS were investigated as a delivery system for SCT to the small intestine.

3.2 SCT encapsulation

The aqueous ethanol method of preparing KEMS encapsulating SCT was more effective at encapsulating SCT than the acetic acid method, 9% bound as opposed to 6% (Table 1). Further, in terms of encapsulation efficiency, the aqueous ethanol prepared KEMS had an encapsulation efficiency of almost double (48%) that of the acetic acid prepared KEMS encapsulating SCT (25%). Encapsulation efficiency of SCT in KEMS by the aqueous ethanol method was similar to that obtained by other workers who encapsulated tea catechins in chitosan-tripolyphosphate nanoparticles (Hu, Pan, Sun, Hou, Ye, Hu & Zeng, 2008) and tannic acid in calcium alginate microspheres (Zhao et al., 2013) using coacervation.

Table 1: The effects of SCT encapsulation in KEMS on their protein content, SCT content and encapsulation efficiency

Material	KEMS protein content[1] (g/100 g, as is)	KEMS SCT content (g/100 g, as is)	SCT encapsulation efficiency[2] (%)
Acetic acid prepared KEMS	[3]80.6[d][4] ± 0.3	NA	NA
Acetic acid prepared KEMS encapsulating SCT	74.2[c] ± 0.1	6.4[a] ± 0.4	24.8[a] ± 1.5
Aqueous ethanol prepared KEMS	80.2[d] ± 0.6	NA	NA
Aqueous ethanol prepared KEMS encapsulating SCT	71.2[b] ± 0.4	9.0[b] ± 0.9	48.4[b] ± 4.9
SCT	1.8[a] ± 0.0	NA	NA

[1]N x 6.25

[2]Encapsulation efficiency = mg SCT bound/mg SCT initially added $\times$ 100

[3]Mean ± standard deviation (n =3)

[4]Values with different superscripts in the same column differ significantly (p <0.05)

NA – Not applicable

The better encapsulation efficiency of the aqueous ethanol prepared KEMS was probably related to the higher coacervation rate used to prepare the aqueous ethanol KEMS (3.6 ml/min), compared to the acetic acid prepared KEMS (only 1.4 ml/min). A faster coacervation rate leads to higher encapsulation efficiency as diffusion of the encapsulated substance is limited (Yeo & Park, 2004). Additionally, SCT were dissolved in aqueous ethanol separately before being added to the kafirin-ethanol mixture. Therefore hydrogen bonding and hydrophobic interactions between SCT and kafirin (Butler et al., 1984) could occur before coacervation, leading to higher encapsulation efficiency. In the acetic acid method, binding and coacervation had to occur simultaneously, probably limiting SCT binding and encapsulation efficiency.

KEMS prepared by the acetic acid method were spherical, porous and ranged in size from 1 to 10 µm (Figure 2 A,B) as previously observed (Taylor et al., 2009a). SCT binding did not affect microparticle size or structure (Figure 2 A,C), but appeared to join some KEMS together (Figure 2 D). The aqueous ethanol method of preparing KEMS had a profound effect on their morphology. These KEMS were aggregated into clumps of about 100 µm (Figure 2 E,G). However, as with the acetic acid prepared KEMS, encapsulation of SCT did not affect the size or structure (Figure 2 G,H).

SCT are strongly osmiophilic and they appear as darkly stained material in TEM with osmium staining (Morrall, Liebenberg & Glennie, 1981). TEM showed with the acetic acid prepared KEMS majority of the SCT bound around the outside the KEMS (Figure 2,D). In contrast, the SCT were embedded inside the aqueous ethanol prepared KEMS (Figure 2,H). Hydrophobic interactions could explain why SCT bound mostly to the outside of the acetic acid prepared KEMS. Insoluble complexes are formed when excess tannins bind protein and

SEM

TEM

Acetic acid

prepared KEMS

Control

Bound SCT

Aqueous ethanol

prepared KEMS

Control
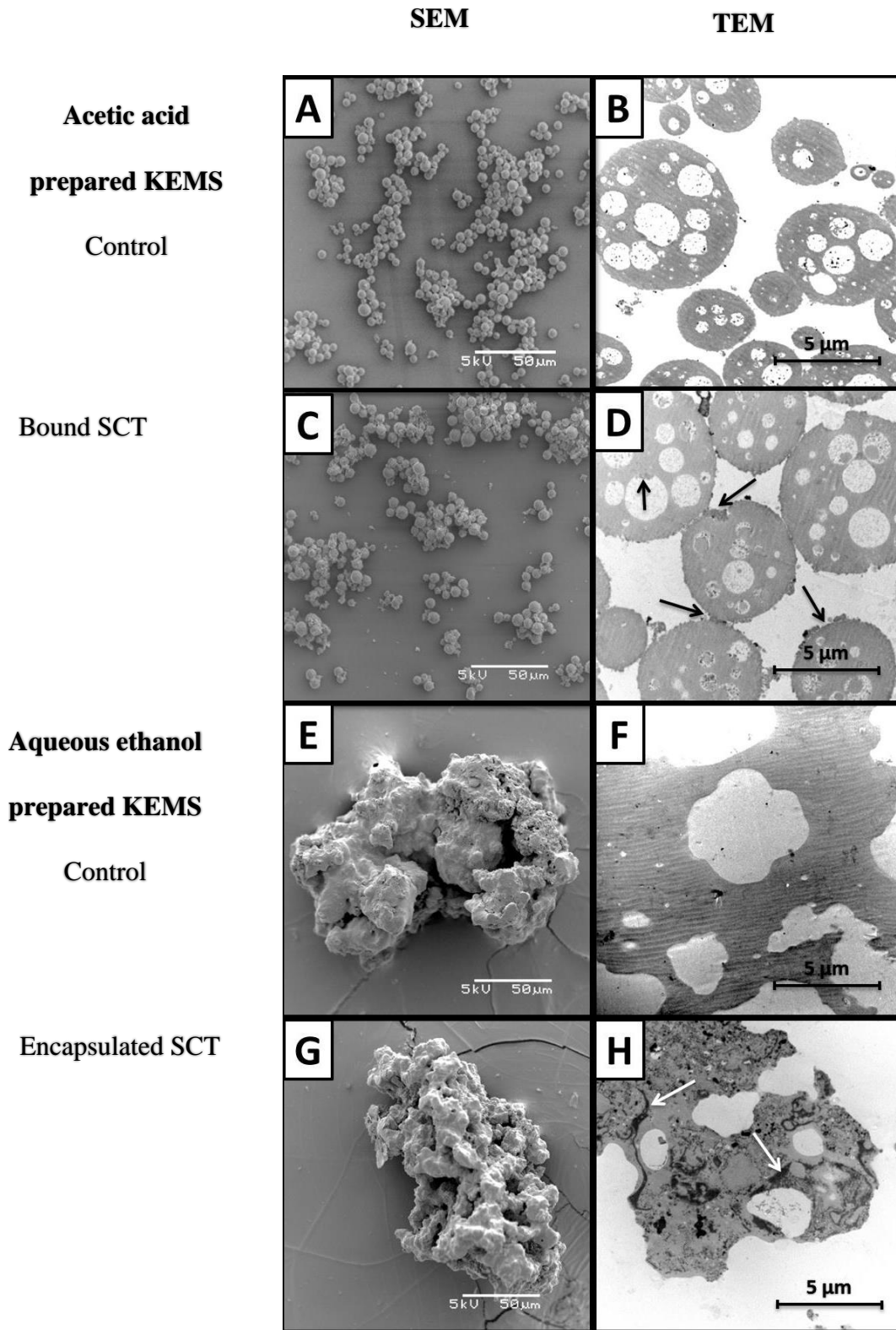
Encapsulated SCT

Figure 2. Electron microscopy showing the effect of KEMS preparation method and SCT encapsulation on KEMS morphology. Black arrows indicate bound SCT, white arrows indicate

lead to the formation of hydrophobic outer layers on the complex surface (Cannas, 2013). The faster precipitation rate, mentioned earlier, could also explain why more SCT were encapsulated in the aqueous ethanol prepared KEMS compared to the acetic acid prepared KEMS. In view of the higher SCT encapsulation of efficiency of the aqueous ethanol prepared KEMS, further work was performed on this material only.

3.3 Simulated gastrointestinal digestion of KEMS

Some degradation of the aqueous ethanol prepared KEMS alone digested with pepsin could be observed by electron microscopy (Figure 3a C,D). Digestion appeared to take place mainly on the surface of KEMS, as the smooth surface (Figure 3a A) was eroded after 120 min of pepsin digestion (Figure 3a C). However, encapsulation of SCT in KEMS substantially reduced the protein digestibility of the KEMS (Table 2). In fact, electron microscopy showed that the KEMS encapsulating SCT were essentially unaffected by pepsin digestion (Figure 3a E-H), as there was no change in the surface (Figure 3a G) or the internal (Figure 3a H) morphology of these KEMS after 120 min of digestion. SCT encapsulation also decreased subsequent trypsin-chymotrypsin digestion of the KEMS (Table 2). However, after 120 min of trypsin-chymotrypsin digestion KEMS encapsulating SCT indicated some signs of degradation along the edges of these KEMS (Figure 3b H) when compared to KEMS only digested for 10 min (Figure 3b F). This digestion pattern is similar to that observed by Taylor et al. (2009a) using the acetic acid method to prepare KEMS encapsulating SCT.

The quantitative digestion of KEMS encapsulating SCT was approx. 7% digestion after pepsin digestion and approx. 12% after pepsin plus trypsin-chymotrypsin digestion (Table 2). In contrast, the KEMS alone were approx. 59% digested after digestion with the proteolytic enzymes. This difference is because SCT associate strongly with kafirin prolamin proteins
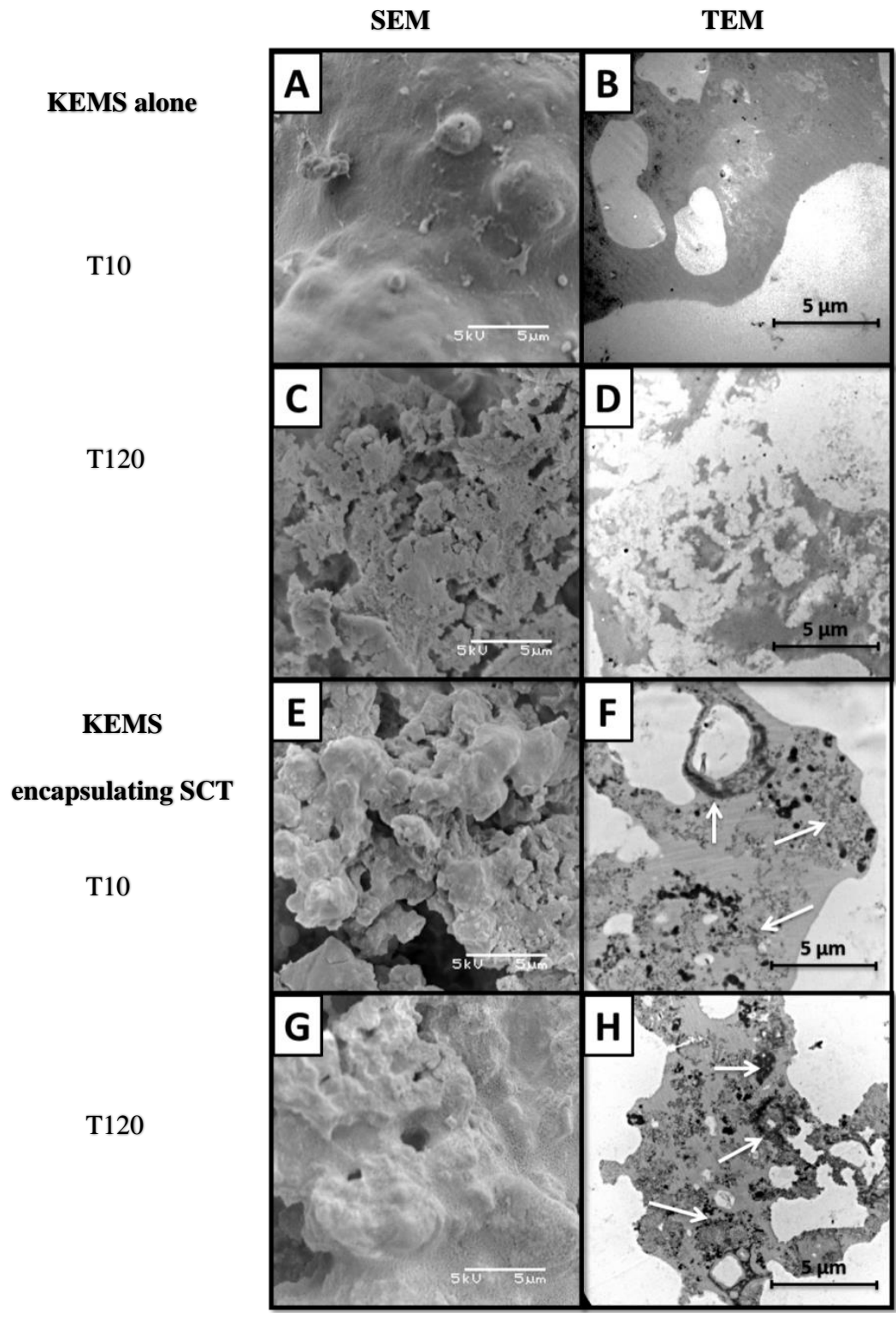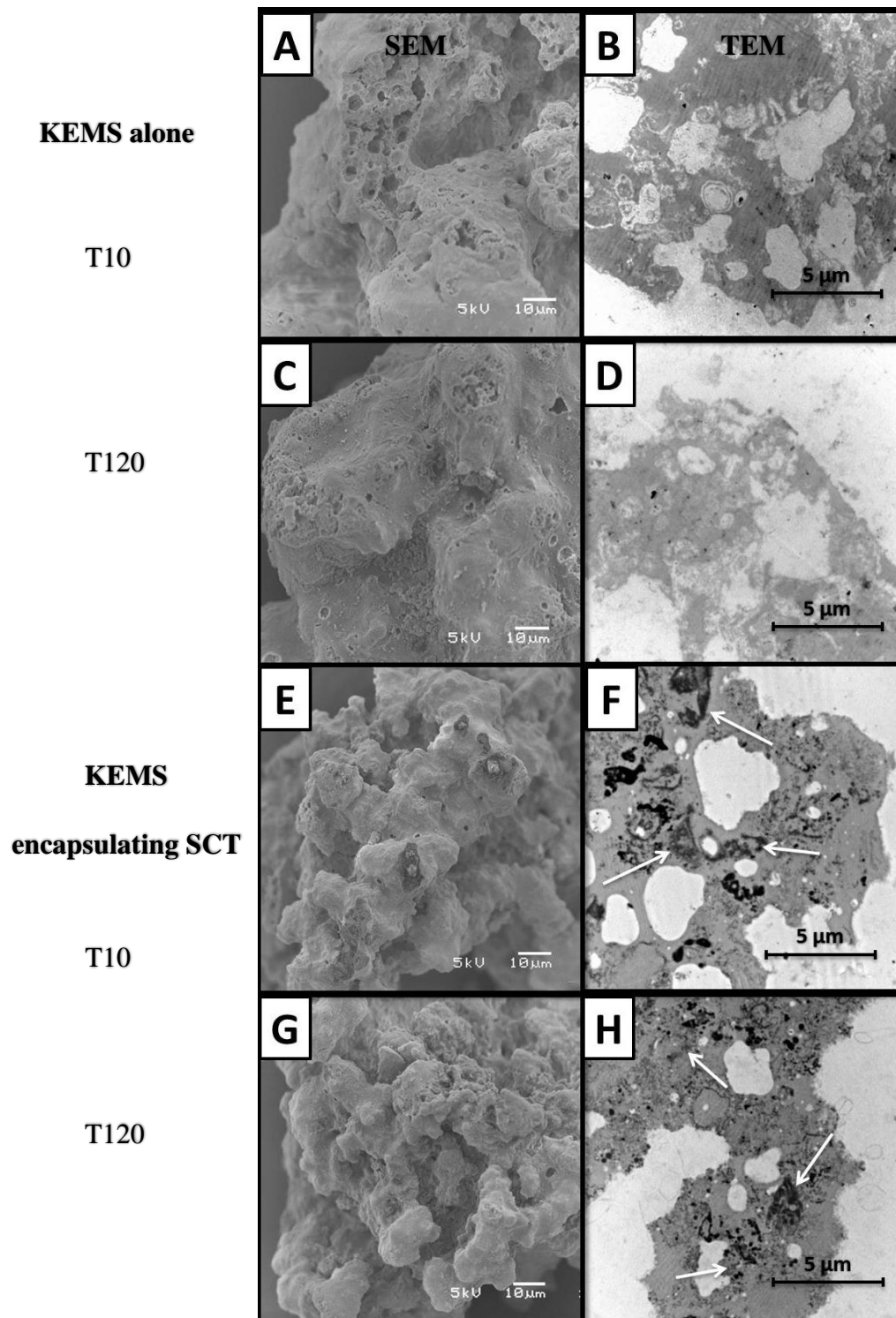
	SEM	TEM

KEMS alone

T10

A — 5kU — 5 μm
B — 5 μm

T120

C — 5kU — 5 μm
D — 5 μm

KEMS

encapsulating SCT

T10

E — 5kU — 5 μm
F — 5 μm

T120

G — 5kU — 5 μm
H — 5 μm

Figure 3a.

Figure 3b

Figure 3. Electron microscopy showing the effect of in vitro pepsin digestion followed by indicate encapsulated SCT. trypsin-chymotrypsin digestion of aqueous ethanol prepared KEMS. T10; digestion time in minutes. Arrows indicate encapsulated SCT. a) Pepsin digestion, b) Trypsin-chymotrypsin digestion following pepsin digestion.

Table 2: The effect of SCT encapsulation in KEMS on the *in vitro* pepsin digestion followed by trypsin-chymotrypsin digestion of aqueous ethanol prepared KEMS

Material	Digestion (%)			
	Pepsin		Trypsin-chymotrypsin	
	10 min	120 min	10 min	120 min
KEMS alone	$^{1}7.2^{b2A3} \pm 1.8$	$33.2^{cB} \pm 1.6$	$25.6^{bA} \pm 1.5$	$39.2^{bB} \pm 0.4$
			$(38.3)^{4}$	(58.7)
KEMS encapsulating SCT	$3.1^{aA} \pm 1.6$	$7.0^{aB} \pm 1.6$	$6.1^{aA} \pm 1.2$	$11.5^{aB} \pm 0.8$
			(6.6)	(12.4)

[1]Mean ± standard deviation (n = 3)

[2]Values with different lower case superscripts in the same column differ significantly ($p < 0.05$) for each enzyme

[3]Values with different capital letter superscripts in the same row differ significantly ($p < 0.05$)

[4]Values in brackets represents calculated total digestion

(Emmambux & Taylor, 2003), resulting in indigestible complexes after pepsin digestion (Butler et al., 1984). SCT also bind and inhibit digestive enzymes and/or reduce enzyme accessibility (Taylor et al., 2009a). The low pepsin digestibility of the SCT encapsulating KEMS is advantageous as SCT needs to bypass gastric digestion without binding to other proteins in the gastrointestinal tract in order to exert inhibition in the small intestine.

3.4 Amylase inhibition by KEMS

To determine whether SCT encapsulated in KEMS retained amylase inhibitory activity after simulated gastrointestinal digestion, the α-amylase and α-glucosidase inhibition of pepsin and pepsin followed by trypsin-chymotrypsin digested KEMS encapsulating SCT were investigated. Interestingly, the KEMS alone inhibited both α-amylase and α-glucosidase (Table 3). Encapsulation of SCT increased the inhibitory activity of KEMS against both enzymes. The increase in inhibition of α-glucosidase due to the encapsulated SCT was substantial up to the end of the pepsin digestion stage, between 92% and 212%, whereas the increase in inhibition against α-amylase up to the end of pepsin digestion was much lower, between 5% and 39%. This is in line with the much lower IC_{50} of SCT against α-glucosidase than α-amylase (Figure 1). With the subsequent trypsin-chymotrypsin digestion step, as the α-glucosidase inhibitory activity of the KEMS alone was so high >96%, there was little increase in α-glucosidase inhibition as a result of encapsulating the SCT. However, the encapsulated SCT increased α-amylase inhibition by 17-23% compared to the KEMS alone.

Pepsin and trypsin-chymotrypsin digestion of KEMS increased inhibitory activity of both KEMS alone and KEMS encapsulating SCT against both amylase enzymes. The increase in inhibitory activity of the KEMS encapsulating SCT is presumably because the digestion of the KEMS by the proteases (Figure 3) partially exposed the encapsulated SCT and enabled

Table 3: Effect of SCT encapsulation on the inhibitory action of KEMS on α-amylase and α-glucosidase after digestion with pepsin followed by digestion with trypsin and chymotrypsin

Enzyme treatment	Digestion time (min)	Material	Enzyme inhibition (%)	
			α-amylase	**α-glucosidase**
None	0	KEMS alone	[1]42.1^{a}[2] ± 1.4	30.1^{b} ± 0.2
		KEMS encapsulating SCT	58.7^{b}[1] ± 1.5 (39%)[3]	63.8^{e} ± 1.8 (112%)
Pepsin	10	KEMS alone	84.7^{fg} ± 0.9	12.9^{a} ± 1.0
		KEMS encapsulating SCT	88.7^{h} ± 2.3 (5%)	40.3^{c} ± 1.6 (212%)
		SCT alone	Not detected	100.6^{f} ± 0.2
	120	KEMS alone	81.1^{de} ± 0.4	29.9^{b} ± 1.0
		KEMS encapsulating SCT	87.4^{gh} ± 1.0 (8%)	57.3^{d} ± 3.6 (92%)
		SCT alone	Not detected	100.7^{f} ± 0.2
Trypsin-Chymotrypsin	10	KEMS alone	67.6^{c} ± 0.9	96.9^{f} ± 0.3
		KEMS encapsulating SCT	83.3^{ef} ± 0.2 (23%)	97.4^{f} ± 1.2 (<1%)
		SCT alone	12.8^{b} ± 4.4	Not detected
	120	KEMS alone	67.6^{c} ± 0.4	99.6^{f} ± 0.3
		KEMS encapsulating SCT	78.8^{d} ± 1.0 (17%)	100.5^{f} ± 0.2 (1%)
		SCT alone	7.0^{b} ± 3.8	Not detected

[1]Mean ± standard deviation (n=2)

[2]Values with different superscripts in the same column are significantly different (p <0.05)

[3]Increase in inhibition due to SCT

them to bind and inhibit more amylase enzymes. The inhibitory activity of KEMS alone was not expected. Clearly, other factors were involved in the α-amylase and α-glucosidase inhibition. The kafirin protein used to prepare KEMS did not exhibit any inhibitory activity (results not shown). However, kafirin undergoes conformation changes when formed into microparticles (Taylor, , Taylor,, Belton & Minnaar, 2009b). Therefore, it can be surmised that these conformational changes caused the amylase enzymes to adsorb to KEMS surface and be inhibited.

In line with the IC_{50} data, the SCT alone did not inhibit the α-amylase during pepsin digestion, but completely inhibited the α-glucosidase (Table 3). This observation is in agreement with the notion that α-amylase and α-glucosidase are both inhibited by plant polyphenols but that the size and structure of the polyphenols determine the degree of inhibition (Lo Piparo, Scheib, Frei & Williamson, 2008; McDougall, Shpiro, Dobson, Smith, Blake & Stewart, 2005). With the SCT alone, there was minimal α-amylase inhibition and no α-glucosidase inhibition after trypsin-chymotrypsin digestion. This suggests that SCT alone were prevented from inhibiting the amylases due to the non-specific binding of the protease proteins to the SCT. This binding would have taken place because the SCT were not encapsulated in the KEMS.

4. Conclusions

The findings indicate that alcoholic extraction of SCT from sorghum bran and their encapsulation in microparticles made from sorghum kafirin protein using a simple alcoholic coacervation technique should substantially reduce carbohydrate digestion in the small intestine when the tannin containing KEMS are orally administrated. This nutraceutical type treatment could enable improved postprandial glycaemic control in persons suffering from

hyperglycaemia associated with T2D. Research is now required to determine the effect of the SCT encapsulated in KEMS on glycaemic response and insulin levels *in vivo*.

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