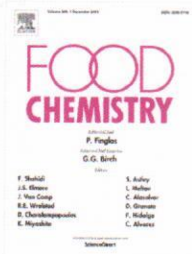

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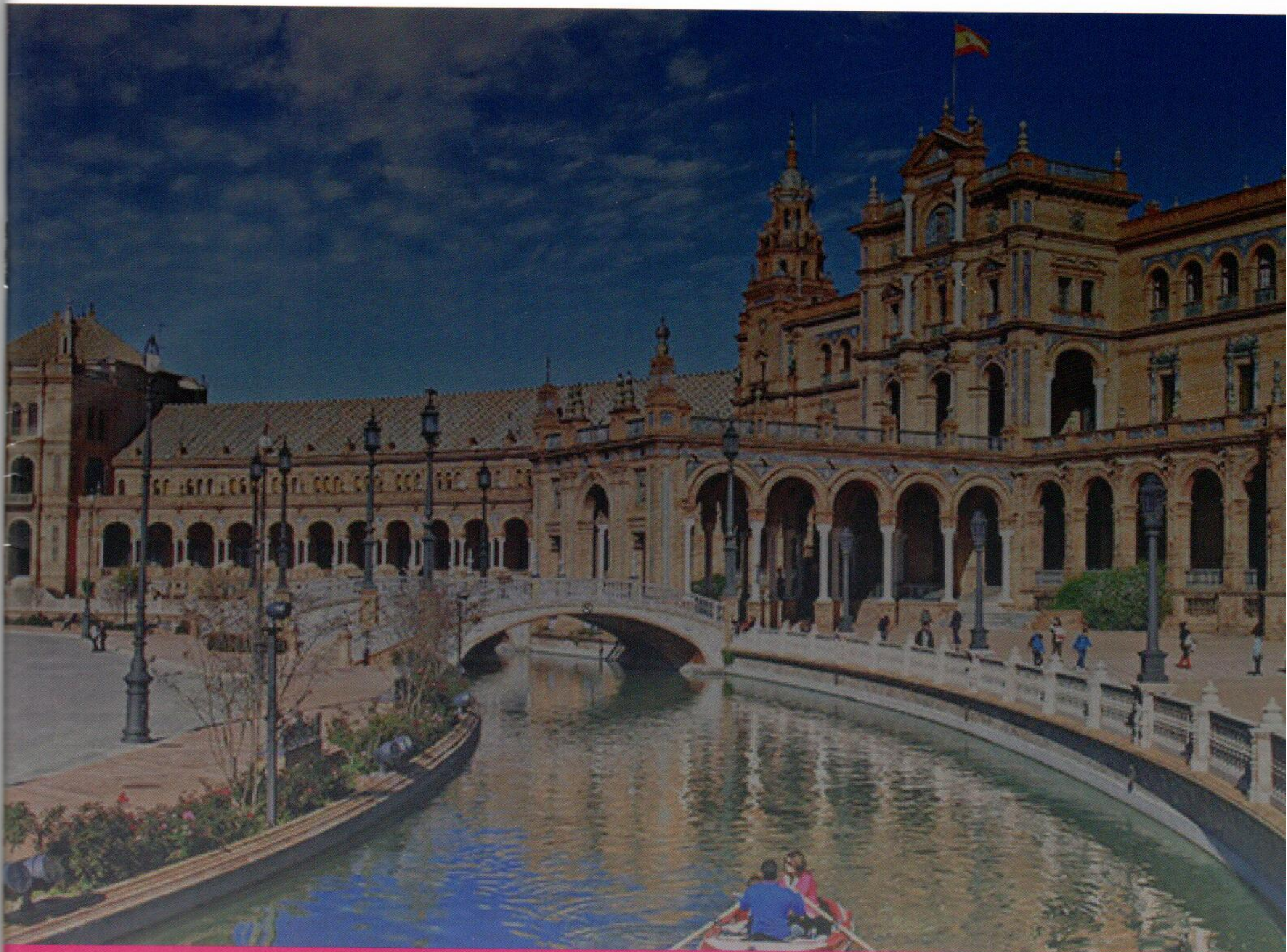

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[P1.2.15] **The functional role of microorganisms during dry fermentation of Australian coffee beans**
D. Mutsla*[1,2], J. Zhao[1], J. Cox[1], *[1]The University of New South Wales, Australia, [2]Institut Teknologi Bandung, Indonesia*

[P1.2.4]

Influence of organic acids on the hydrolysis of oleuropein

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The main objective of any elaboration of table olives is the transformation of the bitter glucoside oleuropein into non-bitter compounds to obtain a palatable product. It can be achieved in a matter of hours by treating the fruit with a dilute NaOH solution. However, consumers are demanding natural and organic olives which are directly placed in brine without any NaOH treatment and the debittering process takes months due to the rapid enzymatic but slow chemical reactions. In this study, the effect of lactic, acetic and citric acid on the chemical hydrolysis of oleuropein was investigated. It was found in model systems that hydrolysis rate of oleuropein was higher in citric and lactic acid solutions than acetic acid, running all the experiments at pH between 3.8-4.1 units. Data were fitted to a first-order kinetic and most of R^2 were greater than 0.95. Temperature also influenced to a large extent the rate of the hydrolysis, the higher this parameter the faster hydrolysis was reached between 10ºC and 30 ºC. These previous results were also confirmed in real systems with olives processed for two seasons at pilot plant scale. Olives of the Manzanilla and Hojiblanca cultivars had a much lower concentration of oleuropein in the pulp after 3-5 months when they were preserved in a lactic acid solution than in brine acidified with acetic acid. These findings will be very useful for the debittering acceleration of natural and organic olives.

Keywords: oleuropein, hydrolysis, acetic acid, lactic acid

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INFLUENCE OF ORGANIC ACIDS ON THE HYDROLYSIS OF OLEUROPEIN

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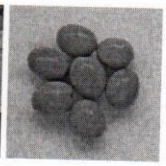
INTRODUCTION

The major phenolic compound in most olive leaves and fruits is oleuropein, a very bitter secoiridoid that makes olive unpalatable. The hydrolysis of this substance is currently made at industrial scale in a matter of hours treating the olives with alkali. However, consumers are demanding natural and organic table olives that which are directly place in brine without any NaOH treatment. In the latter case, the debittering of olives takes many months due to the rapid enzymatic but slow chemical reactions (Ramírez et al., 2016). Hence, the aim of this work was to explore the influence of organic acids (lactic, acetic and citric) on the chemical hydrolysis of oleuropein in vitro and at pilot plant scale.

MATERIALS AND METHODS

In vitro experiments

One liter of 1 mM of oleuropein was splitted in aliquots of 50 mL, and lactic, acetic and citric acids were added to reach concentrations of 0.5, 1, 1.5 and 2 % (w/v). NaOH 4M was employed to adjust the pH to 4.1 units. Finally, the solutions were distributed in 2 mL centrifuge tubes that were incubated at 10, 20 and 30 ºC in a thermostatic chamber for one year. Phenolic compounds were analyzed by HPLC (Ramírez et al., 2016).



Pilot plant experiments

Fruit of the Manzanilla (green and black) an Hojiblanca (green) cultivars were processed as natura olives during the season 2017/2018. Olives were pu in 8.5 L vessels and covered with either 6 % NaCl an 1 % acetic acid (Preservation A) or only 1.5 % lacti acid (Preservation B). Chemical and microbia analyses of olive solutions and physica measurements of olives were made as describe elsewhere (De Castro et al., 2007).

RESULTS AND DISCUSSION

The hydrolysis of oleuropein at pH 4.1 was a very slow reaction that took almost one year to reduce half of its initial content under 0.5% acetic acid solution but it was much faster with lactic and citric acids. Moreover, the data obtained from the oleuropein hydrolysis in vitro fitted to an apparent first-order kinetic (Table 1).

Table 1. Influence of the type of organic acid on the first-order kinetic constant of the oleuropein hydrolysis at pH 4.1.

	10ºC		20ºC		30ºC	
	k (d^{-1})	R^2	k (d^{-1})	R^2	k (d^{-1})	R^2
Lactic 0.5	0.0006	0.40	0.0040	0.98	0.0093	0.99
Lactic 1.0	0.0006	0.30	0.0052	0.86	0.0068	0.92
Lactic 1.5	0.0008	0.94	0.0047	0.96	0.0090	0.99
Lactic 2.0	nd	nd	0.0039	0.93	0.0075	0.99
Acetic 0.5	nd	nd	0.0022	0.94	nd	nd
Acetic 1.0	nd	nd	0.0022	0.96	nd	nd
Acetic 1.5	nd	nd	0.0021	0.93	nd	nd
Acetic 2.0	nd	nd	0.0027	0.94	nd	nd
Citric 0.5	nd	nd	0.0069	0.83	nd	nd
Citric 1.0	nd	nd	0.0065	0.97	nd	nd
Citric 1.5	nd	nd	0.0049	0.94	nd	nd
Citric 2.0	nd	nd	0.0049	0.93	nd	nd

nd, not determined; R^2, regression coefficient

From the experiments with lactic acid, it can be deduced that the hydrolysis rate was almost seven times higher at 20ºC than 10 ºC, and twice as high at 30ºC than 20ºC. It is interesting to note that the range of temperature 20-30 ºC is currently achieved in most Mediterranean table olive factories during processing of natural olives.

As expected from in vitro assays, the concentration of oleuropein was much higher in the pulp of olives preserved for 5 months under current industrial practice (6% NaCl, 1 % acetic acid) than only in 1.5 % lactic acid (Table 2). This effect was observed irrespective of the cultivar studied (Manzanilla and Hojiblanca). Furthermore, the content of hydroxytyrosol was lower in the pulp of olives fermented under acetic acid than lactic acid, which confirmed the higher hydrolysis rate of oleuropein in the presence of lactic than acetic acid.

Table 2. Influence of the type of preservation on the content of oleuropein and hydroxytyrosol in the pulp of olives after 5 months. Preservation A, 6% NaCl and 1 % acetic acid; Preservation B, 1.5 % lactic acid.

	Oleuropein (mg/kg)		Hydroxytyrosol (mg/kg)	
	A	B	A	B
Hojblanca (green)	728±68a	94±20b	628±11b	1002±3a
Manzanilla (green)	3990±181a	2058±28b	885±28b	1282±72a
Manzanilla (black)	1144±159a	362±62b	1293±136a	1561±140a

Row values for each phenolic compound followed by the same letter did not differ at the 5% level according to Duncan´s multiple-range test.

CONCLUSIONS

The results obtained in the study have demonstrated that the type of organic acid has a great influence in vitro and in vivo on the hydrolysis rate of oleuropein. Hence, the use of lactic acid could be an interesting and promising method for the processing of natural and organic olives in the future instead of acetic acid and NaCl.

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