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Comparison of extraction methods for analysis of flavonoids in onions

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Introduction

Phenolic compounds are known to occur widely in the plant kingdom as secondary metabolites [1]. They are considered having health-promoting effects due to their antioxidant properties and have been proposed to decrease the risk of heart diseases and cancer [2].

Onions are widely consumed and contain flavonoids, which is a group of phenolic compounds [3]. Environmental and cultural conditions as well as genotype are expected to affect the content of flavoniods [2]. Hence, it is of interest to develop a fast and reliable analytical method. The aim of the study was to compare the efficiency and reproducibility of conventional and classical extraction methods with faster and/or automatic methods.

Materials and Methods

A freeze-dried and homogenized onion bulb sample (*Allium cepa* var. *zittauer*) was used for comparison of the following extraction methods, where the extraction solvent was 60% aqueous methanol:

• **Ultrasonication** (0.1 g sample, 5 ml extraction solvent, room temp., 60 min, 120 Watt) [4]

• **Shaking-water bath** (0.5 g sample, 50 ml extraction solvent, 30°C, 60 min) followed by filtration, evaporation *in vacuo* (30°C) and redissolvation in 5 ml solvent

• **Microwave-assisted extraction** (0.1 g sample, 5 ml extraction solvent, 60°C, 2 min, 15 Watt)

• **Ultrasonication using a Ultrasonic Liquid Processor** (0.1 g sample, 5 ml extraction solvent, room temp., 30 sec, 10 Watt)

• **Accelerated solvent extraction** (0.1 g sample, 20 ml extraction solvent, 40°C, 1500 psi, pre-heating time: 2 min, static extraction time: 1 min, static cyles: 2, flush volume: 100%, purge time with N_2: 60 s)

The extracts were filtrated and injected into a HPLC-UV used for quantification of selected flavonoids. The identification and verification of the compounds was performed by comparison with available standards and/or exact mass measurements on a high resolution mass spectrometer.

Results

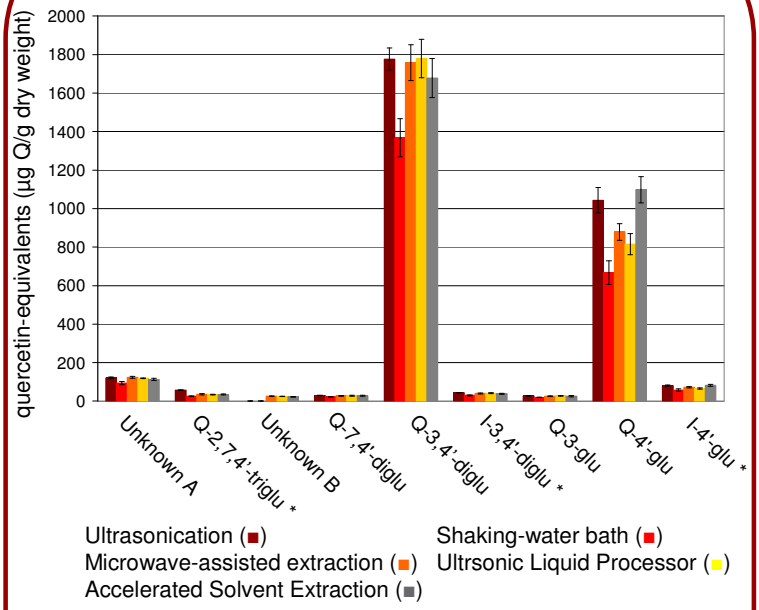

Figure 1. Efficiency (expressed in quercetin equivalents) of the tested extraction methods. Compounds marked with a star (*) are only identified tentatively and need further confirmation. Abbreviations: Q: quercetin, I: isorhamnetin and glu: glucoside.

The two most abundant flavonoids in onions are quercetin-4'-glucoside and quercetin-3,4'-diglucoside as shown by Bonaccorsi *et al.* [5].

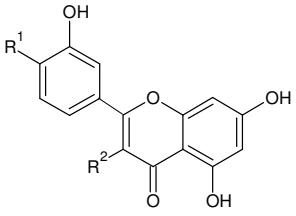

Figure 2. Chemical structure of quercetin-4'-glucoside (R^1: glu, R^2: OH) and quercetin-3,4'-diglucoside (R^1: glu, R^2: glu)

Conclusions

The results of the efficiency show that the conventional water bath extraction method is not the preferred method of application, while the other four show equal efficiencies. The reproducibility of the extraction methods was acceptable and in similar range (RSD: 1-11%).

It is recommended to perform an accelerated solvent extraction due to difficulties experienced with the filtration of the extracts from the ultrasonication and microwave extraction. Furthermore, it is automatic as well as performed in an inert atmosphere and protected from light, which decreases the risk of degradation during sample preparation.

References: [1] Shahidi F. and Ho C.T. (2005) *In* Shahidi F. and Ho C.T. (Eds), American Chemical Society Inc., Washington DC, pp. 1-8; [2] Parr A.J. and Bolwell G.P. (2000). J. Sci. Food Agri. 80: 985-1012; [3] Sellapan S. and Akoh C.C. (2002). J. Agric. Food Chem. 50: 5338-5342; [4] Lin L.-Z. and Harnly J.M. (2007). J. Agric. Food Chem. 55: 1084-1096; [5] Bonaccorsi P. *et al.* (2005). J. Agric. Food Chem. 53: 2733-2740; Picture of onions: downloaded from www.madweb.kvik.dk (110308)