Supporting information

Methods

Effect of simulated intestinal media on the detection of H_2O_2 by the AAP-DCHBS-HRP method

Solutions of H_2O_2 at final concentrations up to 15 μM were prepared in different media by diluting a commercial solution of H_2O_2. AAP (0.1 mM final concentration), DCHBS (1 mM final concentration), HRP (3.6 units/mL, final concentration) were then added and incubated for 15 min at 25 °C. For each sample, absorbances at 515 nm and at 750 nm were read. The differences were calculated and subtracted by the value calculated in the sample not containing H_2O_2.

Production of H_2O_2 by DAO incubated with pancreatin

DAO (0.15 mg of solid/mL) was incubated for 24 h at 37 °C in SIF pH 6.8 containing 1% pancreatin Aliquots were withdrawn, supplemented or not with 2000 units/mL bovine liver catalase and incubated for 15 min at 37 °C. Samples were then diluted to a final concentration of 0.6 $x10^{-3}$ mg of solid/mL into a solution containing AAP, DCHBS, HRP at the concentrations indicated above and incubated for 15 min at 25 °C. Absorbance at 515 nm of the adduct generated from the reaction of DCHBS with AAP (oxidized by H_2O_2 in the presence of HRP) was read, and after subtraction of the background absorbance (at 750 nm), it was corrected with the value obtained in the absence of DAO; the content of H_2O_2 was calculated using 2.6×10^4 $M^{-1}cm^{-1}$ as the extinction coefficient.

Figure captions

Figure S1: Effect of simulated intestinal media on the detection of H_2O_2 by the AAP-DCHBS-HPR method. Solutions of H_2O_2 were prepared at the indicated final concentrations in PB at pH 7 (♦-♦), SIF at pH 6.8 (**x-x**), FaSSIF at pH 6.8 (●-●), FaSSIF at pH 6.5 (▲-▲), FeSSIF at pH 5.5 (■-■). AAP, DCHBS, HRP were then added and absorbance recorded and corrected as reported in Material and Methods (means +/- SD, n=3 different experiments).

Figure S2: Michaelis-Menten kinetics for the oxidation of benzylamine by DAO in different simulated intestinal media. DAO (0.18 mg of solid/mL final concentration) was added to PB at pH 7 (♦-♦), SIF at pH 6.8 (**x-x**), FAS SIF at pH 6.8 (●-●), FAS SIF at pH 6.5 (▲-▲), FES SIF at pH 5.5 (■-■) at 25 °C in the presence of benzylamine and the formation of benzaldehyde measured (means +/- SD, n=3 different experiments).

Figure S3: Effect of CA on DAO activity in the presence of proteases. DAO (0.15 mg of solid/mL final concentration) was incubated at 37 °C in SIF at pH 6.8 alone (control); in the presence of 3 mM CA; in the presence of 1% pancreatin; in the presence of 3 mM CA and 1% pancreatin; in the presence of 1% trypsin; in the presence of 3 mM CA and 1% trypsin (from the darkest to lightest shade). At the indicated times, aliquots were withdrawn, diluted in the incubation buffer and the activity measured by the AAP-DCHBS-HRP method. Reported values of DAO activity refer to the enzymatic activity in the incubation media (means +/- SD, n=3 different experiments).

Figure S4: Production of H_2O_2 during the incubation of pancreatin with DAO. DAO (0.15 mg of solid/mL final concentration) was incubated at 37 °C in SIF pH 6.8 containing 1% pancreatin. After 24 h of the incubation samples were diluted in SIF at pH 6.8 to a final concentration of $0.6x10^{-3}$ mg of solid/mL, then added with AAP, DCHBS, HRP and H_2O_2 concentration measured. Samples not incubated with bovine liver catalase (dark shade); samples incubated for 15 min with bovine liver catalase (2000 units/mL) (light shade) before dilution and addition of AAP, DCHBS and HRP. Reported values refer to DAO activity in the incubation media (means +/- SD, n=3 different experiments).

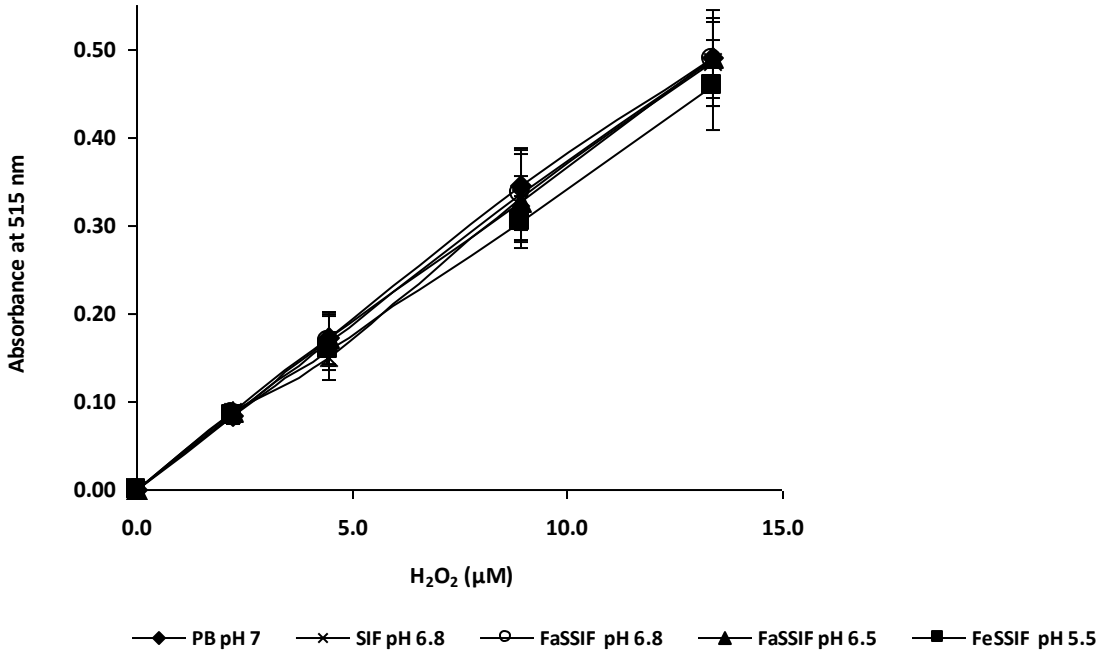
Absorbance at 515 nm
0.50
0.40
0.30
0.20
0.10
0.00
0.0
5.0
10.0
15.0
H_2O_2 (µM)
PB pH 7
SIF pH 6.8
FaSSIF pH 6.8
FaSSIF pH 6.5
FeSSIF pH 5.5

Fig. S1

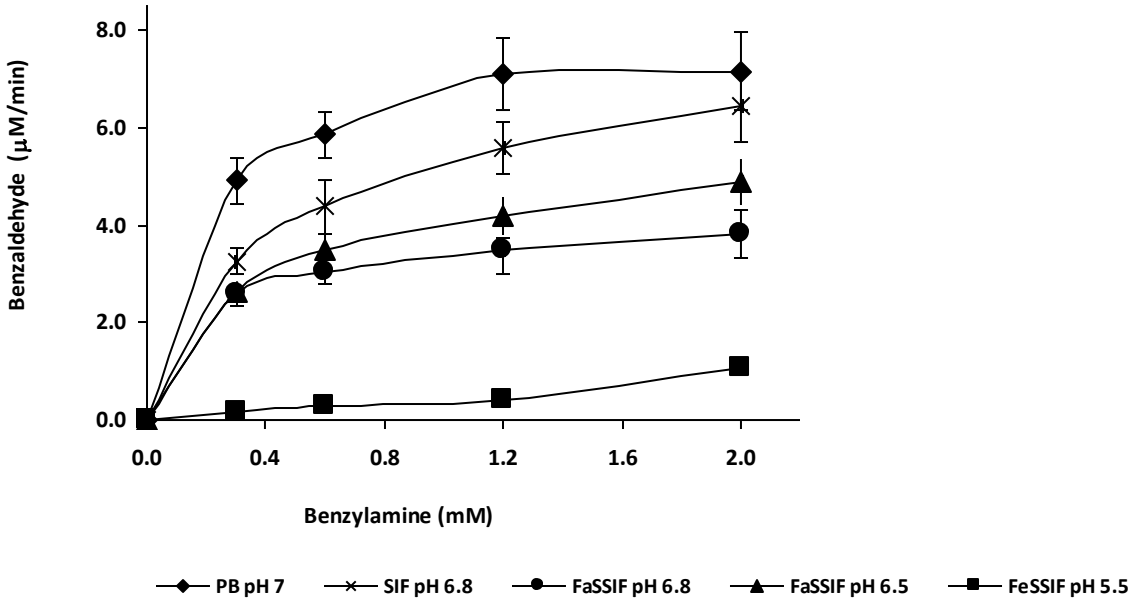
Benzaldehyde (µM/min)
8.0
6.0
4.0
2.0
0.0
0.0
0.4
0.8
1.2
1.6
2.0
Benzylamine (mM)
PB pH 7
SIF pH 6.8
FaSSIF pH 6.8
FaSSIF pH 6.5
FeSSIF pH 5.5

Fig. S2

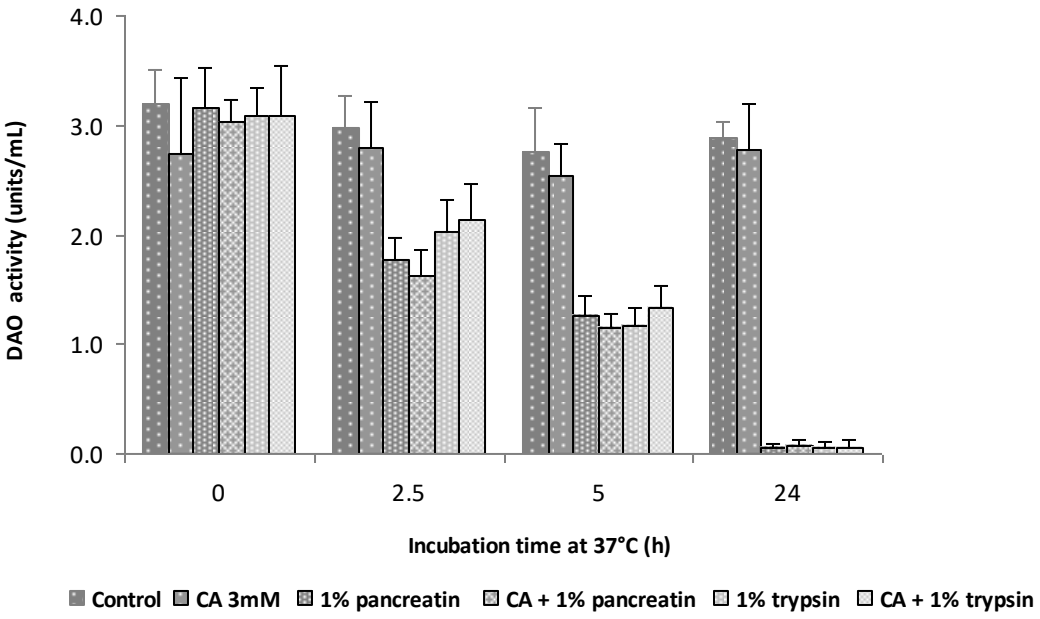
4.0
3.0
2.0
1.0
0.0
DAO activity (units/mL)
0
2.5
5
24
Incubation time at 37°C (h)
Control
CA 3mM
1% pancreatin
CA + 1% pancreatin
1% trypsin
CA + 1% trypsin

Fig. S3

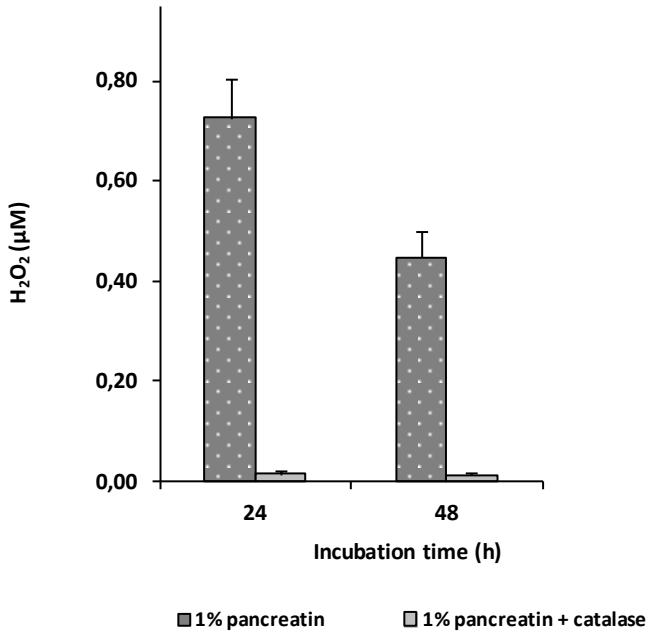
0,80
0,60
0,40
0,20
0,00
H_2O_2 (µM)
24
48
Incubation time (h)
1% pancreatin
1% pancreatin + catalase

Fig. S4