Evaluation of the Anti-Obesity Potential of Polyphenols through Pancreatic Lipase Inhibition

Maria C. Lobo[1], Sílvia Rocha[1], Marco Zanchetta[2], Vera LM Silva[2], Artur MS Silva[2], Eduarda Fernandes[1], Ana T. Rufino[1]

[1] *LAQV, REQUIMTE, Laboratory of Applied Chemistry, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal*

[2] *LAQV, REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal*

INTRODUCTION

OBESITY is characterised by an overproduction and accumulation of triglicerydes into the adipose cells, due to an imbalance between energy intake and expenditure [1].

Lipids from diet are important for the onset and development of obesity

↓

Their absorption can be explored for obesity treatment

PANCREATIC LIPASE → Responsible for the degradation of 50 to 70% of the triglycerides from diet → **IMPORTANT TARGET FOR OBESITY TREATMENT**

The known inhibitor of pancreatic lipase, **Orlistat**, is associated with **low efficacy** and **undesirable side effects** [2].

↓

POLYPHENOLS are natural occurring and structurally diverse compounds with diferent biological activities, so they could be explored to be **potential anti-obesity molecules** [3].

WORK AIMS

1) Optimization of the experimental conditions for the meausure of pancreatic lipase activity, using a colorimetric low-cost microanalysis system based on the enzymatic metabolization of *p*-nitrophenyl butyrate (Figure 1).

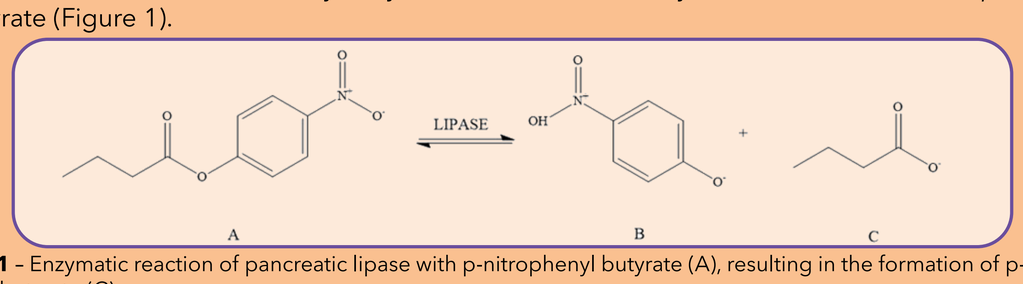
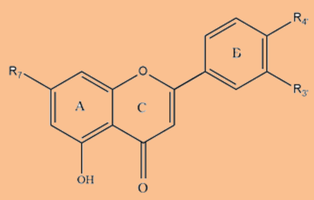
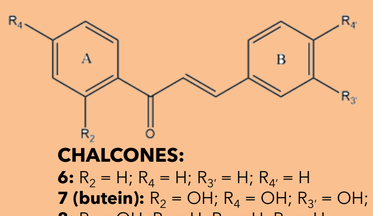


Figure 1 – Enzymatic reaction of pancreatic lipase with p-nitrophenyl butyrate (A), resulting in the formation of p-nitrophenol (B) and butyrate (C).

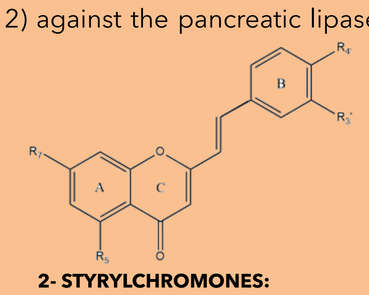
2) Explore the inhibitory influence of a panel of 16 polyphenols (figure 2) against the pancreatic lipase activity. Whenever possible a structure-activity relationship was explored.



FLAVONOIDS:
1: $R_{3'}$ = H; $R_{4'}$ = H; R_5 = H; R_7 = H
2 (luteolin): $R_{3'}$ = OH; $R_{4'}$ = OH; R_5 = OH; R_7 = OH
3: $R_{3'}$ = OH; $R_{4'}$ = OH; R_5 = OH; R_7 = H
4: $R_{3'}$ = H; $R_{4'}$ = H; R_5 = OH; R_7 = H
5: $R_{3'}$ = H; $R_{4'}$ = Cl; R_5 = H; R_7 = H



CHALCONES:
6: R_2 = H; R_4 = H; $R_{3'}$ = H; $R_{4'}$ = H
7 (butein): R_2 = OH; R_4 = OH; $R_{3'}$ = OH; $R_{4'}$ = OH
8: R_2 = OH; R_4 = H; $R_{3'}$ = H; $R_{4'}$ = H
9: R_2 = OH; R_4 = H; $R_{3'}$ = Cl; $R_{4'}$ = H
10: R_2 = H; R_4 = H; $R_{3'}$ = H; $R_{4'}$ = Cl



2- STYRYLCHROMONES:
11: R_5 = H; R_7 = H; $R_{3'}$ = H; $R_{4'}$ = H
12: R_5 = OH; R_7 = OH; $R_{3'}$ = OH; $R_{4'}$ = OH
13: R_5 = OH; R_7 = H; $R_{3'}$ = OH; $R_{4'}$ = OH
14: R_5 = OH; R_7 = H; $R_{3'}$ = H; $R_{4'}$ = H
15: R_5 = OH; R_7 = H; $R_{3'}$ = Cl; $R_{4'}$ = H
16: R_5 = H; R_7 = H; $R_{3'}$ = H; $R_{4'}$ = Cl

Figure 2 – Chemical Structure of the tested polyphenols.

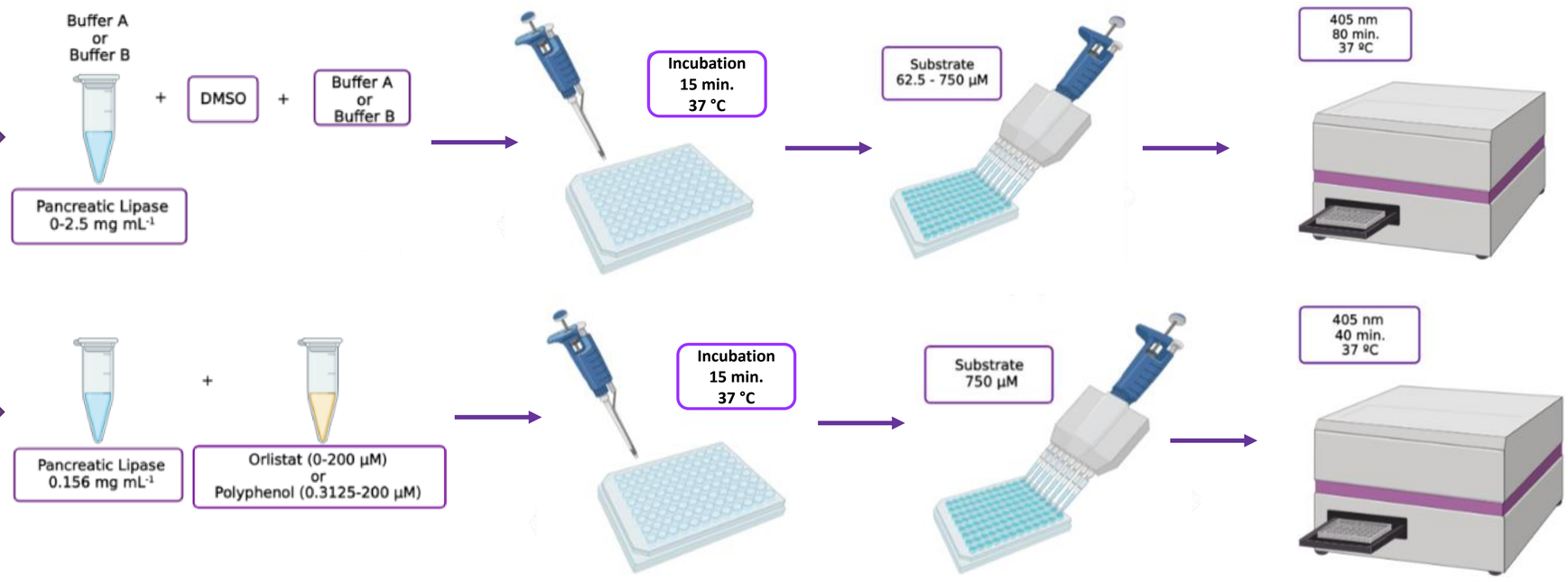
METHODS

***In vitro* Optimization of the pancreatic lipase enzyme activity**

Optimised conditions:

- **Buffer Composition**:
  A - Tris 100 mM and $CaCl_2$ 5 mM pH 7.4
  B - PBS w/ Tween 80 (0.1 %) pH 7.45
- **pH**: 7.2 and 7.4
- **Substrate concentration**:
  62.5; 125; 250; 500; 750 µM
- **Enzyme concentration**:
  0.156; 0.3125; 0.625; 1.25; 2.5 mg mL^{-1}

Evaluation of the inhibitory effect of the polyphenols on pancreatic lipase activity



RESULTS

In vitro Optimization of the pancreatic lipase enzyme activity

Buffer Composition:

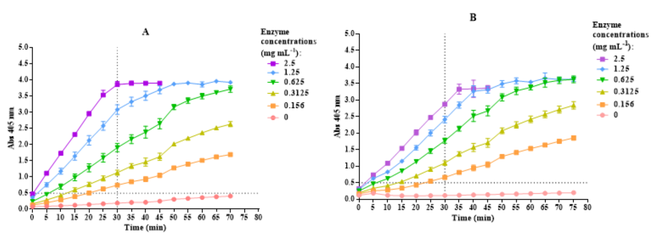


Figure 3 - Absorbance (405 nm) versus time (min) as result of the variation of the buffer. Graph A shows the activity of pancreatic lipase using the buffer A (100 mM Tris and 5 mM $CaCl_2$ pH 7.4). Graph B shows the activity of pancreatic lipase using buffer B (PBS w/ Tween 80 (0.1%) pH 7.45). Values are given as mean ± SEM (n ≥ 3).

The Buffer A (100 mM Tris and 5 mM $CaCl_2$ pH 7.4) was chosen because it demonstrated a more linear response in the Abs readings in comparison with Buffer B.

pH:

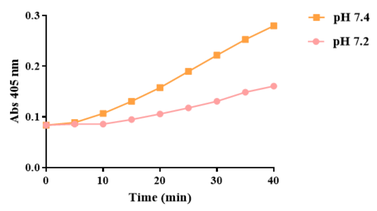


Figure 4 - Absorbance (405 nm) versus time (min) as result of the variation of the pH. Values are given as mean ± SEM.

The enzyme showed a better activity at the **pH of value of 7.4** when compared with the pH of 7.2.

Substrate and enzyme concentration:

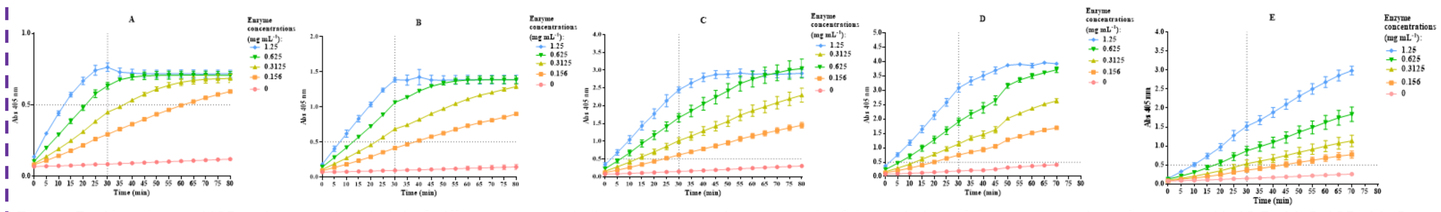


Figure 5 - Absorbance (405 nm) versus time (min) of different pancreatic lipase concentrations as result of the variation of the concentration of substrate (A-62.5 µM; B-125 µM; C-250 µM; D-500 µM and E-750 µM) of *p*-nitrophenyl butyrate. Values are given as mean ± SEM (n ≥ 3).

The substrate concentration of 750 µM, presented an intermediate reaction rate, maintaining the absorbance readings in an ideal variation rate for testing the compounds under study was chosen and the **enzyme concentration of 0.156 mg mL^{-1}** was chosen since it was the first to reach a satisfactory activation of the enzyme, showing an absorbance value of approximately 0.5, at the linear region of the Abs vs time curve.

Evaluation of the inhibitory effect of the polyphenols on pancreatic lipase activity

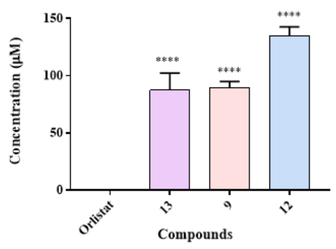
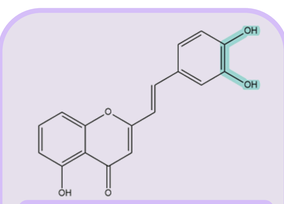
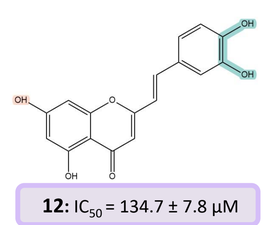


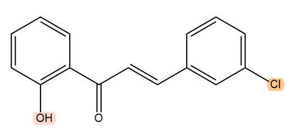
Figure 6 - Representative graph of the IC50 values of the tested compounds with inhibitory activity against pancreatic lipase. The IC50 values with * are significantly different from the IC50 value of corresponding positive control, orlistat (*p<0.05 ; ** p <0.005; ***p <0.0005; ****p <0.0001. Values are given as mean ± SEM (n≥3).



13: IC_{50} = 87.3 ± 15.0 µM



12: IC_{50} = 134.7 ± 7.8 µM



9: IC_{50} = 89.6 ± 5.4 µM

DISCUSSION

- The group of 2-SC seems to be the one showing a higher inhibitory activity when compared to the corresponding flavonoids and chalcones.
- In the case of chalcones, the -Cl substituent on B-ring and the -OH substituent on A-ring seem to be relevant to the inhibitory activity of chalcones, but not of 2-SC.
- The catechol group present in B-ring of 2-SC seem to be relevant to the inhibitory activity, as previously reported for other enzymes and flavonoids.

Although some compounds have shown some potential for pancreatic lipase inhibition, further studies are needed to disclose its inibition kinetics

ACKNOWLEDGMENTS

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References

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