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FLUORESCENCE POLARIZATION IMMUNOASSAY (FPIA) FOR THE DETERMINATION OF ANTIAMYLASE ACTIVITY OF PLANT PHENOLIC COMPOUNDS

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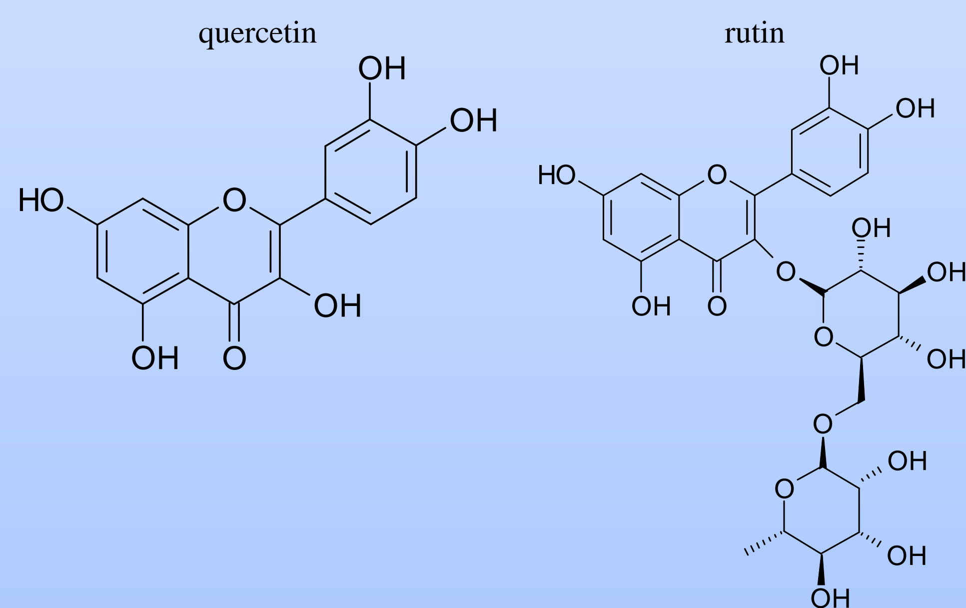
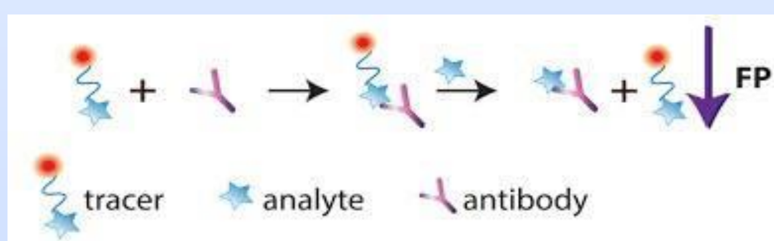
Introduction

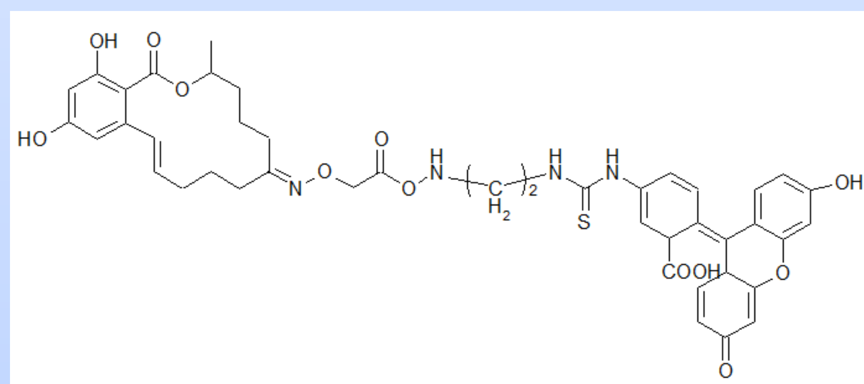
The digestive enzyme inhibitory activity of phenolic compounds of plant origin is investigated for the last few decades. Inhibition of starch-degrading enzymes is one approach to the therapy and prevention of type II diabetes. In vitro studies have determined that flavonoids effectively inhibit amylase. Rutin and quercetin are the most common flavonoids found in plants worldwide. It is noted that quercetin and rutin has high anti-amylase activity comparable to acarbose.
The main approach to quantify the anti-amylase activity of phenolic compounds is spectrophotometric determination based on colour reactions. These methods involve several stages of incubation, making the test time-consuming. Immuno-biological methods take minutes, require microlitres of sample and do not use toxic reagents.
The aim of this study was to develop a rapid, simple, easy-to-use FPIA method to evaluate the anti-amylase activity of flavonoids.



Materials and methods

FPIA principle

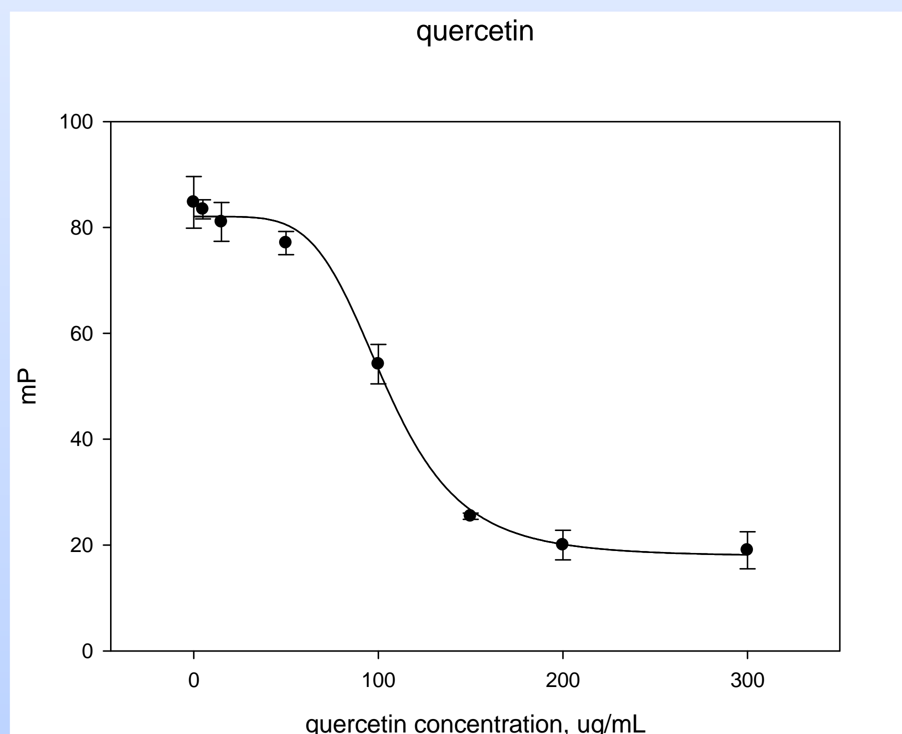




ZEA tracer – zearalenone bonded with fluorofore

The method of fluorescence-polarization immuno-assay is based on the interaction of fluorophore-labelled antigen and antibody. Zaralenone is a mycotoxin found in cereals. Possessing phenolic hydroxyls, zearalenone binds to amylase in the same way as flavonoids. Fluorescein-labelled zearalenone (ZEN-EDF) was used as a tracer.
The intensity of polarization emitted by the fluorophore labelled compound depends on its molecular weight. Tracer has a low molecular weight and therefore low polarization. After interaction with the enzyme, a high molecular weight complex is formed and the polarization increases. Inhibitors (quercetin, rutin) competitively bind to amylase, free tracer is present in solution, and polarization is reduced.
Fluorescence immunoassay was perfomed on Sentry-200.

Determination of anti-amylase activity of quercetin

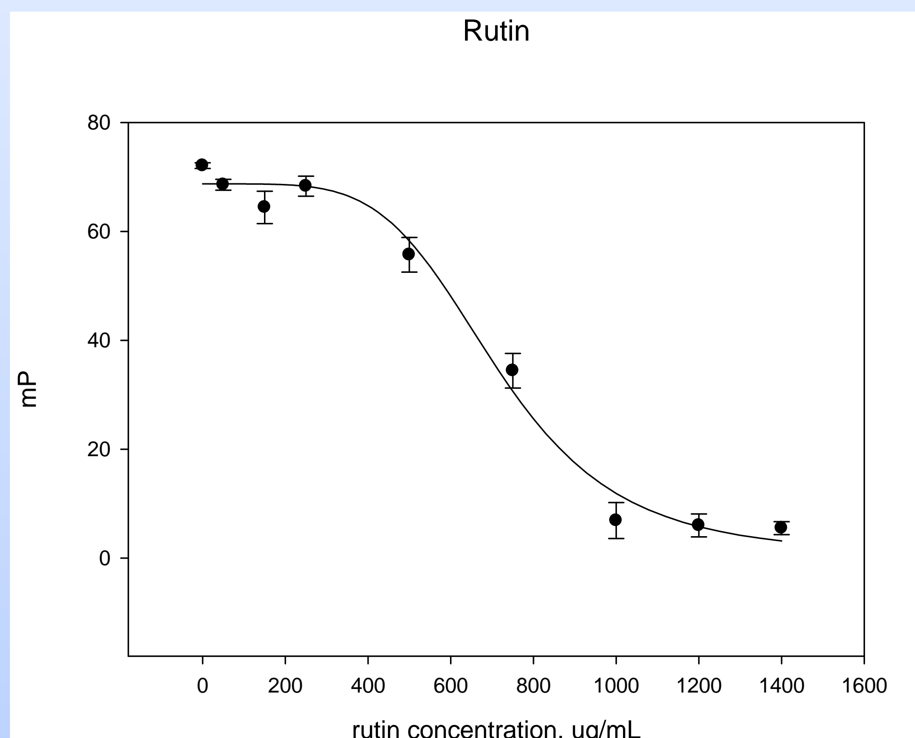


The method is characterised by the equation

$$y = 17.8 + \frac{64.2}{1 + (\frac{x}{104.2})^{5.06}}$$

IC_{50} is 104.2 µg/mL
Limit of detection is 63.6 µg/mL,
limit of quantification 74.4 µg/mL;
Detection range (20-80% inhibition) 74.4 – 120.7 µg/mL
R^2 value is 0.9988, $p<0.05$

Determination of anti-amylase activity of rutin



The method is characterised by the equation

$$y = 0.53 + \frac{68.2}{1 + (\frac{x}{714.2})^{4.8}}$$

IC_{50} is 714.2 µg/mL
Limit of detection is 350.1 µg/mL,
limit of quantification 436.9 µg/mL;
Detection range (20-80% inhibition) 436.9 – 885.9 µg/mL
R^2 value was 0.9944, $p<0.05$

Conclusion

1. A rapid, simple, non-toxic FPIA method for determination of anti-amylase activity of quercetin and rutin was developed.
2. Quercetin has greater inhibitory activity (IC_{50} 104.2 µg/mL) then rutin (IC_{50} 714.2 µg/mL), which corresponds to the literature data.
3. This method can be further used for screening of plant-derived amylase inhibitors.