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Original Research Article

Optimization of the extraction of apple monomeric phenolics based on response surface methodology: Comparison of pressurized liquid–solid extraction and manual-liquid extraction

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ABSTRACT

Response surface methodology (RSM) was performed in order to evaluate the optimal extraction conditions for flavan-3-ol monomers ((+)-catechin and (−)-epicatechin), phloridzin, chlorogenic acid, hyperoside, isoquercitrin, quercitrin, ideain and total phenolic content (TPC) from Braeburn freeze-dried apples, using a pressurized liquid solid (PLS) extractor. The aim of this study was to evaluate the effect of four independent extraction factors, including nature of extraction solvent (pure methanol, acetone–water (70:30, v/v)), sample mass (50–550 mg), extraction duration (1–15 min) and number of extraction cycles (1–3), on these polyphenol concentrations (HPLC–DAD analysis). Methanol was the most suitable extraction solvent for the studied phenolic compounds. Optimal conditions varied with phenolic compound and must be chosen as a compromise. For an extraction in favour of chlorogenic acid, hyperoside, quercitrin and ideain, we recommended the following experimental conditions using the pressurized liquid solid extraction (PLE) method: two successive extractions with pure methanol from 50 mg freeze-dried apple-samples for 15 min, using three extraction cycles. In comparison with manual methods, benefits from the increase of polyphenol concentrations, the reduction of extraction time and organic solvent amounts were observed with the PLE method.

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1. Introduction

Consumption of fruits and vegetables has been shown to contribute effectively in the prevention of chronic diseases. Apples are the most consumed fruits in France, commonly eaten during the whole growth period, and thus constitute one of the main sources of phenolic compounds in the French diet (Brat et al., 2006).

Previous studies revealed that apple polyphenols could be classified into hydroxycinnamic acids, flavan-3-ol monomers, flavan-3-ol polymers also called procyanidins, dihydrochalcones, flavonols and anthocyanidins (Fig. 1). Generally, procedures used to extract these compounds from vegetable material are solid–liquid extractions with various organic solvents, such as pure methanol, acetone, ethanol or mixtures of these with water (Coseteng and Lee, 1987; Burda et al., 1990; Escarpa and Gonzáles, 1998; Kondo et al., 2002; Will et al., 2008). In a study on cider apples, Guyot et al. (1998) reported the selectivity of methanol for the extraction of procyanidin oligomers, hydroxycinnamic acid derivatives, dihydrochalcones and catechins, whereas 70% (v/v) acetone solvent selectively extracts procyanidin polymers. Consequently, two successive extractions with each of these solvents would probably extract the majority of apple phenolic compounds both in monomeric and polymeric forms. Varying the percentage of methanol in the extraction solvent applied to 'Golden Delicious' apples, Alonso-Salces et al. (2001) demonstrated that the most powerful extraction solvent would be a hydroalcoholic mixture that would contain about two-thirds methanol. However, slight differences were observed in extracted analytes when pure methanol was used. Although water was a suitable solvent for the extraction of phenolic compounds which are generally hydrosoluble, the yield from water extraction was low, because

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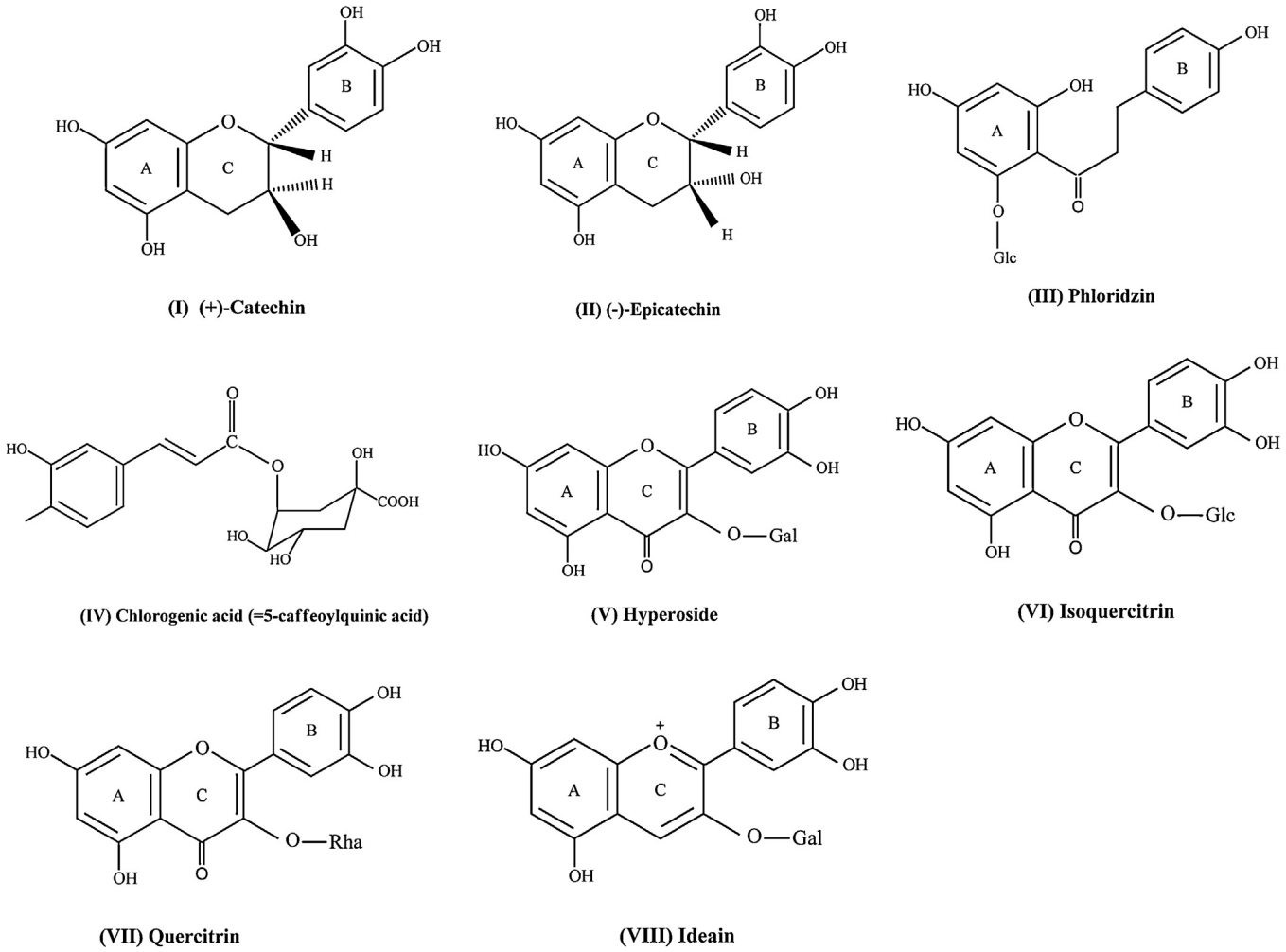

Fig. 1. Chemical structures of studied phenolic compounds. *Legend*: Glc: Glucosyl radical; Gal: Galactosyl radical; Rham: Rhamnosyl radical.

of procyanidins with high polymerisation degree were mainly bind to cell wall polysaccharides by hydrogen and hydrophobic bonds (Renard et al., 2001; Le Bourvellec et al., 2009).

Among the existing extraction procedures, pressurized liquid solid extraction (PLE) seems to be a very promising technique. This method combines elevated pressure with solvents in order to increase the extraction time and efficiency (Carabias-Martínez et al., 2005). The PLE method is interesting because it is adapted to the preservation of compounds combining high pressure and acceptable temperature. Moreover, by reducing extraction time and operator interventions, this automatic extraction leads to quick and reliable determination of sample preparations. In comparison with manual extraction methods, the amounts of organic solvents used for the PLE method and their contact with operators are reduced, better for both the environment and human health. This extraction method has already been tested for the extraction of molecules in apples (Alonso-Salces et al., 2001, 2005), tea leaves and parsley (Jacques et al., 2008; Luthria, 2008) and other food samples (Carabias-Martínez et al., 2005).

In order to improve the extraction of phenolic compounds (catechins, phloretin glycosides, quercetin glycosides and hydroxycinnamic acids) from Golden Delicious apple pulp and peel, Alonso-Salces et al. (2001) tested different experimental conditions, such as proportion of methanol in the extraction solvent (20, 50, 70 and 100%), temperature (40, 60, 80 and 100 °C), static extraction time (5, 10 and 15 min), and pressure (1000, 1250 and 1500 p.s.i.). Selected optimal parameters for phenolic extraction were 40 °C, 1000 p.s.i. and 5 min respectively, using two extraction cycles. As for the choice of the solvent, pure methanol was the most successful. Under the following operating conditions – pressure, 1000 p.s.i.; static duration, 5 min; and extraction solvent, pure methanol – 13 main apple phenolic compounds were quantified with recoveries higher than 80% and good accuracy.

In a further study developed on cider apple cultivars (Alonso-Salces et al., 2005), the PLE method was compared to a solid–liquid extraction (SLE) method, and this latter method had higher recoveries and limits of detection than the previous, except for daily repeatability. Moreover, Luthria (2008) studied other pressurized liquid extraction parameters such as particle size, flush volume and solid-to-solvent ratio. Among the 6 parameters tested, temperature, particle size, and solid-to-solvent ratio showed the most significant impact on extraction yields of phenolic compounds from parsley extracts.

In our paper, new extraction conditions were explored with PLE from Braeburn apple sample mass (50, 300 or 550 mg) by comparing the extraction efficiency of pure methanol, 70% (v/v) acetone and combinations of these two solvents, combined trough experimental designs with different extraction durations (1, 8 or 15 min) and different extraction cycles (1 to 3). Experiments were performed on Braeburn apples because they present high phenolic and antioxidant potential (Imeh and Khokhar, 2002), and the focus was on the extraction of major compounds from these fruits (Markowski and Plocharski, 2006): hydroxycinnamic acids and flavonoids (with the exception of procyanidins).

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