Assessment of the influence of processing conditions on the antioxidant potential of extracts obtained from olive oil industry byproducts

ABSTRACT

The olive oil industry generates an important number of byproducts, such as olive leaves and olive pomace. It has been demonstrated that these vegetable wastes are rich in the same phenolic compounds which are also present in the olive oil. Nevertheless, olive oil byproducts have not yet been exploited on an industrial scale, for example as sources of bioactive compounds. For this purpose, it is necessary to thoroughly study how processing conditions (raw material pretreatment, extraction, etc.) affect the bioactive potential, as well as to explore novel applications in the food industry. Therefore, the main goal of this Thesis was to determine the influence of the main processing stages involved in obtaining natural extracts with high antioxidant potential from byproducts originating in the olive oil industry.

Firstly, the effect of freezing and/or the drying methods applied to olive oil byproducts on the polyphenol content and antioxidant capacity of the extracts subsequently obtained was addressed. For this purpose, two byproducts were considered: olive leaves and olive pomace. On the one hand, olive leaves (fresh, conventionally frozen at -28 °C or frozen in liquid N_2) were hot air dried at two different temperatures, 70 or 120 °C, or freeze dried. On the other hand, olive pomace drying was analyzed at different temperatures (from 50 to 150 °C) and mathematically described by means of diffusion and Weibull models.

Secondly, the feasibility of intensifying the extraction of olive leaf polyphenols by means of a new technology, such as power ultrasound, was approached taking both compositional and kinetic issues into account. For this purpose, the influence of some of the main process parameters (the electric power supplied, emitter surface and temperature) was assessed. The extraction kinetics were mathematically described by Naik's model.

Thirdly, how the processing conditions (drying and extraction) influence the extract's stability was evaluated. Thus, on the one hand, a set of experiments was carried out with extracts obtained from dried olive leaves (hot air dried at 70 and 120 °C, and freeze dried) by means of conventional or ultrasound assisted extraction. These extracts were subjected to *in vitro* digestion. On the other hand, in another set of experiments, fresh, hot air dried at 120 °C and freeze dried olive leaves were used to obtain different kinds of extracts. One part of these extracts was kept in liquid state and another was dehydrated at 120 °C or vacuum dehydrated at 55 °C until a powder product was obtained. All the extracts (liquids and powders) were stored at 4, 25 and 35 °C for 4 weeks.

Finally, the possibility of obtaining a dried vegetable matrix (apple) rich in olive leaf phenolic compounds was explored by addressing the influence of apple pretreatments (blanching and freezing) and drying on the final retention of infused phenolics. Raw and blanched apple cubes were initially air dried (60 °C) or freeze dried. In this last case, the samples were previously frozen by using different freezing methods: conventional (-28 °C), blast freezing (-30 °C) and liquid N_2 (-196 °C). Then, dried apples were impregnated with the phenolic extract. Once the polyphenolic infusion was completed, samples were dried for the final stabilization by means of three different methods: hot air drying at 60 °C with and without ultrasound application and freeze drying.

The antioxidant potential of extracts and the retention of infused polyphenols in apple were evaluated by means of the total phenolic content and antioxidant capacity analysis, as well as the identification and quantification of the main olive leaf polyphenols by HPLC-DAD/MS–MS. Moreover, in apple samples, the polyphenol oxidase and peroxidase activity and microstructure were also analyzed.

The experimental results highlighted that both drying and freezing methods significantly ($p<0.05$) influenced the concentration of the main polyphenols identified in the olive leaf extracts. Thus, drying at the highest temperature tested (120 °C) was the best processing condition in which to obtain extracts with high antioxidant capacity and phenolic content. This effect of the drying conditions was

less relevant when olive pomace was used as the phenolic source, since the antioxidant potential of extracts was only mildly influenced by the drying temperature. However, it was not only the highest temperature tested (150 °C) but also long drying times, leading to the sample overheating, which significantly ($p<0.05$) increased the antioxidant potential of olive pomace extracts. Olive leaves ended up being a more promising source than olive pomace from which to obtain natural extracts rich in phenolic compounds.

Ultrasound application was found to be a relevant, non-thermal way of speeding-up the antioxidant extraction from olive leaves. Thus, by appropriately tuning-up the process variables, the ultrasonic assisted extraction shortened the extraction time from the 24 h needed in conventional extraction to 15 min, without modifying either the extract composition or the antioxidant potential. However, it is important to remark that not all the studied process variables had the same influence on the extraction process. Thus, both the electric power supplied and the emitter surface were relevant factors in the improvement of the extraction performance, whereas the influence of temperature was not clear at the tested values.

As far as extract stability is concerned, the processing conditions used for obtaining the olive leaf extracts did not have a meaningful influence on bioaccessibility. In every case, the phenolic content was significantly reduced ($p<0.05$) by the digestion. Oleuropein and verbascoside practically disappeared at the end of the *in vitro* simulation. Nevertheless, luteolin-7-O-glucoside showed a good stability to *in vitro* digestion (43 % bioaccessibility).

Unlike what was observed during the *in vitro* digestion, the processing conditions did affect the extract stability during storage. Thus, the drying of olive leaves influenced not only the initial composition of the extracts but also their bioactive potential evolution. Regardless of the method used, stabilizing the extracts by means of dehydration only reduced both the antioxidant capacity and the total phenolic content by around 10 %. Moreover, storage conditions (temperature and extract form: liquid or powder) did not show a significant ($p<0.05$) effect on the antioxidant potential of the extracts for 28 days of storage.

Abstract

A stable dried product (apple), rich in natural phenolic compounds (from olive leaves or tea extracts), was obtained by combining drying–impregnation–drying steps. However, it should be considered that the role of fresh apple drying on the retention of infused olive leaf polyphenols was more important than the further drying of the impregnated apple. Thus, the structure and oxidative enzymatic activity of samples obtained after the fresh apple drying played a key role on the retention of phenolic compounds in the final product.

In overall terms, olive leaves can be considered a potential source of natural phenolic compounds. Notwithstanding this, the previous drying and freezing steps applied in the raw material processing are decisive factors in the obtaining of natural extracts with high antioxidant potential. Moreover, enhancing the extraction by applying power ultrasound was stated as a non-thermal way of shortening processing times. The stability of olive polyphenols during storage and *in vitro* digestion was closely related to the individual component considered. Finally, the exploitation of olive leaf extracts as a means of enriching solid foodstuffs requires the use of porous solid matrices free of oxidative enzymes.