LWT - Food Science and Technology 79 (2017) 27–33

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

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

Roberto Rodríguez Madrera*, Rosa Pando Bedriñana, Belén Suárez Valles

*Área de Tecnología de los Alimentos, Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA), E-33300 Villaviciosa, Asturias, Spain*

ARTICLE INFO

*Article history:*
Received 5 September 2016
Received in revised form
23 December 2016
Accepted 8 January 2017
Available online 9 January 2017

*Keywords:*
Apple pomace
Dietary fibre
Protein
Antioxidant activity
Fatty acids

ABSTRACT

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.



## 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

* Corresponding author.
*E-mail address:* rrodriguez@serida.org (R. Rodríguez Madrera).

http://dx.doi.org/10.1016/j.lwt.2017.01.021
0023-6438/

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 ± 0.2 $^{a}$ | 8.6 ± 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 ± 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 ± 0.2 $^{a}$ | 8.7 ± 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 ± 0.2 $^{a}$ | 8.7 ± 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 ± 0.3 $^{a}$ | 8.9 ± 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 ± 0.2 $^{a}$ | 8.8 ± 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 ± 0.3 $^{a}$ | 8.1 ± 0.5$^{a}$ | 60.7 ± 1.0b$^{c}$ | 17.7 ± 0.1$^{bc}$ | 78.3 ± 0.9$^{bcd}$ |
| *H. v.* 43 | 4.5 ± 0.2$^{ab}$ | 2.1 ± 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 ± 0.1 $^{a}$ | 8.6 ± 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 ± 0.1 $^{a}$ | 8.7 ± 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 ± 0.2 $^{a}$ | 8.7 ± 0.2$^{a+}$ | 59.1 ± 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.

*2.4.2. Implantation capacity*

The implantation of the strains in the fermentations was evaluated after 7 days of inoculation by analysis of 10 isolated colonies. The number of inoculated strains was expressed as a percentage. The isolates were analysed by mtDNA-RFLP (Querol, Barrio, & Ramón, 1992) for the tanks inoculated with *Saccharomyces* strains and by RAPD (Bujdosó, Egli & Heninck-Kling, 2001) for the experimental units inoculated with non-*Saccharomyces* strains.

*2.5. Chemical analysis*

The following analyses were carried out. Sugar content and alcoholic strength were determined by HPLC analysis (Blanco, Gutiérrez, Mangas, & Noval, 1988) and NIR (Alcoholyzer Wine, Anton Paar, GmgH, Graz, Austria), respectively, subsequent to extraction in water as performed by Pina and Hogg (1999). Crude protein was determined via the Kjeldahl method, total fat was quantified gravimetrically after Soxhlet extraction and dry matter was determined gravimetrically as the residue remaining after drying (AOAC methods, 2005). Soluble, insoluble and total dietary fibres were estimated, under near-physiological conditions, following the enzymatic-gravimetric method described by Goñi, Díaz-Rubio, Pérez-Jiménez, and Saura-Calixto (2009). Fatty acids, previously derivatized to FAME, were analysed by GC-MS according to Rodríguez Ruiz, Belarbi, García Sánchez and López Alonso (1998). Total phenolic content and low molecular weight polyphenols were determined in acetone/water (70/30) extracts by Folin's method and HPLC-DAD, respectively, following Diñeiro García, Suárez Valles and Picinelli Lobo (2009).

*2.6. Treatment of data*

Significant differences in the nutritional components between each inoculation and the unfermented apple pomace were evaluated using a Student's *t*-test at the 95% confidence level. Significant differences in nutritional values among inoculations were detected a Duncan's test at the 95% confidence level. The program used was SPSS version 11.5 (SPSS Inc, Chicago, IL).

## 3. Results and discussion

*3.1. Fermentation and microbiological determinations*

Taking into account the results obtained in previous studies, the apple pomaces were fermented for 7 days at 25 °C in a thermostat cabinet (Rodríguez Madrera, Pando Bedriñana & Suárez Valles, 2015).

Microbiological counts and the implantation capacity of the inoculated yeast species showed that the yeasts under study successfully carried out alcoholic fermentation, finding yeast concentrations above $10^8$ cfu/mL and a degree of implantation equal to or higher than 90% in all cases (Table 1).

The sugar content in unfermented apple pomace (47 g/kg) was depleted by three (*S. c.* 32, *S. c.* C6 and *H. u.* 62) of the 11 yeast strains inoculated within 7 days of the trial, although another 6 species consumed up to 85% of the fermentable sugars in this period (Table 1). These findings are consistent with previous results on the use of autochthonous yeasts for fermenting apple pomace (Rodríguez Madrera, Pando Bedriñana & Suárez Valles, 2015).

In contrast, sugar consumption by the strains *M. p.* 302 and *P. g.* 388 was slower, consuming only 61 and 65% of the sugars, respectively. Hence, these species do not seem appropriate for alcoholic fermentation, at least under the conditions employed in

**Table 3**
Fatty acids in fermented apple pomaces. Expressed as mg/kg dry matter (mean of three experimental units ± standard deviation).

| Strain | Caprylic (C8) | Capric (C10) | Lauric (C12) | Myristic (C14) | Palmitic (C16) | Palmitoleic (C16:1) | Stearic (C18) | Oleic (C18:1) | Linoleic (C18:2) | Arachidic (C20) | Linolenic (C18:3) | Behenic (C22:0) | Lignoceric (C24:0) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Unfermented | 17 | 40 | 31 | 38 | 2605 | 77 | 684 | 3139 | 8051 | 283 | 1166 | 183 | 98 |
| *S. c.* 32 | $32 \pm 3^{d}$ | $57 \pm 4^{d}$ | $52 \pm 4^{ef}$ | $56 \pm 3^{df}$ | $3613 \pm 369^{cd+}$ | $194 \pm 9^{c}$ | $988 \pm 91^{cd+}$ | $3910 \pm 1095^{ab+}$ | $9759 \pm 2270^{ad+}$ | $412 \pm 47^{cd+}$ | $1509 \pm 101^{df}$ | $282 \pm 7^{de}$ | $144 \pm 17^{ab}$ |
| *S. c. 3′* | $32 \pm 2^{d}$ | $68 \pm 3^{e}$ | $56 \pm 3^{ef}$ | $58 \pm 2^{ef}$ | $3847 \pm 317^{d}$ | $190 \pm 27^{c}$ | $1063 \pm 99^{d+}$ | $4675 \pm 562^{bc+}$ | $11.300 \pm 1110^{cd+}$ | $457 \pm 43^{d}$ | $1599 \pm 115^{f}$ | $296 \pm 21^{e+}$ | $177 \pm 23^{c}$ |
| *S. b.*C6 | $33 \pm 4^{d}$ | $66 \pm 3^{e}$ | $57 \pm 2^{ef}$ | $61 \pm 4^{ef}$ | $3272 \pm 152^{ac}$ | $150 \pm 10^{b+}$ | $850 \pm 43^{ab}$ | $3528 \pm 523^{a+}$ | $8958 \pm 1051^{ac+}$ | $352 \pm 29^{ac+}$ | $1387 \pm 25^{bd}$ | $227 \pm 4^{ab}$ | $140 \pm 18^{ab+}$ |
| *S. l.* 180 | $18 \pm 1^{a+}$ | $46 \pm 4^{ab+}$ | $36 \pm 3^{a+}$ | $47 \pm 5^{ab+}$ | $2931 \pm 308^{a+}$ | $138 \pm 26^{b+}$ | $779 \pm 84^{a+}$ | $3414 \pm 853^{a+}$ | $8530 \pm 1523^{ab+}$ | $329 \pm 39^{ab+}$ | $1283 \pm 104^{ab+}$ | $211 \pm 24^{a+}$ | $122 \pm 3^{a}$ |
| *H. u.* 62 | $21 \pm 2^{ab+}$ | $42 \pm 1^{a+}$ | $41 \pm 1^{bc}$ | $52 \pm 2^{be}$ | $3275 \pm 99^{ac}$ | $127 \pm 4^{ab}$ | $874 \pm 27^{ac}$ | $3739 \pm 462^{ab+}$ | $9421 \pm 918^{ac+}$ | $381 \pm 11^{ac}$ | $1394 \pm 6^{bd}$ | $260 \pm 18^{cd}$ | $142 \pm 4^{ab}$ |
| *H. u.* 283 | $22 \pm 1^{ab+}$ | $46 \pm 1^{ac}$ | $47 \pm 1^{d}$ | $57 \pm 3^{df}$ | $3279 \pm 200^{ac+}$ | $142 \pm 10^{b}$ | $849 \pm 60^{ab+}$ | $3593 \pm 332^{a+}$ | $9104 \pm 698^{ac+}$ | $368 \pm 21^{ac}$ | $1395 \pm 102^{bd+}$ | $245 \pm 15^{bc}$ | $145 \pm 6^{ab}$ |
| *H. v.* 185 | $22 \pm 1^{ab+}$ | $49 \pm 2^{bc}$ | $43 \pm 2^{cd}$ | $54 \pm 3^{ce}$ | $3541 \pm 398^{cd+}$ | $131 \pm 12^{b}$ | $984 \pm 114^{cd+}$ | $5166 \pm 45^{c}$ | $10,654 \pm 2615^{bd+}$ | $412 \pm 67^{cd+}$ | $1438 \pm 69^{ce}$ | $265 \pm 31^{ce+}$ | $151 \pm 23^{b+}$ |
| *H. v.* 43 | $22 \pm 2^{bc+}$ | $49 \pm 6^{bc+}$ | $45 \pm 5^{cd+}$ | $55 \pm 1^{ce}$ | $3458 \pm 155^{bd}$ | $133 \pm 10^{b}$ | $959 \pm 47^{bd}$ | $3412 \pm 343^{a+}$ | $9013 \pm 605^{ac+}$ | $394 \pm 21^{c}$ | $1574 \pm 104^{ef}$ | $279 \pm 18^{de}$ | $151 \pm 9^{b}$ |
| *P. g.* 388 | $20 \pm 0^{ab}$ | $51 \pm 4^{bc+}$ | $38 \pm 2^{ab}$ | $46 \pm 2^{a+}$ | $2956 \pm 94^{a}$ | $103 \pm 1^{a}$ | $780 \pm 30^{a+}$ | $3441 \pm 355^{a+}$ | $8027 \pm 569^{a+}$ | $318 \pm 17^{a+}$ | $1231 \pm 20^{a+}$ | $210 \pm 5^{a}$ | $133 \pm 5^{ab}$ |
| *M. p.* 302 | $20 \pm 2^{ab+}$ | $64 \pm 3^{e}$ | $37 \pm 2^{ab+}$ | $50 \pm 2^{ac}$ | $3061 \pm 141^{ab+}$ | $128 \pm 23^{ab+}$ | $796 \pm 33^{a}$ | $3769 \pm 449^{ab+}$ | $8405 \pm 946^{ab+}$ | $324 \pm 20^{ab+}$ | $1307 \pm 9^{ac}$ | $222 \pm 12^{ab}$ | $130 \pm 14^{ab+}$ |
| *S. c.* Levuline | $25 \pm 1^{c}$ | $52 \pm 2^{cd}$ | $48 \pm 2^{de}$ | $52 \pm 4^{ad}$ | $3417 \pm 113^{bd}$ | $135 \pm 4^{b}$ | $943 \pm 26^{bd}$ | $3799 \pm 150^{ab}$ | $9527 \pm 386^{ac}$ | $384 \pm 13^{bc}$ | $1457 \pm 59^{de}$ | $241 \pm 14^{ac}$ | $144 \pm 13^{ab}$ |

$^{+}$: Difference is not significant compared to the unfermented apple pomace.
Difference letters mean significant differences among inoculations ($p < 0.05$).

this study. Furthermore, the ethanol yield (g ethanol/g consumed sugar) of *M. p.* 302 and *P. g.* 388 was the lowest of those obtained. Different factors could explain this result. On the one hand, the inhibitory effect of ethanol on *M. pulcherrima* even at low levels has been previously reported (Clemente-Jiménez, Mingorance-Cazorla, Martínez-Rodríguez, Las Heras-Vázquez, & Rodríguez-Vico, 2004). On the other hand, as both *M. pulcherrima* and *P. guilliermondii* are species with aerobic metabolism (Jolly, Augustyn, & Pretorius, 2006), they produce low levels of alcohol and high volatile ester contents. Furthermore, the existence of Crabtree negative-strains of these species with the capacity of consuming sugars via the respiratory route, thus reducing the alcohol content in fermented products, has recently been reported (Qi, Zhong, & Xia, 2014; Quirós, Rojas, Gonzalez & Morales, 2014).

### 3.2. Chemical composition

For all tested strains, fermentation produced significant changes in the nutritional and functional parameters under study compared to the unfermented apple pomace.

Significant increases in crude protein, total fat and total dietary fibre contents were detected in all fermentations (Table 2).

The highest increase in protein was detected in fermentations using *H. v.* 185 (46%), and the lowest, for the strains *M. p.* 302 and *P. g.* 388 (23%), contributing to enhancing the nutritional value of apple pomace.

Dietary fibre is one of the major functional components in fruits and vegetables. It comprises the edible parts of the plant that are resistant to hydrolysis by digestive enzymes in humans and includes an insoluble fraction (consisting mainly of cellulose, lignin and insoluble hemicellulose) and soluble fibre (pectin, gums, mucilages, and soluble hemicellulose) and other non-starch polysaccharide components with beneficial health properties (Elleuch et al., 2011). A fibre-rich diet is associated with good digestion, lower risk of coronary heart disease, and lower possibility of colorectal cancer, in addition to helping manage obesity and diabetes (Anderson et al., 2009). Several authors have put forward apple pomace as an interesting source of dietary fibre, with advantages over cereals and legumes due to its better soluble (SDF)/insoluble (IDF) ratio (Figuerola, Hurtado, Estévez, Chiffelle, & Asenjo, 2005; Sudha, Baskaran, & Leelavathi, 2007), the absence of phytic acid (Masoodi, Shiarma, & Chauhan, 2002), and the presence of bioactive molecules such as polyphenols with a potent antioxidant capacity (Auclair et al., 2008; Boyer & Liu, 2004).

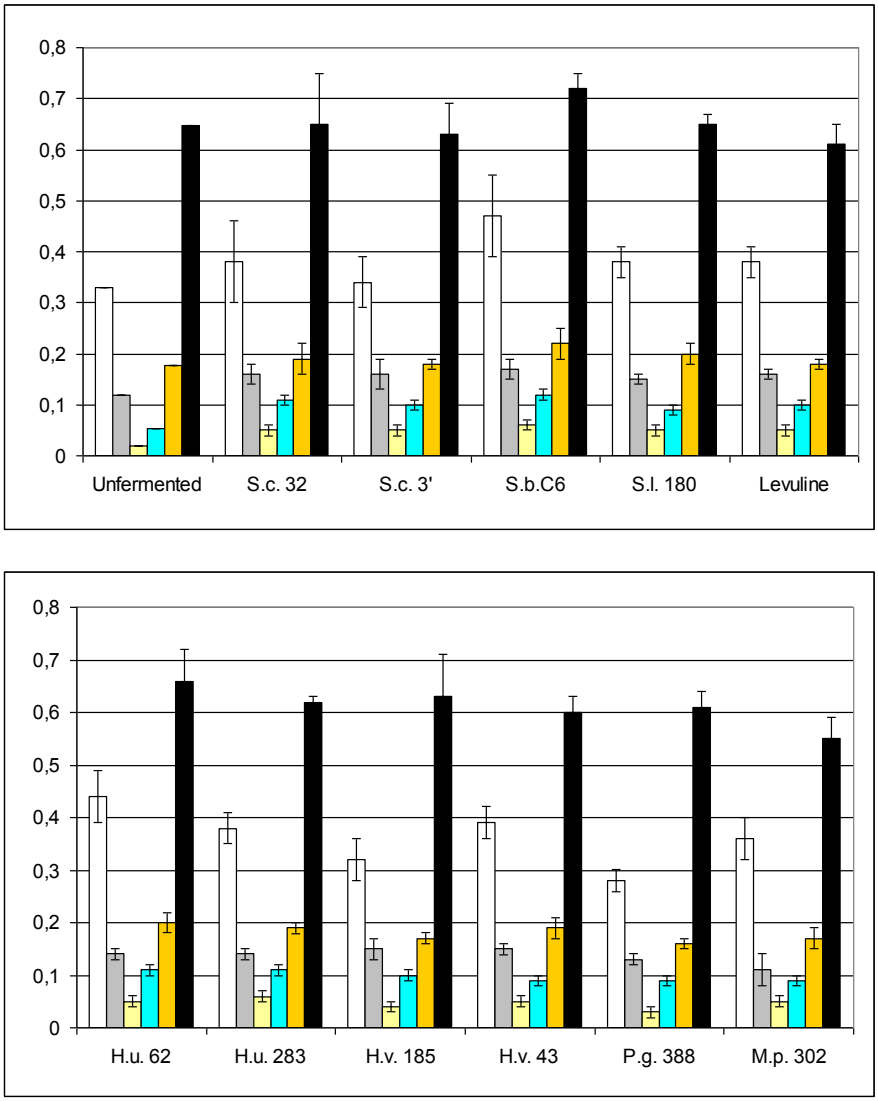


**Fig. 1.** Phenolic acids and dihydrochalcones in apple pomaces (mean of three experimental units and standard deviation). Acids: □ Chlorogenic acid (mg/g), ▒ Protocatechuic acid (mg/g); dihydrochalcones (expressed as mg/g of phloridzine): □ Unknown PG-1, ▒ Unknown PG-2, ▒ phloretin-2′-xyloglucoside, ■ Phloridzine (mg/g).

In this study (Table 2), unfermented apple pomace showed soluble and insoluble dietary fibre contents of 20.1 and 35.8 (% dry matter), respectively, in line with the findings of other authors (Bhushan, Kaliaa, Sharmaa, Singha, & Ahujaa, 2008; Sato et al., 2010). After 7 days of fermentation, significant increases in total dietary fibre levels were detected in all fermentations, ranging from 30% (*M. p.* 302) to 41% (*S. c.* 32 and *H. u.* 283). However, it should be noted that this increase took place in the portion of insoluble fibre, with an average increase of 66%, whereas the levels of soluble fibre decreased by 13% on average. As is well known, pectin is the main component of the soluble fibre dietary content of apples. Hence, the action of distinct pectinase activities reported for *Saccharomyces* and non-*Saccharomyces* yeasts (Romano, Capece, & Jespersen, 2006) could explain this decrease.

It is worth noting that the levels of fibre achieved by fermentation (total dietary fibre higher than 72%) make it possible to produce dietary products by incorporating only 9% fermented apple pomace in their formulation. These products can be labelled as "high fibre" according to Regulations (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006.

A significant increase in total fat (ether extract) content was detected in all inoculations (Table 2), with an average increase of 24% compared to unfermented pomace. As regards fat composition, it was observed in all cases that the fat consisted mainly of fatty acids, which accounted for between 78% (*S. l.* 180) and 93% (*S. c.* 32 and *S. c.* 3′) of the total fat content (Table 3). It is well known that fatty acids form part of the composition of glycolipids, phospholipids and triglycerides, and contributing to flavour as being precursors of volatile compounds and the nutritive components of food (Chistensen, Edelenbos, & Kreutzmann, 2007). Unsaturated acids predominate over saturated acids, with the C18 family accounting for more than 70% of total fatty acid compounds. The major fatty acids were linoleic and oleic acids, belonging to the omega-6 and omega-9 series, respectively, representing more than 60% of the total fraction of the lipidic extract. The saturated/unsaturated fatty acid ratio is around the optimum value of 30/70 suggested by Rogez et al. (2004). Previous reports on the composition of apple and apple seed oil showed linoleic acid to be the most prevalent fatty acid, followed by palmitic and oleic acids (Lu & Foo, 1998; Walia, Rawat, Bhushan, Padwa, & Singh, 2014; Wu et al., 2007). Moreover, these results show that the total fatty acid content in fermented apple pomace is higher than in unfermented pomace,

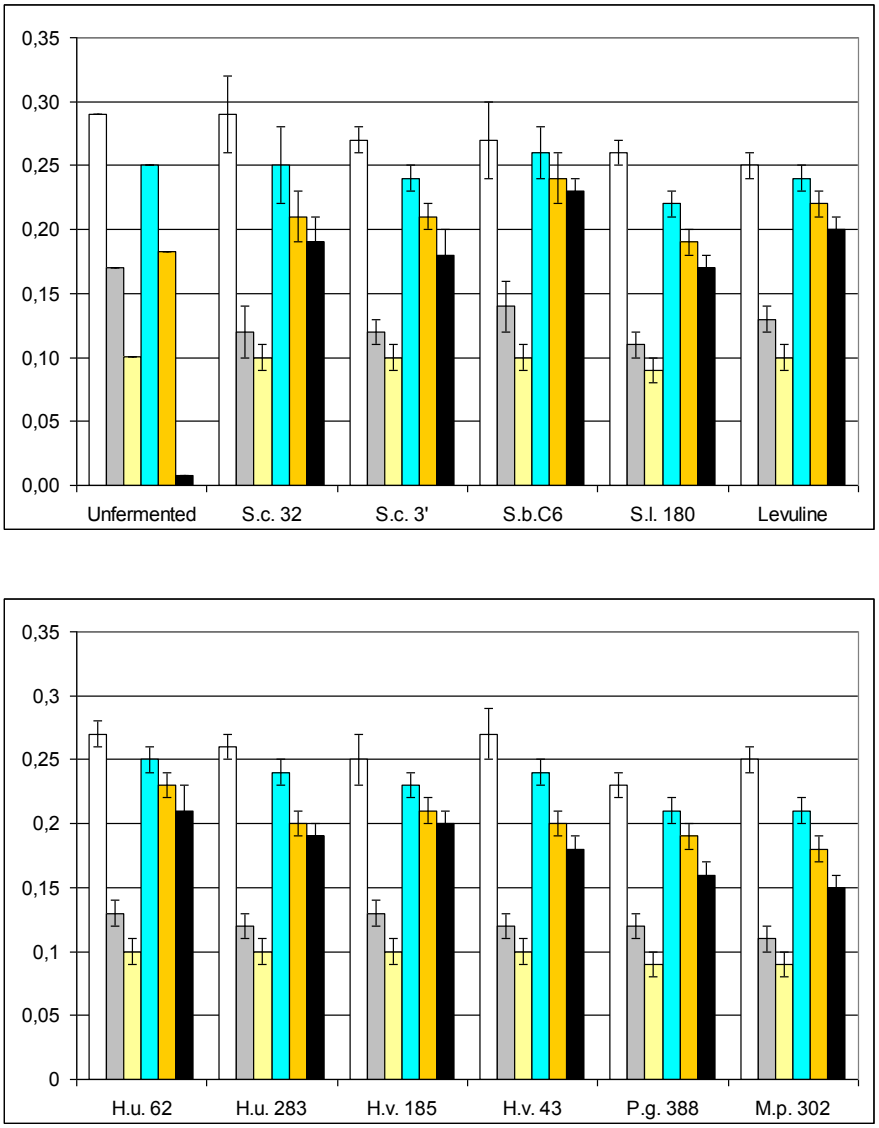


**Fig. 2.** Flavonols in apple pomaces (mean of three experimental units and standard deviation). Quercetin glycosides (expressed as mg/g quercitrin): □ Hyperine, ▒ Rutin + isoquercitrin, □ Reynoutrin, ■ Avicularin, ■ Quercitrin; ■ Quercetin (mg/g).

ranging from 6 to 45%, which means it can be considered a potential source of edible oil. The profiles found for the medium-chain saturated fatty acids (octanoic, decanoic and dodecanoic acids) for strains *S.c.* 3′, *H.u.* 283, *H.v.* 43, and Levuline were in line with those detected for these strains in a previous study after 7 days of fermentation (Rodríguez Madrera, Pando Bedriñana & Suárez Valles, 2015).

No significant differences in total phenolic content were detected among the 11 inoculated strains (Table 2). However, the levels of this parameter when fermented with *S. c.* 3′, *H.v.* 185, and *H.v.* 43 were lower than that of the unfermented apple pomace. In any case, total phenolic contents were higher than those reported in different fresh fruits and vegetables (Cieslik, Greda, & Adamus, 2006; Dodevska, Sobajic, & Djordjevic, 2015), some of which, such as blueberries, spinach and broccoli, are usually labelled as important sources of phenolic compounds. From a functional point of view, polyphenolic compounds from apples show antioxidant activity, a free-radical scavenging capacity, and anticarcinogenic properties, besides helping prevent coronary heart disease (Chinnici, Bendini, Gaiani, & Riponi, 2004; Dupont, Bennet, Mellon, & Williamson, 2002; Hertog, Hollman, & Van de Putte, 1993; Leontowicz et al., 2002).

Among the low molecular weight polyphenols, the presence of phenolic acids, dihydrochalcones and flavonol derivatives is worth noting (Figs. 1 and 2). None of the major phenolic acids analysed (chlorogenic and protocatechuic acids) showed significant differences compared to their concentrations in the unfermented apple pomace (Fig. 1). Phloridzin was the major dihydrochalcone detected in all the samples, with average contents of around 0.6 mg/g, followed by phloretin-2'xylogluside (Fig. 1). Two unknown dihydrochalcones, PG-1 and PG-2, increased significantly during fermentation in all cases, while phloridzin increased significantly in the fermentation using *S. b.* C6 and decreased significantly when using *M. p.* 302. The unidentified dihydrochalcones are probably the result of partial oxidation of phloridzin and phloretin-2'xylogluside (Lu & Foo, 1998). In several studies carried out in recent years, phloridzin and phloretin derivatives have been shown to lower blood glucose, provide a potential treatment for type 2 diabetes mellitus, and act as effective antioxidants (Fromm, Loos, Bayha, Carle, & Kammerer, 2013; Rana, Gupta, Rana, & Bhushan, 2015). Five quercetin glycosides were detected (Fig. 2), hyperin and avicularin representing more than 40% of total flavonols (Lu & Foo, 1997; Diñeiro García, Suárez Valles & Picinelli Lobo, 2009; Suárez et al, 2010). As regards the flavonol family, the significant decrease in hyperine, rutine and isoquercitrin and significant increase in the aglycone quercetin are worth noting. This finding is valuable because the antioxidant functionality of the glycosylated form is reduced compared to the aglycone (Vattem & Shetty, 2002).

The information is relevant for revalorizing fermented apple pomace for producing adapted products enriched with proteins, fat, dietary fibre and phenolic compounds for target consumers (diabetic, obese and celiac individuals). Likewise, the enhancement in the nutritional and functional properties of apple pomace enables the production of foodstuffs using a residual fruit base tailored to a particular segment of the elderly population.

Studies on the formulation of bakery products in collaboration with a company (ADPAN S.L, El Berrón, Asturias, Spain) and sensory tests with consumers and trained panels should be carried out to optimize the use of fermented apple pomace (Dos Santos, Bastianello Campagnol, da Cruz, Galvao et al, 2015; Dos Santos, Campagnol, da Cruz, Morgano, et al, 2015).

## 4. Conclusions

Fermentation of apple pomace using autochthonous cider yeasts can increase its protein, fat and dietary fibre contents, providing benefits from a nutritional point of view. Also worth noting is the high percentage of fatty acids and phenolic compounds thus obtained, mainly quercetin and phloretin derivatives, which undoubtedly contribute to enhancing the nutritional and bioactive properties of apple pomace. The proper selection of yeast and fermentation conditions permits the consumption of sugars present in the raw material, which could broaden the scope of application of apple pomace in the food sector. Adding small amounts of fermented apple pomace to processed foodstuffs could be an appropriate way of obtaining products labelled as “high fibre”.

## Acknowledgments

Financial support for this work was managed by the National Institute of Research and Agro-Food Technology (INIA) and co-financed with ERDF and ESF funds (project RTA2013-00110-00-00). The authors wish to thank ADPAN S.L. and Martínez Sopeña Hermanos S.L. for their collaboration in this study.

## References

Álvarez, A., Melón, S., Dalton, K. P., Nicieza, I., Roque, A., Suárez, B., et al. (2012). Apple pomace, a by-product from the Asturias cider industry, inhibits herpes simplex virus types 1 and 2 in vitro replication: Study of its mechanisms of action. *Journal of Medicinal Food, 15*, 581–587.

Anderson, J. W., Baird, P., Davis, R. H., Ferreri, S., Knudtson, M., Koraym, A., et al. (2009). Health benefits of dietary fiber. *Nutrition Reviews, 67*, 188–205.

Auclair, S., Silberberg, M., Gueux, E., Morand, C., Mazur, A., Milenkovic, D., et al. (2008). Apple polyphenols and fibers attenuate atherosclerosis in apolipoprotein E-deficient mice. *Journal of Agricultural and Food Chemistry, 56*, 5558–5563.

Berovic, M., & Ostroversnik, H. (1997). Production of *Aspergillus niger* pectolyctic enzymes by solid state bioprocessing of apple pomace. *Journal of Biotechnology, 53*, 47–53.

Bhushan, S., Kaliaa, K., Sharmaa, M., Singha, B., & Ahujaa, P. S. (2008). Processing of apple pomace for bioactive molecules. *Critical Reviews in Biotechnology, 28*(4), 285–296.

Blanco, D., Gutiérrez, M. D., Mangas, J. J., & Noval, A. (1988). Determination of sugars and alcohols in apple juice and cider by HPLC. *Chromatographia, 25*, 701–706.

Boyer, J., & Liu, R. H. (2004). Apple phytochemicals and their health benefits. *Nutrition Journal, 3*. http://dx.doi.org/10.1186/1475-2891-3-5.

Bujdosó, G., Egli, C. M., & Henick-Kling, T. (2001). Characterization of *Hanseniaspora (Kloeckera)* strains isolated in finger lakes wineries using physiological and molecular techniques. *Food Technology and Biotechnology, 39*, 83–91.

Chinnici, F., Bendini, A., Gaiani, A., & Riponi, C. (2004). Radical scavenging activities of peels and pulps from cv. Golden Delicious apples as related to their phenolic composition. *Journal of Agricultural and Food Chemistry, 52*, 4684–4689.

Chistensen, L. P., Edelenbos, M., & Kreutzmann, S. (2007). Fruits and vegetables of moderate climate. In R. G. Berger (Ed.), *Flavours and fragrances* (pp. 135–188). Berlin: Springer.

Cieslik, E., Greda, A., & Adamus, V. (2006). Contents of polyphenols in fruit and vegetables. *Food Chemistry, 94*, 135–142.

Clemente-Jiménez, J. M., Mingorance-Cazorla, L., Martínez-Rodríguez, S., Las Heras-Vázquez, F., & Rodríguez-Vico, F. (2004). Molecular characterization and oenological properties of wine yeasts isolated during spontaneous fermentation of six varieties of grape must. *Food Microbiology, 21*, 149–155.

Diñeiro García, Y., Suárez Valles, B., & Picinelli Lobo, A. (2009). Phenolic and antioxidant composition of by-products from the cider industry. *Food Chemistry, 117*, 731–738.

Dodevska, M., Sobajic, S., & Djordjevic, B. (2015). Fiber and polyphenols of selected fruits, nuts and green leafy vegetables used in Serbian diet. *Journal of the Serbian Chemical Society, 80*, 21–33.

Dos Santos, B. A., Bastianello Campagnol, P. C., da Cruz, A. G., Galvao, M. T. E. L., Monteiro, R. A., Wagner, R., et al. (2015). Check all that apply and free listing to describe the sensory characteristics of low sodium dry fermented sausages: Comparison with trained panel. *Food Research International, 76*, 725–734.

Dos Santos, B. A., Campagnol, P. C. B., da Cruz, A. G., Morgano, M. A., Wagner, R., & Pollonio, M. A. R. (2015). Is there a potential consumer market for low-sodium fermented sausages? *Journal of Food Science, 80*, S1093–S1099.

Dupont, M. S., Bennet, R. N., Mellon, F. A., & Williamson, G. (2002). Polyphenols from alcoholic apple cider are absorbed, metabolized and excreted by humans. *Journal of Nutrition, 132*, 172–175.

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C., & Attia, H. (2011). Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food Chemistry, 124*, 411–421.

Figuerola, F., Hurtado, M. L., Estévez, A. M., Chiffelle, I., & Asenjo, F. (2005). Fibre

concentrates from apple pomace and citrus peel as potential fibre sources for food enrichment. *Food Chemistry, 91*, 395–401.
Fromm, M., Loos, H. M., Bayha, S., Carle, R., & Kammerer, D. R. (2013). Recovery and characterisation of coloured phenolic preparations from apple seeds. *Food Chemistry, 136*, 1277–1287.
Goñi, I., Díaz-Rubio, M. E., Pérez-Jiménez, J., & Saura-Calixto, F. (2009). Towards an update methodology for measurement of dietary fiber, including associated polyphenols, in food and beverages. *Food Research International, 42*, 840–846.
Gullón, B., Falqué, E., Alonso, J. L., & Parajó, J. C. (2007). Evaluation of apple pomace as a raw material for alternative applications in food industries. *Food Technology and Biotechnology, 45*, 426–433.
Hang, Y. D., & Woodams, E. E. (1995). β–Frucofuranosidase production by *Aspergillus niger* species from apple pomace. *Lebensmittel-Wissenschaft & Technologie, 28*, 340–342.
Hertog, M. G. L., Hollman, P. C. H., & Van de Putte, B. (1993). Content of potentially anticarcinogenic flavonoids of tea infusions, wines and fruit juices. *Journal of Agricultural and Food Chemistry, 41*, 1242–1246.
Jolly, N. P., Augustyn, O. P. H., & Pretorius, I. S. (2006). The role and use of non-*Saccharomyces* yeasts in wine production. *S Afr J Enol Vitic, 27*(1), 8–14.
Joshi, V. K., & Devrajan, A. (2008). Ethanol recovery from solid state fermented apple pomace and evaluation of physico-chemical characteristics of the residue. *Natural Product Radiance, 7*, 127–132.
Kolodziejczyk, K., Markowski, J., Kosmala, M., Król, B., & Plocharski, W. (2007). Apple pomace as a potential resource of nutraceutical products. *Polish Journal Of Food And Nutrition Sciences, 57*, 291–295.
Leontowicz, H., Gorinstein, S., Lojej, A., Leontowicz, M., Ciz, M., Soliva-Fortuny, R., et al. (2002). Comparative content of some bioactive compounds in apples, peaches and pears and their influence on lipids and antioxidant capacity in rats. *The Journal of Nutritional Biochemistry, 13*, 603–610.
Lu, Y., & Foo, Y. (1997). Identification and quantification of major polyphenols in apple pomace. *Food Chemistry, 59*, 187–194.
Lu, Y., & Foo, Y. (1998). Composition of some chemical components of apple seed. *Food Chemistry, 61*, 29–33.
Masoodi, F. A., Shiarma, B., & Chauhan, G. S. (2002). Use of apple pomace as a source of dietary fibre in cakes. *Plant Foods for Human Nutrition, 57*, 121, 1218.
May, C. D. (1990). Industrial pectins: Sources, production and applications. *Carbohydrate Polymers, 12*, 79–99.
Pina, C. G., & Hogg, T. A. (1999). Microbial and chemical changes during the spontaneous ensilage of grape pomace. *Journal of Applied Microbiology, 86*(5), 777–784.
Qi, K., Zhong, J.-J., & Xia, X.-X. (2014). Triggering respirofermentative metabolism in the crabtree-negative yeast *Pichia guilliermondii* by disrupting the CAT8 gene. *Applied and Environmental Microbiology, 80*, 3879–3887.
Querol, A., Barrio, E., & Ramón, D. A. (1992). Comparative study of different methods of yeast strain characterization. *Systematic and Applied Microbiology, 15*, 439–446.
Quirós, M., Rojas, V., González, R., & Morales, P. (2014). Selection of non-*Saccharomyces* yeast strains for reducing alcohol levels in wine by sugar respiration. *International Journal of Food Microbiology, 181*, 85–91.
Rana, S., Gupta, S., Rana, A., & Bhushan, S. (2015). Functional properties, phenolic constituents and antioxidant potential of industrial apple pomace for utilization as active food ingredient. *Food Science and Human Wellness, 4*, 180–187.
Rodríguez Madrera, R., Pando Bedriñana, R., & Suárez Valles, B. (2015). Production and characterization of aroma compounds from apple pomace by solid-state fermentation with selected yeasts. *Lebensmittel-Wissenschaft und-Technologie, 64*(2), 1342–1353.
Rodríguez Madrera, R., & Suárez Valles, B. (2011). Determination of aroma compounds in apple pomace by stir bar sorptive extraction and gas chromatography-mass spectrometry (SBSE-GC-MS). *Journal of Food Science, 76*, C1326–C1334.
Rodríguez Ruiz, J., Belarbi, E. H., García Sánchez, J. L., & López Alonso, D. (1998). Rapid simultaneous lipid extraction and transesterification for fatty acid analysis. *Biotechnology Techniques, 12*, 689–691.
Rogez, H., Buxant, R., Mignolet, E., Souza, J. N. S., Silva, E. M., & Larondelle, Y. (2004). Chemical composition of the pulp of three typical Amazonian fruits: Araçá-boi (*Eugenia stipitata*), bacuri (*Platonia insignis*), and cupuaçu (*Theobroma grandiflorum*). *European Food Research and Technology, 218*, 380–384.
Romano, P., Capece, A., & Jespersen, L. (2006). Taxonomic and ecological diversity of food and beverage yeasts. In A. Querol, & G. Fleet (Eds.), *Yeasts in food and beverages* (pp. 13–53). Berlin: Springer.
Sato, M. F., Vieira, R. G., Zardo, D. M., Falcão, L. D., Nogueira, A., & Wosiacki, G. (2010). Apple pomace from eleven cultivars: An approach to identify sources of bioactive compounds. *Acta Scientiarum Agronomy, 32*, 1.
Schieber, A., Hilt, P., Streker, P., Endreb, H. U., Rentschler, C., & Carle, R. (2003). A new process for the combined recovery of pectin and phenolic compounds from apple pomace. *Innovative Food Science and Emerging Technologies, 4*, 99–107.
Suárez, B., Álvarez, A., Diñeiro García, Y., del Barrio, G., Picinelli Lobo, A., & Parra, F. (2010). Phenolic profiles, antioxidant activity and in vitro antiviral properties of apple pomace. *Food Chemistry, 75*, 339–342.
Sudha, M. L., Baskaran, V., & Leelavathi, K. (2007). Apple pomace as a source of dietary fiber and polyphenols and its effect on the rheological characteristics and cake making. *Food Chemistry, 104*, 686–692.
USDA Foreign Agricultural Service. (2016) [Accessed 08.November.16] http://apps.fas.usda.gov/psdonline/circulars/fruit.pdf.
Vattem, D. A., & Shetty, K. (2002). Solid-state production of phenolic antioxidants from cranberry pomace by Rhizopus oligosporus. *Food Biotechnology, 16*, 189–210.
Walia, M., Rawat, K., Bhushan, S., Padwa, Y., & Singh, B. (2014). Fatty acid composition, physicochemical properties, antioxidant and cytotoxic activity of apple seed oil obtained from apple pomace. *Journal of the Science of Food and Agriculture, 94*, 929–934.
Wu, J., Gao, H., Zhao, L., Chen, F., Wang, Z., & Hu, X. (2007). Chemical compositional characterization of some apple cultivars. *Food Chemistry, 103*, 88–93.