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DEVELOPMENT OF A TECHNIQUE FOR QUANTITATIVE DETERMINATION OF QUERCETIN IN ONION PEEL (ALLIUM SATIVUM L.) BY FLUORESCENCE-POLARIZATION ANALYSIS

Mina Babazadeh, 5th year student, Svetlana M. Filimonova, Associate Professor, Lilia I. Mukhametova, Professor, Sergei A. Eremin, Professor

Mina Babazadeh

Institute of Pharmacy, I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia
Lomonosov Moscow State University, Faculty of Chemistry, Moscow, Russia

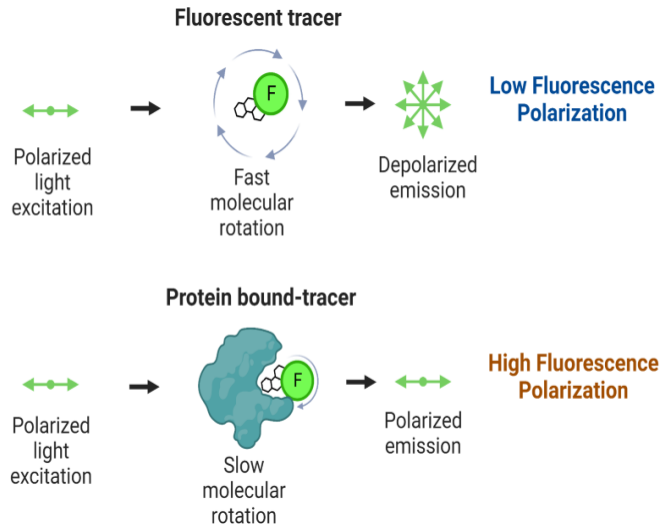
INTRODUCTION

The capacity of flavonoids to bind to and inhibit the digestive enzyme α-amylase has been documented, suggesting their potential role as adjunctive therapies for the treatment and prevention of diabetes. This study employs fluorescence polarization analysis (FPA) to examine the interaction between quercetin, one of the most prevalent flavonoids, and α-amylase. The FPA method assesses the rotational dynamics of a fluorescently labeled hapten; specifically, it compares the rapid rotation of free hapten with the slower rotation of the hapten when complexed with protein. An increase in molecular weight of the complex results in slower rotation and elevated polarization values (mP).

GOAL

While monoclonal antibodies are commonly utilized in this analysis, their high costs can be prohibitive. Our approach explores the use of readily available α-amylase as an economical high molecular weight component that binds to quercetin, potentially lowering assay expenses. Furthermore, the FPA method delivers rapid results, requiring only minutes for completion, and eliminates the need for multi-step sample preparation or toxic reagents.

FLUORESCENCE POLARIZATION METHOD



METHOD

Device: Sentry 200 from Ellie (USA)
Active substances:
Quercetin monohydrate Acros Organics (Belgium),
Tracer was synthesized by activation of the keto group followed by addition of ethylenediaminfluorescein (EDF), Onion peel (local market, Russia)
Solvent: ethanol (Uvasol® Merck
Darmtadt, Germany)

Method:
*Method was used to measure quercetin in onion peel extract (0.1 g of onion peel + 1 mL of EtOH, heating in water bath at 80°C for 10 min).

*Concentration of quercetin in the extract was determined by calibration graph (Fig.1).
standard solutions of quercetin were used to construct the calibration curve.

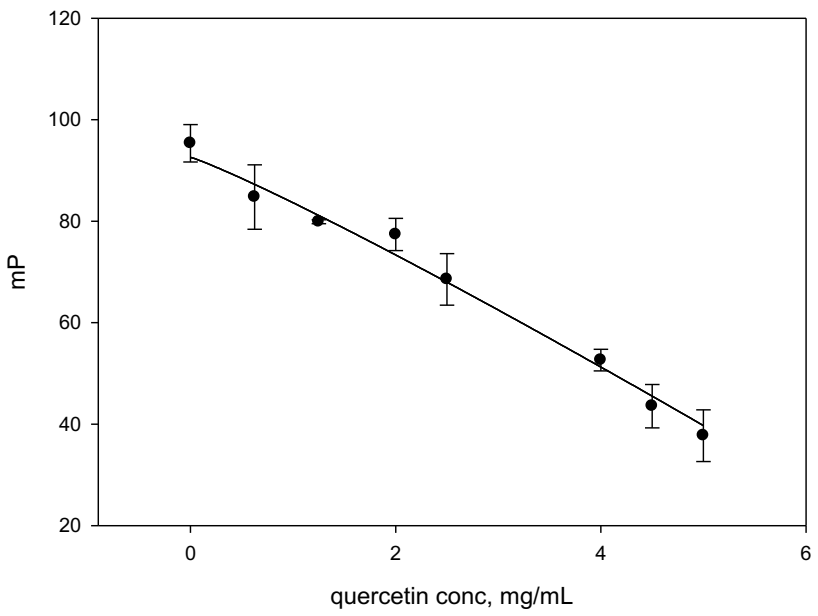


Figure 1: Calibration plot of polarization (mP) dependence on quercetin concentration

RESULTS

Statistic characteristics:

IC_{50} = 2,58 mg/mL

LOD (limit of detection) = 0.715 mg/mL

LOQ (limit of quantification) = 1.13 mg/mL

Detection range = 1.13-4.03 mg/mL

*Specificity: no cross-reactivity with dihydroquercetin was observed

CONCLUSION

A quantification method based on the binding of quercetin and amylase using FPA was developed.
Tracer quercetin-EDF was obtained.
The basic statistical performance of the method is described.

* Result of the quantification of quercetin in the onion peel extract is 1.89 mg/mL.