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Enhancement of the nutritional properties of apple pomace by fermentation with autochthonous yeasts

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This paper focuses on the enhancement of the nutritional composition and phenolic compounds of apple pomace by solid state fermentation with autochthonous cider yeasts. Under the tested fermentation conditions (7 days, 25 °C), 3 yeast strains (*S. cerevisiae*, ref: 32; *S. bayanus*, ref: C6; and *H. uvarum*, ref: 62) were able to deplete fermentable sugars. Significant increases in protein (23–49%), fat (17–39%) and dietary fibre (30–41%) were detected in all cases with respect to unfermented apple pomace. The biotransformation increased the content of phenolic compounds, mainly quercetin and phloretin derivatives, as well as that of oleic and linoleic acids. The information derived from this study is relevant to revalorize apple pomace as a nutritive and functional foodstuff allowing the production of enriched foods.

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1. Introduction

Increased environmental awareness in recent decades has led to changes in legislation and new regulations, including different taxes on diverse waste management. Waste from agri-food industries will foreseeably be included in these regulations in the future, resulting in increased costs and a loss of competitiveness in the market. It is therefore necessary to find ways to reuse food industry by-products that contribute to their economic and environmental sustainability.

Apples constitute a major global source of fruit, with an estimated world production in 2014/2015 of 76.5 million tons (USDA Foreign Agricultural Service, 2016). Around 15% of this production is intended for the manufacture of beverages, mainly juice and cider. The waste from this process, known as apple pomace, comprises between 20 and 30% of the initial apple weight and is made up of skin, pulp and seeds.

Different studies have been carried out on the composition and properties of apple pomace, revealing that it is an interesting raw material due to its content in phytochemicals such polyphenols with antioxidant and antiviral activities (Diñeiro García, Suárez Valles & Picinelli Lobo, 2009; Suárez et al, 2010; Álvarez, Melón, Dalton, Nicieza, Roque, Suárez, B & Parra, 2012).

In this respect, far from being classified as a waste, apple pomace should actually be considered a by-product with added value. Several authors have suggested interesting ways in which it may be used. These include using it to obtain malic acid, ethanol, flavours, pectin or nutraceuticals, in the synthesis of different enzymes, or for growing mushrooms (May, 1990; Hang & Woodams, 1995; Berovic & Ostroversnik, 1997; Schieber et al., 2003; Joshi & Devrajan, 2008; Diñeiro García, Suárez Valles & Picinelli Lobo, 2009; Kolodziejczyk, Markowski, Kosmala, Król, & Plocharski, 2007; Rodríguez Madrera & Suárez Valles, 2011; Rodríguez Madrera, Pando Bedriñana & Suárez Valles, 2015). Its industrial use, however, is limited to the production of pectin (Gullón, Falqué, Alonso, & Parajó, 2007).

The aim of this study was to enhance the nutritional composition and functional properties of apple pomace by solid state fermentation (SSF) with autochthonous cider yeasts.

2. Material and methods

2.1. Apple pomace

The apple pomace used throughout this study was obtained from a mixture of cider apples characterized as mildly bitter from the Martínez Sopeña Hermanos S.L. cellar (Villaviciosa, Asturias, Spain). The apple pomace came from a 15,000 kg capacity industrial

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hydraulic press after 36 h of pressing producing a juice yield of 78%. The apple pomace (56.4 kg) was dried in an oven with air circulation at 60 °C for 48 h. The moisture content of the pomace was 75.9%, which yielded 13.6 kg of dry apple pomace, prepared in 250 g batches for subsequent fermentation. The batches were kept protected from the light and moisture in sealed bags until used.

2.2. Growth of strains and inoculum population

Eleven yeast strains were used in this study. Of these, 10 belong to the SERIDA collection of pure cultures (*Saccharomyces cerevisiae*, ref: 3′ and 32; *Saccharomyces bayanus*, ref: C6; *Saccharomycodes ludwigii*, ref: 180; *Hanseniaspora uvarum*, ref: 62 and 283; *Hanseniaspora valbyensis*, ref: 43 and 185; *Metschnikowia pulcherrima*, ref: 302; *Pichia guilliermondii*, ref: 388), while the other was a commercial dry wine yeast (*Saccharomyces cerevisiae*, Levuline CHP, OenoFrance, France).

The active dry yeast strain (Levuline CHP) was inoculated at 40 g/hL. Before use, dry yeast was gently mixed in 10 times its volume of water at 35 °C and left to hydrate for 20 min.

Pure cultures of autochthonous yeasts stored at −80 °C were used as inocula. First, all strains were grown in GPY broth (4% glucose, 0.5% peptone, 0.5% yeast extract) and shaken for 16 h at 30 °C. Five hundred μL of these cultures were streaked onto GPY agar plates and incubated for 48 h at 30 °C. After this time, surface growth from four plates was transferred to 350 mL GPY broth and incubated with stirring for 72 h (*Hanseniaspora* strains) or 24 h (other strains). Finally, the cultures were washed twice with sterile water and adjusted to an OD_{660} of 0.5 (~10^9 cfu/mL). Each fermentation tank was inoculated with 5 mL of these cultures.

2.3. Fermentation

Each batch of 250 g dry pomace was rehydrated in 700 mL of deionised and sterile water, adding the corresponding inoculum to this water. Inoculations were carried out in triplicate, resulting in 33 experimental units. Fermentations took place in 1 L capacity polypropylene food grade containers equipped with an air lock at 25 ± 0.2 °C for 7 days.

After this time, an aliquot was taken for microbiological analysis and determination of sugars and alcoholic strength. The rest was dried (60 °C, 48 h), milled (particle size 0.5 mm) and kept at 20 °C, preserved from the light until the time of chemical analysis.

2.4. Microbiological analysis

2.4.1. Microbiological counting

Samples (10 g) were taken from each container at the beginning and after 7 days of fermentation. These samples were then homogenized with 90 mL Ringer serum for 1 min in a Masticator 0410 (IUL Instrument). Several 1:10 (v/v) dilutions were performed in Ringer's solution and plated for yeast counts in Wallerstein Laboratory Nutrient medium supplemented with 25 mg/L penicillin G potassium salt and 100 mg/L streptomycin sulphate to inhibit bacterial growth. Plates were incubated for colony development at 30 °C for two days.

Table 1
Yeasts counts, residual sugars and alcoholic strength in fermented apple pomaces.

Strain	Yeasts[a] (0 days)	Yeasts[a] (7 days)	Implantation capacity (%)	Residual sugars[b]	Alcoholic strength (% w/w)
S. c. 32	5.0E+07	3.8E+09	100	0.0 ± 0.0	2.3 ± 0.1
S. c. 3′	2.2E+07	6.9E+08	100	5.2 ± 1.4	1,9 ± 0.1
S. b. C6	3.3E+07	4.7E+08	100	0.0 ± 0.0	2.2 ± 0.0
S. l. 180	1.0E+06	3.4E+08	90	2.6 ± 0.8	2.1 ± 0.1
H. u. 62	3.2E+07	1.3E+08	90	0.0 ± 0.0	2.2 ± 0.1
H. u. 283	5.2E+06	1.9E+08	90	1.9 ± 1.8	2.0 ± 0.1
H. v. 185	4.1E+07	4.7E+08	90	3.5 ± 1.6	2.0 ± 0.1
H. v. 43	1.2E+07	2.0E+08	100	6.8 ± 2.0	1.8 ± 0.2
P. g. 388	4.2E+06	1.0E+09	90	16.4 ± 2.5	1.3 ± 0.2
M. p. 302	2.5E+07	3.4E+08	90	18.3 ± 2.7	1.2 ± 0.0
S.c. Levuline	1.2E+07	1.0E+08	100	2.3 ± 0.9	2.1 ± 0.0

[a] cfu/mL.
[b] Sum of sucrose, glucose and fructose (g/kg).

Table 2
Nutritional and functional properties of fermented apple pomaces. Expressed as % dry matter (mean of three experimental units ± standard deviation).

Strain	Crude protein	Total fat	TPC*	Dietary fiber		
				Insoluble	Soluble	Total
Unfermented	3.5	1.8	9.5	35.8	20.1	55.9
S. c. 32	4.9 ± 0.4^{b}	$2.2 \pm 0.2\ ^{a}$	$8.6 \pm 0.7^{a+}$	61.6 ± 0.2^{c}	17.5 ± 0.4^{bcd}	79.1 ± 0.6^{d}
S. c. 3′	5.0 ± 0.1^{b}	$2.5 \pm 0.1\ ^{a}$	8.6 ± 0.3^{a}	61.5 ± 0.5^{c}	16.6 ± 0.4^{a}	78.1 ± 0.9^{cd}
S. b. C6	4.7 ± 0.4^{ab}	$2.2 \pm 0.2\ ^{a}$	$8.7 \pm 0.4^{a+}$	60.5 ± 0.4^{c}	18.3 ± 0.3^{d}	78.9 ± 0.6^{d}
S. l. 180	4.8 ± 0.5^{ab}	$2.3 \pm 0.2\ ^{a}$	$8.7 \pm 0.3^{a+}$	61.4 ± 1.7^{c}	17.2 ± 0.5^{abc}	78.6 ± 1.4^{d}
H. u. 62	4.9 ± 0.3^{b}	$2.4 \pm 0.3\ ^{a}$	$8.9 \pm 0.5^{a+}$	57 ± 0.5^{ab}	17.5 ± 0.7^{bcd}	74.6 ± 0.4^{ab}
H. u. 283	4.9 ± 0.0^{ab}	$2.2 \pm 0.2\ ^{a}$	$8.8 \pm 0.3^{a+}$	61.3 ± 1.2^{c}	17.8 ± 0.4^{bcd}	79.1 ± 0.9^{d}
H. v. 185	5.1 ± 0.5^{b}	$2.4 \pm 0.3\ ^{a}$	8.1 ± 0.5^{a}	$60.7 \pm 1.0b^{c}$	17.7 ± 0.1^{bc}	78.3 ± 0.9^{bcd}
H. v. 43	4.5 ± 0.2^{ab}	$2.1 \pm 0.1\ ^{a}$	8.4 ± 0.6^{a}	61 ± 1.2^{c}	16.9 ± 0.7^{ab}	77.9 ± 1.4^{cd}
P. g. 388	4.3 ± 0.2^{a}	$2.1 \pm 0.1\ ^{a}$	$8.6 \pm 0.6^{a+}$	55.7 ± 0.5^{ab}	17.7 ± 0.8^{cd}	73.4 ± 0.3^{bc}
M. p. 302	4.3 ± 0.3^{a}	$2.1 \pm 0.1\ ^{a}$	$8.7 \pm 0.3^{a+}$	55 ± 0.9^{a}	17.6 ± 0.1^{bcd}	72.5 ± 1.0^{a}
S. c. Levuline	4.9 ± 0.1^{b}	$2.3 \pm 0.2\ ^{a}$	$8.7 \pm 0.2^{a+}$	$59.1 \pm 1.4b^{c}$	17.8 ± 0.4^{bcd}	76.9 ± 1.3^{bcd}

*Total phenolic content, expressed as g tannic acid/kg dry pomace.
Different letters mean significant differences among inoculations ($p < 0.05$).
[+]Difference is not significant compared to the unfermented apple pomace.

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