Identification and quantification of flavonol aglycones in cactus pear (*Opuntia ficus indica*) fruits using a preparation of cellulase and pectinases

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Introduction

Recent studies indicate a positive relation between consumption of plant foods and reducing the risks of diseases such as cancer, cardiovascular diseases, or diabetes [1,2]. This relation is in most cases attributed to high contents of flavonoids and their respective antioxidant activity [3]. Flavonols are widely distributed flavonoids in many fruits and vegetables, but there is a lack in data on flavonols in fruits like cactus pear.

Cactus pear *O. ficus indica* fruits contain many bioactive compounds such as vitamin C, betalains, and flavonols [4]. However, literature on cactus pear flavonols is not consistent: while some studies showed that cactus pear *Opuntia ficus indica* fruit primarily contains isorhamnetin derivatives [5], it is mainly characterized with quercetin derivatives in another study [6].

Because of the lack of authentic standards, flavonol glycosides are in most cases hydrolyzed to their respective aglycones prior to analysis. The acidic hydrolysis using hydrochloric acid in aqueous methanol under continuous heating is the main and traditional method, but it is affected by many factors such as the nature of the plant matrix and the flavonol composition [7]. Therefore, it is difficult to follow a single protocol for acidic hydrolysis of flavonol glycosides in the different plant materials.

A promising alternative hydrolysis of flavonol glycosides might be an enzymatic hydrolysis, without producing any degradation products such as protocatechuic acid. Hydrolysis of the flavonol glycosides using a preparation of cellulase and commercial pectolytic enzymes was successful in case of cactus pear fruits, onions and isorhamnetin-3-*O*-rutinoside and quercetin-3,4′-*O*-diglucoside as model compounds [8].

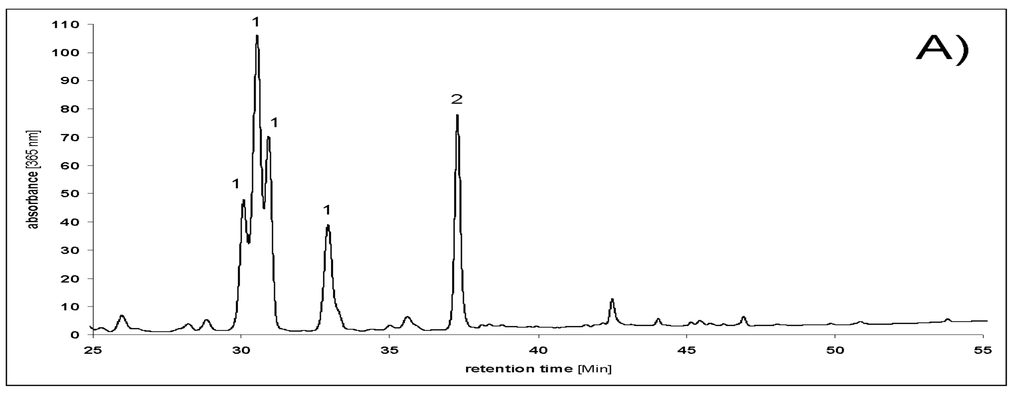

Fig. 1 A-C. HPLC profile of cactus pear (*O. ficus indica*) for different enzymatic hydrolysis periods, (A) after 0 h, (B) after 2 hours, and (C) after 16 h; Code: (1) Isorhamnetin glycosides, (2) isorhamnetin-3-*O*-rutinoside, (3) neo-formed non-identified isorhamnetin derivative, (4) isorhamnetin-4-*O*-glucoside, (5) isorhamnetin.

This work aims to identify and quantify the flavonol aglycones in different cactus pear fruits using a preparation of pectolytic enzymes and cellulase as a gentle alternative of acidic hydrolysis.

Materials and Methods

Fresh fruits of different *Opuntia ficus indica* varieties were collected during seasons from Sicily, Egypt and South Africa. Both pulp and peel were lyophilized, ground and stored in closed bottles at -18ºC until analysis. Prior to HPLC-DAD analysis, 100 mg of dried cactus pear pulp or peels were extracted with 1mL MeOH:Water (50:50), sonicated, vortexed, centrifuged at 10000 x g for 5 min and then supernatant was filtrated through nylon filters 0.45 µm. Detection was at the three wavelengths 365, 325 and 280 nm.

Enzymatic hydrolysis of flavonol glycosides was carried out as described by [8], 100 mg of dried material in 1mL of an enzyme mixture of RAPIDASE C80MAX (DSM, Heerlen, Netherlands) and cellulase (Fluka Chemie AG, Switzerland). Samples were incubated and vortexed at 50 ºC. After complete hydrolysis (16 hours), samples were diluted with pure methanol to a final concentration of 80% prior to HPLC-DAD analysis.

Results and Conclusion

Results showed that cactus pear peels are characterized by mainly isorhamnetin glycosides (Fig. 1A and Fig.2), with isorhamnetin-3-*O*-rutinoside as the main derivative with more than 25% of total isorhamnetin derivatives. These bioactive compounds have been found only in the peels of the all fruit samples, while the pulps had no flavonols. These facts were confirmed by preliminary investigations with HPLC-MS analysis (data not shown).

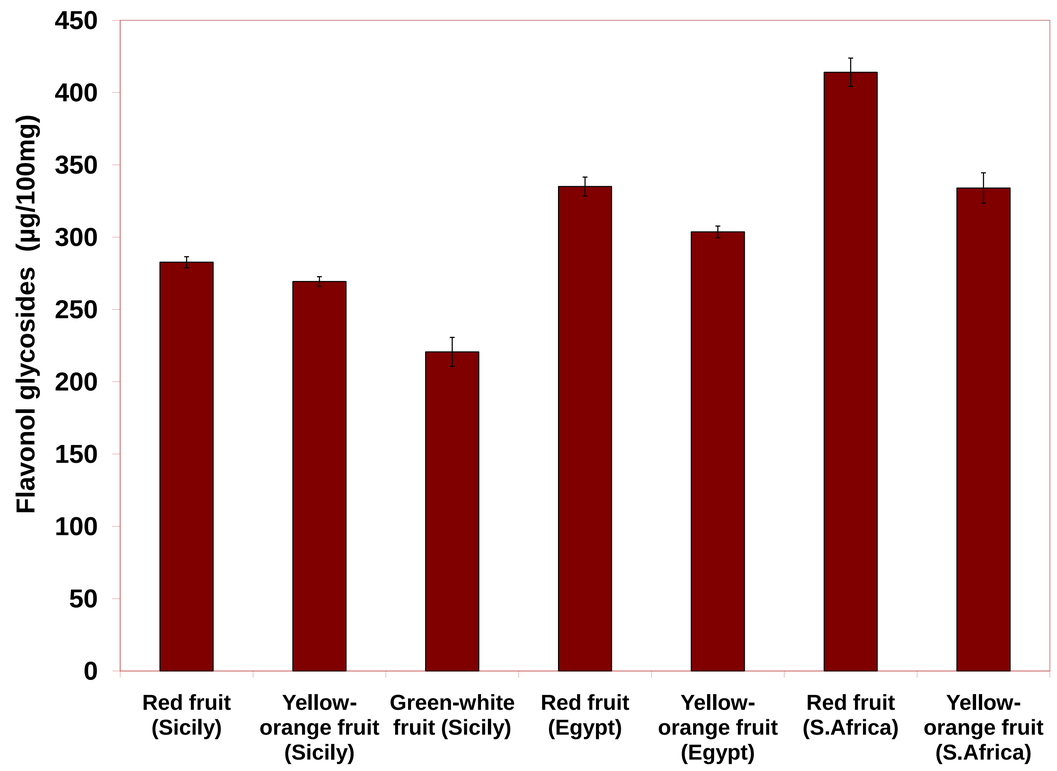

Figure 2. Total flavonol glycosides in fruit peels of different varieties of cactus pear (*Opuntia ficus indica*) peels expressed as isorhamnetin 3-rutinoside (µg/100mg).

Hydrolysis of flavonol glycosides using pectolytic enzymes and cellulase produced isorhamnetin as the only flavonol aglycone (Fig. 1B&C and Fig. 3). Cellulase did not cause any hydrolysis effect on flavonol glycosides in cactus matrices, but enhance the extraction of these bioactive compounds (data not shown).

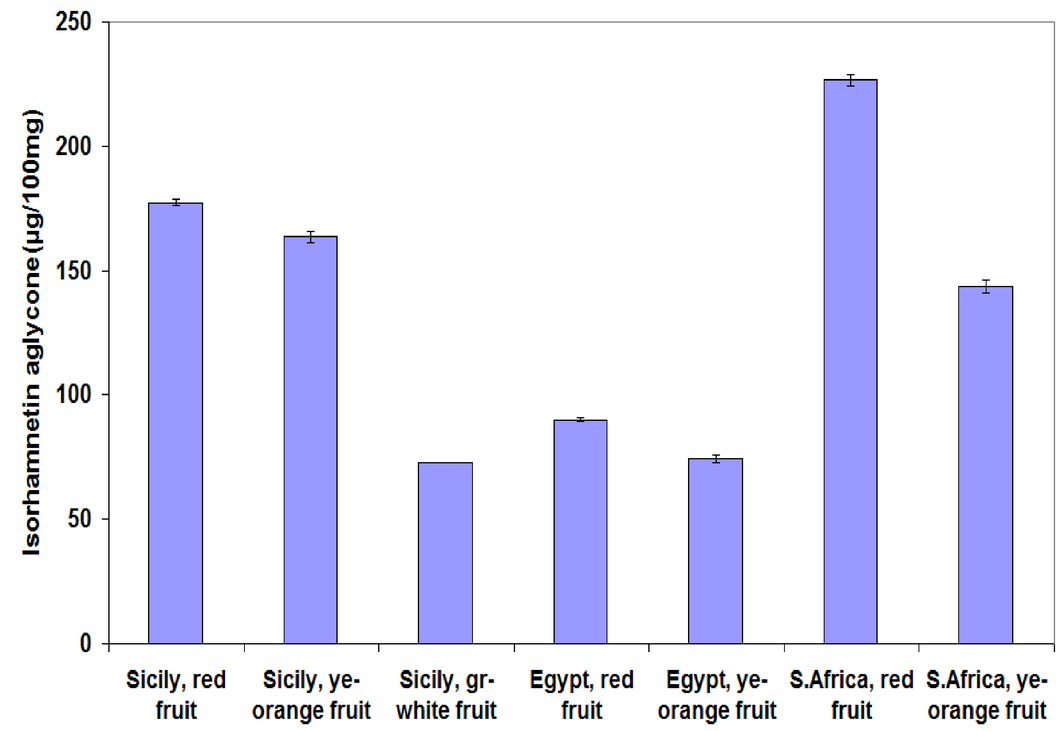

Figure 3. Isorhamnetin aglycone of different varieties of cactus pear (*Opuntia ficus indica*) peels (µg/100mg).

In addition to total phenolic content, peels of all varieties have a higher antioxidant activity than pulps which may correlate to the flavonols (data not shown).

In conclusion, isorhamnetin glycosides (a group of compounds contributes to antioxidant activity of fruits and vegetables) may be considered as markers for *Opuntia ficus indica* fruits, whereas all investigated varieties have the same profile of these bioactive compounds. It is highly recommended to involve the fruit's peel in producing of cactus pear products such as juices, cereal-based extrudates, ice cream, etc.

References

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