



# The antioxidant activity of encapsulated polyphenols following *in vitro* gastrointestinal simulated digestion

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## Introduction

Polyphenols have strong antioxidant and anti-inflammatory properties with potential for prevention and alleviation of oxidative stress associated diseases. Low bioaccessibility and poor bioavailability are major limiting factors for use of polyphenols in therapies. However, encapsulation of polyphenols have been shown to improve bioaccessibility and activity by preventing degradation during digestion (Radünz *et al.*, 2021).

## Aim

This study aimed to explore encapsulation of catechin (CAT), gallic acid (GA) and epigallocatechin gallate (EGCG) alone and in combination in  $\beta$ -cyclodextrin ( $\beta$ CD) to improve their bioactivity for potential use as therapy to oxidative stress propagated diseases.

## Materials and methods

The polyphenols were encapsulated in  $\beta$ CD with subsequent lyophilisation. Inclusion complexation was confirmed using tandem mass spectrometry. Encapsulation yield was calculated and efficiency determined using the Folin–Ciocalteu assay.

Samples underwent simulated *in vitro* gastrointestinal digestion (GID). The antioxidant activity was quantified using the oxygen radical absorbance capacity assay. Cellular antioxidant activity was determined using the dichlorofluorescein-diacetate assay on Caco-2 cells. Antiglycation activity was determined using the bovine serum albumin (BSA) model with methylglyoxal (MGO) and fructose (FRU) glycating agents. Samples were tested at 100  $\mu$ M.

## Results and discussion

Encapsulation was confirmed by identifying ions with a mass to charge ratio corresponding to complexes of  $\beta$ CD and each polyphenol (Table 1). This data confirmed the formation of 1:1 inclusion complexes between  $\beta$ CD and each polyphenol encapsulated.

The lyophilisation method produced high encapsulation yield and efficiency (Table 1). The results were consistent with previous studies (Ho *et al.*, 2017). To date, this is the first study to report the encapsulation efficiency for EGCG and co-encapsulated CAT/GA/EGCG triple combination in  $\beta$ CD with lyophilisation.

Table 1: Mass spectrometry ions, encapsulation efficiency and yield of lyophilised polyphenol inclusion complexes with  $\beta$ CD.

Samples	Inclusion complex ion	Mass to charge ratio (Da)	Encapsulation efficiency (%)	Encapsulation yield (%)
CAT+ $\beta$ CD	[CAT+ $\beta$ CD-H] <sup>-</sup>	1424.5	96.62 $\pm$ 0.61 <sup>b</sup>	91.27 $\pm$ 2.90 <sup>a</sup>
GA+ $\beta$ CD	[GA+ $\beta$ CD-H] <sup>-</sup>	1304.5	95.65 $\pm$ 1.34 <sup>b</sup>	93.36 $\pm$ 5.45 <sup>a</sup>
EGCG+ $\beta$ CD	[EGCG+ $\beta$ CD+H] <sup>+</sup>	1594.2	98.16 $\pm$ 0.56 <sup>b</sup>	92.49 $\pm$ 3.25 <sup>a</sup>
CAT/GA/EGCG+ $\beta$ CD	–	–	104.42 $\pm$ 0.78 <sup>a</sup>	94.45 $\pm$ 3.05 <sup>a</sup>

$\beta$ CD – beta cyclodextrin, CAT – catechin, GA – gallic acid, EGCG – epigallocatechin gallate, Da – Dalton. Different superscript letters in each column represent significant differences ( $p < 0.05$ ).

Chemical antioxidant activity was maintained following encapsulation (Figure 1A). Similar results have been previously reported (Ho *et al.*, 2017). There was a slight loss in antioxidant activity seen for EGCG ( $p < 0.005$ ) and a more significant loss in the triple combined sample ( $p < 0.0001$ ) post digestion. These results are in agreement with previous studies (Chait *et al.*, 2020).

Cellular antioxidant activity was only seen in undigested free and encapsulated CAT, EGCG and the triple combination (none with GA), with complete losses in activity post digestion (Figure 1B). Cellular antioxidant activity has been previously reported for polyphenols. Moreover, *in vitro* digestion has been shown to significantly decrease cellular antioxidant activity of polyphenols (Moyo *et al.*, 2020).

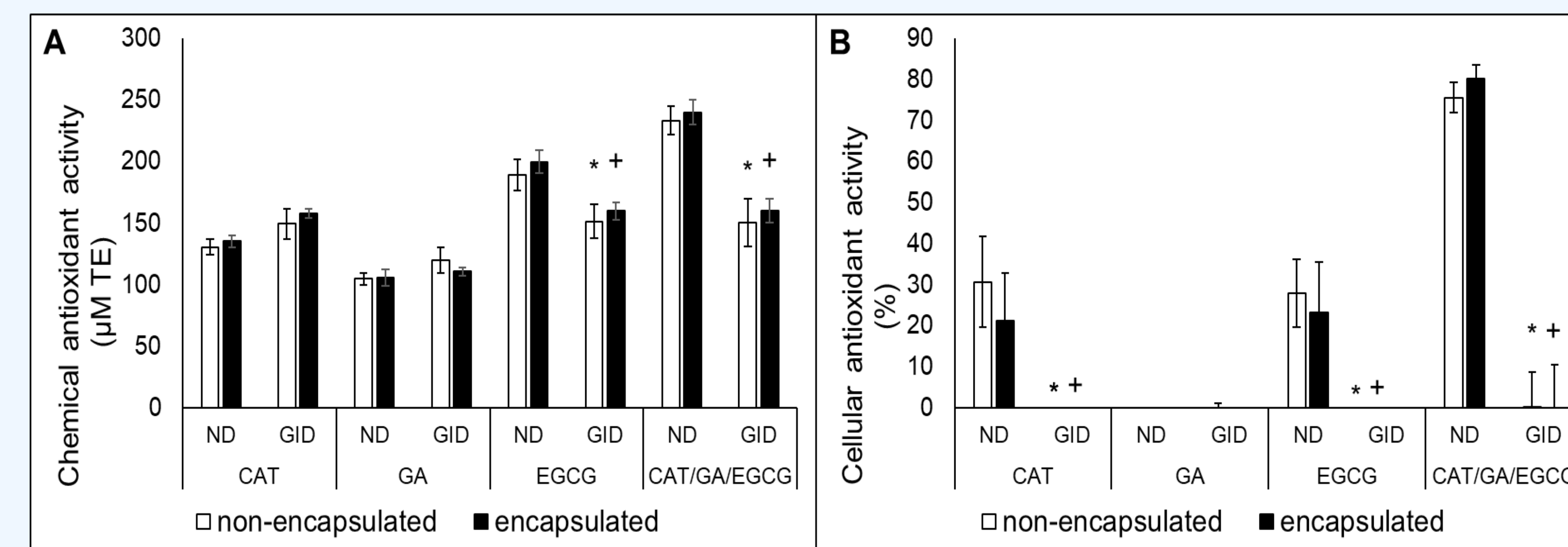


Figure 1: Chemical (A) and cellular (B) antioxidant activity of single and combination polyphenol samples at 100  $\mu$ M. The data is represented as mean  $\pm$  SEM. ND – non-digested, GID – gastrointestinal digested, CAT – catechin, GA – gallic acid, EGCG – epigallocatechin gallate,  $\mu$ M TE – micromolar Trolox equivalents. The \* and + represent significant ( $p < 0.05$ ) difference from non-encapsulated and encapsulated ND, respectively.

There was higher antiglycation activity in the BSA-FRU model compared to the BSA-MGO model (Figure 2A and B), with CAT, GA and the triple combined sample gaining activity post digestion. The antiglycation activity observed in both BSA-MGO and BSA-FRU model is in agreement with results of previous studies (De Lima-Júnior *et al.*, 2021). However, in previous reports, *in vitro* digestion decreased antiglycation of polyphenol-rich extracts (Spínola *et al.*, 2020).

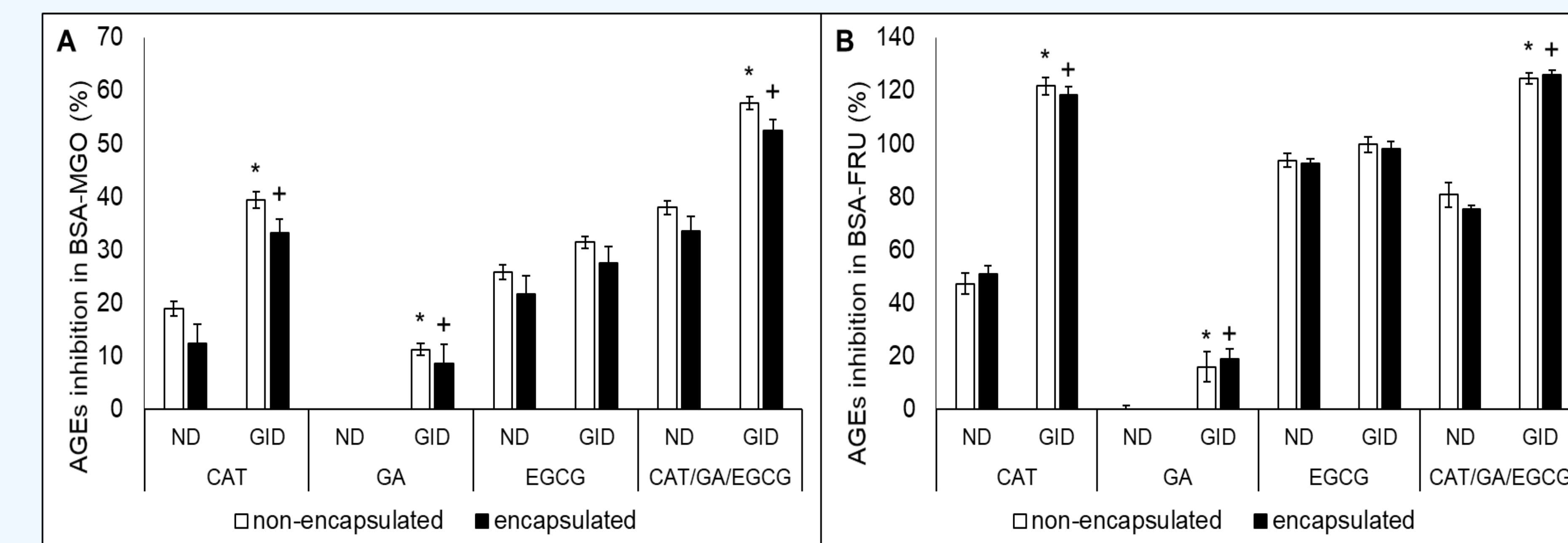


Figure 2: Inhibition of AGEs in the BSA-MGO (A) and BSA-FRU (B) by single and combination polyphenol samples at 100  $\mu$ M. The data is represented as mean  $\pm$  SEM. ND – non-digested, GID – gastrointestinal digested, CAT – catechin, GA – gallic acid, EGCG – epigallocatechin gallate, AGEs – advanced glycation end-products. The \* and + represent significant ( $p < 0.05$ ) difference from non-encapsulated and encapsulated ND, respectively.

## Conclusion

$\beta$ CD formed 1:1 inclusion complexes with tested polyphenols. Lyophilisation produces high encapsulation efficiency and yield. Chemical and cellular antioxidant activity was maintained following encapsulation in  $\beta$ CD with slight losses for some polyphenols post *in vitro* digestion. In contrast, antiglycation activity was increased post *in vitro* digestion. Further studies are needed to confirm the bioavailability results in an *in vivo* environment.

## References

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