Ultrasound as a pretreatment for malaxation: effect on enzymatic activity

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Introduction

Endogenous olive enzymes are directly responsible for the stability and quality of virgin olive oil (VOO). β-glucosidase (β-GLU), poliphenol oxidase (PPO) and peroxidase (POX) are the main enzymes responsible for the phenolic composition of VOO while sensory quality is influenced by the enzymes of the lipoxygenase pathway (lipoxygenase - LOX, hydroperoxide lyase - HPL, alcohol dehydrohenase - ADH), which catalyse the formation of the desirable volatile components of VOO. The activity of these enzymes is highly influenced by the genetic characteristics of the fruit, but can also be modified by the conditions during the production process, especially during malaxation.

Ultrasound has been proposed for virgin olive oil production due to its mechanical and thermal action, which reduces the malaxation time in the production process. The mechanical action of cavitation, which causes the breaking of cell walls still intact after the crushing phase in the crusher, is considered responsible for facilitating the extraction of the oil and its minor components. However, the thermal effect of ultrasound may alter the activity of endogenous enzymes, thus changing the nutritional value and sensory quality of the oil.

The aim of this study was to determine the effect of ultrasound on the endogenous olive enzymes, β-glucosidase and lipoxygenase.

Materials and Methods

Model systems of commercial enzymes (β-GLU and LOX) and their substrates (p-nitropheniyglucopyranoside - PNPG and linolenic fatty acid - ALA) were indirectly sonicated in an ultrasonic bath for 1, 2, 5, 8 and 12 min at 20, 50 and 100% ultrasonic power, after which the treated enzyme/substrate solution was incubated for 30 min at 25 °C to simulate a 30-min malaxation process. The enzymatic activity of both enzymes was determined after sonication and after the simulated malaxation process according to the methods of Romero-Segura et al. (2009) for β-GLU and a method of Pérez et al. (1999) for LOX. The activity of both enzymes was also determined during the 60-min incubation period without ultrasound pretreatment.

Results

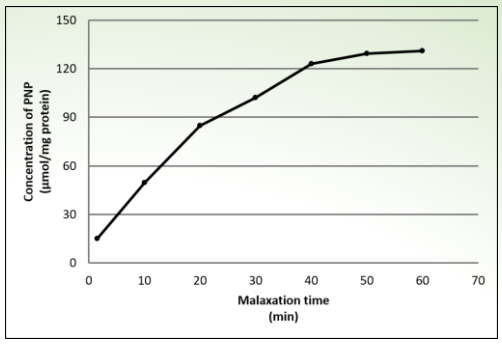

Fig. 1 Activity of β-glucosidase during the 60-min incubation period at 25°C expressed as concentration of p-nitrophenol (PNP).

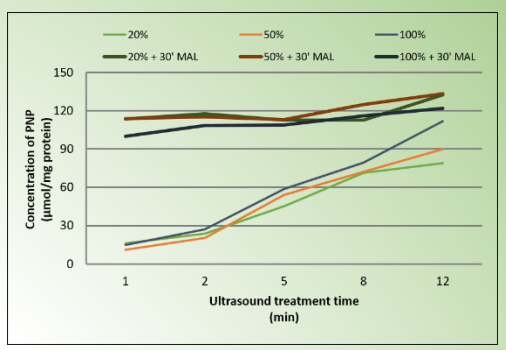

Fig. 2 Effect of ultrasound on β-glucosidase activity. The thin lines show the activity after sonication and the bold lines show the enzyme activity after an additional 30-min incubation period at 25°C (+ 30' MAL), expressed as concentration of p-nitrophenol (PNP).

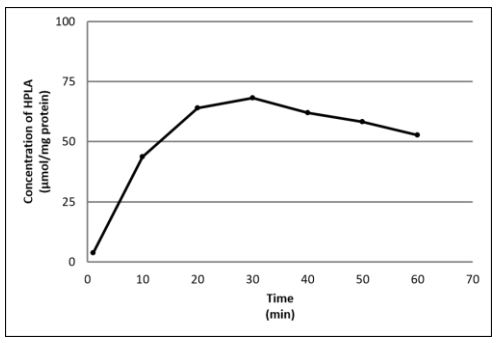

Fig. 3 Activity of lipoxygenase during the 60-min incubation period at 25°C expressed as concentration of hydroperoxy linolenic acid (HPLA).

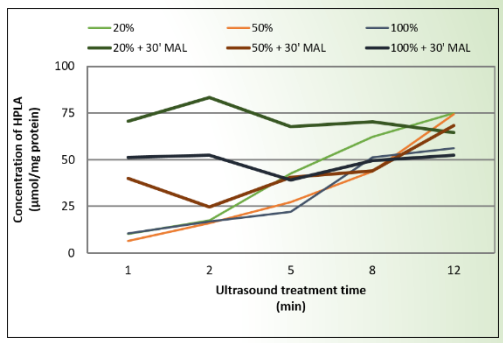

Fig. 2 Effect of ultrasound on lipoxygenase activity. The thin lines show the activity after sonication and the bold lines show the enzyme activity after an additional 30-min incubation period at 25°C (+ 30' MAL), expressed as concentration of hydroperoxy linolenic acid (HPLA).

Conclusions

Both time and power had an effect on enzyme activity.

β-glucosidase

12 minutes of ultrasound increased activity by 2-fold (at 20 and 50% sonication power) and almost 3-times (at 100% power) when compared to standard malaxation process at 25 °C

Additional malaxation of the sonicated samples resulted in a significant increase in enzyme activity.

Samples sonicated for 12 min had the same activity after an additional 30 min incubation at 25 °C as samples incubated for 60 min at 25 °C without the sonication pretreatment.

Lipoxygenase

Activity of the enzyme doubled during the sonication at the lower powers compared to the activity of untreated solutions.

Longer treatment (8 and 12 min) at higher power (50 and 100%) probably resulted in inactivation of the enzyme, as additional incubation of the samples did not change the concentration of hydroperoxides formed.

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