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Fermentative Production of Volatile Metabolites Using *Brettanomyces bruxellensis* from Fruit and Vegetable By-Products

Melodie A. Lindsay [1], Ninna Granucci [1], David R. Greenwood [1] and Silas G. Villas-Boas [1,2,*]

[1] School of Biological Sciences, University of Auckland, Auckland 1010, New Zealand
[2] Luxembourg Institute of Science and Technology, L-4422 Belvaux, Luxembourg
* Correspondence: silas.villas-boas@list.lu

Abstract: Natural sources of flavour and aroma compounds are highly sought by the modern consumer; however, traditional sources are often low-yielding, and global supply is often outstripped by consumer demand. Fermentation is a favourable route by which natural flavours and fragrances can be produced. A non-*Saccharomyces* yeast, *Brettanomyces bruxellensis*, was investigated for its fermentative potential for the production of flavour and aroma metabolites from juice industry by-products: apple pomace, carrot pomace, and orange pomace. Submerged solid-substrate fermentations were carried out using sterile by-products without nutrient supplementation. Gas chromatography–mass spectrometry was used for volatile metabolite profiling of fermented substrates. One compound of interest, phenylethyl alcohol (rose fragrance), was extracted and quantified using GC-MS at a yield of 2.68 g/kg wet carrot pomace weight. This represents a novel, natural production strategy for phenylethyl alcohol compared to the traditional steam distillation of *Rosa domascus* sp. petals.

Keywords: *Dekkera (Brettanomyces) bruxellensis*; natural flavours; natural fragrances; SPME headspace; phenylethyl alcohol; volatilome; 2-phenylethan-1-ol

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1. Introduction

Strong consumer pressure on traditional supply chains of natural fragrances and flavours has created a demand for alternative, sustainable sources of natural compounds. While many natural fragrances and flavours are historically sourced from herbs, spices, flowers, plants, and animals, global demand often outstrips supply. A combination of adverse weather events, political events, and unpredictable harvests makes the prices of these chemicals unstable and volatile. As synthetic alternatives have been rejected by the consumer base, there is an opportunity to fulfil demand with alternative natural sources of these compounds.

For centuries, microorganisms have been used to produce and enhance flavours in foods and beverages such as wine, cheese, chocolate, and beer [1,2]. There is a recent resurgence in the use of microorganisms to produce consumer goods, including flavour and fragrance compounds [3]. However, rather than using traditional sugar feedstocks or fermented food products to add flavour, as in wine fermentation or cheese making, there is an increasing awareness of underutilised agro-industrial by-products. While some are diverted to animal feed, many by-products are still directed to the landfill, often at a cost to the producer. This makes these fruit and vegetable pomaces a promising candidate for cheap, sustainable alternative feedstock. These by-products have been investigated to produce or extract high-value compounds, including dietary supplements, polyphenols, pectins, enzymes, antioxidants, and antimicrobials [4–6]. As many by-products are chemically complex and often have a high content of fermentable sugars, fibre, proteins, vitamins, and minerals, they are an ideal candidate as a feedstock for the production of natural flavour and aroma compounds [7].

Previously, the authors noted a pleasant aroma from a by-product fermentation with *Brettanomyces bruxellensis* Kufferath and von Laer (the anamorph of *Dekkera bruxellensis*), which is a yeast species commonly used in beer fermentation [8]. This yeast species also has a high potential for producing flavour and aroma compounds—both desirable and undesirable—depending on the fermentation substrate. *B. bruxellensis* was the first patented microorganism for its role in beer fermentations in the early 1920s [8] and is the main fermenting organism in fruity, Lambic-style Belgian beers. It also has a key role in the production of sourdough, olives, and the fermented beverage kombucha [9].

On the other hand, *B. bruxellensis* has been identified as a spoilage yeast associated with the specific aroma compounds 2-ethyltetrahydropyridine and 2-acetyltetrahydropyridine in spoiled wine [8,10]. These compounds give off a characteristic medicinal, metallic, spiced, and/or "mousy" off-odour in wine [10,11]. *B. bruxellensis* is well-adapted to survive the harsh environment of the wine-making process, which includes low pH; high osmotic stress; high acidity; nitrogen limitation; and high ethanol content. These properties are often encountered in industrial-scale fermentations and are characteristic of the agro-industrial by-products in the current screening experiment [12,13]. Therefore, in this study, we used *B. bruxellensis* to ferment three different common agro-industrial by-products of the juice industry to investigate the yeast fermentative potential for the production of flavour and aroma metabolites.

2. Materials and Methods

2.1. By-Products from New Zealand Industries

The three fruit and vegetable by-products were sourced from local beverage industries in New Zealand: Frucor Beverages Limited (apple pomace), Simply Squeezed Limited (orange pomace), and R2D International Limited (carrot pomace). The fresh pomaces were stored at −20 °C and protected from light and air in food-grade, resealable freezer bags. In preparation for fermentation, the substrates were thawed at 4 °C overnight. Comprehensive chemical analyses of these substrates were carried out as part of a PhD project and are reported by Granucci, 2018 [14].

2.2. Yeast Strain

The yeast *Brettanomyces bruxellensis* CCT 3469 (GenBank accession OP216733), previously erroneously identified as *Candida utilis* [15], was re-identified based on its 5.8S rDNA sequence using the internal transcribed spacer (ITS) sequencing with forward and reverse primers (Supplementary Table S1), following a previously described methodology [16]. This yeast strain was obtained from the Tropical Culture Collection (CCT) of the Fundação André Tosello in Brazil.

2.3. Submerged Solid-Substrate and Liquid Fermentations

Three submerged solid-substrate fermentations were carried out using apple, orange, and carrot pomaces based on previously established methods [14]. Briefly, *B. bruxellensis* was maintained on MYPD plates containing malt extract (3 g/L), yeast extract (3 g/L), peptone (6 g/L), glucose (10 g/L), and agar (15 g/L) at 30 °C. Preinoculum was prepared by inoculating 200 mL of MYPD broth with a single colony of *B. bruxellensis* in 1 L Erlenmeyer flasks fitted with cotton stoppers. The flasks were left at 30 °C with agitation at 200 rpm until the end of the exponential growth phase was reached (OD_{600} of approximately 10). Aliquots (50 mL) of preinoculum were aseptically transferred to 50 mL falcon tubes and centrifuged (3000× *g*, 5 min) to obtain a yeast cell pellet. Residual media were discarded, and the cell pellet was washed with sterile saline (0.9 % NaCl) twice.

For the fermentations, sterile wet substrate (150 g) was weighed into 1 L Erlenmeyer flasks along with sterile, distilled water (350 mL); sterilised by autoclavation (121 °C, 20 min); and inoculated with the preinoculum resuspended in 10 mL saline, as described above. These were incubated at 30 °C under continuous agitation (200 rpm). Five replicates for each substrate and corresponding sterile, negative controls were incubated together.

Liquid shake-flask fermentations in triplicate using 200 mL synthetic culture medium (glucose 40 g/L, KH_2PO_4 15 g/L, $MgSO_4\_7H_2O$ 0.5 g/L, YNB 0.2 g/L, thiamine 3 mg/L, pH 6.5), supplemented and non-supplemented with L-phenylalanine (7 g/L), were carried out to assess the production efficiency of phenylethyl alcohol by *B. bruxellensis* in synthetic media.

Samples (10 mL) were taken at the beginning of the fermentations (Time = 0 h) and every 24 h for 72 h. Yeast growth was estimated based on either cell numbers counted using a Neubauer chamber at each time point (submerged substrate fermentation) or on dry weight (synthetic media). Contamination was checked using light microscopy, and reducing soluble sugars were quantified using the DNS method, as outlined by Miller [17].

After 72 h, samples (20 mL) were aseptically transferred into glass vials and flushed with nitrogen to prevent oxidation of volatile compounds. The vials were sealed and rapidly frozen at −20 °C until sample preparation for GC-MS analysis.

2.4. Headspace Solid-Phase Microextraction (HS-SPME) Coupled with Gas Chromatography–Mass Spectrometry (GC-MS)

Stored solid-substrate fermentation samples were first thawed at 4 °C overnight. Samples (2 mL) were transferred into amber SPME headspace vials (20 mL) and immediately fitted with silicone/PTFE septum caps. All analyses were carried out using a 1 cm 50/30 µm DVB/CAR/PDMS (Supelco, St. Louis, MO, USA) fibre, as previously described [18]. Preincubation was carried out for 10 min at 60 °C and was immediately followed by a 10 min extraction. The temperature selected for the incubation was 60 °C, as this yielded more volatiles than a 30 °C incubation. Desorption was performed for 1 min at 250 °C, operating in splitless mode. Following desorption, the fibre was held for a further 5 min at 250 °C to clean the fibre in preparation for the next sample.

Volatile compounds entrapped by the fibre were analysed by GC-MS by desorbing into a Shimadzu QP2010 Plus GC-MS system via a CTC Analytics Combi PAL autosampler. The column used was a fused silica HP-5MS, 30 m long, 250 µm (internal diameter), 0.25 µm stationary phase (Agilent). The inlet temperature was fixed at 250 °C. Ultra-high-purity-grade helium was used as carrier gas at a column flow rate of 1 mL/min. The GC oven temperature was set initially at 35 °C, which was immediately ramped at a rate of 10 °C/min to 80 °C and then ramped again at a rate of 2 °C/min to 160 °C, followed by a final ramp of 10 °C/min to 260 °C. The transfer line to the mass selective detector was maintained at 250 °C, the ion source at 200 °C, and the quadrupole at 200 °C. The ion source was operated in electron impact ionisation mode at 70 eV. Compounds were detected using mass spectra acquired in scan mode in the range of 33 to 400 m/z.

2.5. SPME-GCMS Data Processing

Volatile compounds detected by GC-MS were identified by their retention times and mass spectra using a suite of libraries, software, and in-house R packages. First, a subset of the NIST2014 library was created using Enhanced Chemstation (Agilent, Santa Clara, CA, USA). Then, AMDIS (Automated Mass Spectral Deconvolution and Identification system) was used to deconvolute and assign identifications to each peak. Automated peak integration was performed using an in-house R package, "MassOmics", and manual corrections were made to remove false identifications and retention time corrections. Compounds that increased in abundance or were produced *de novo* and had a match factor of over 90% to the NIST2014 library spectra were considered for further characterisation experiments. Unless confirmed by comparison to authentic standards, compounds were considered tentatively identified (putative identification).

The fold change increase in volatile metabolites after fermentation was determined by dividing the analyte peak areas by the total ion count (TIC) of the corresponding data file. The peak abundances relative to the TIC were then divided by the raw peak area of hexamethyl cyclotrisiloxane (external standard). The resulting data were log-transformed and Pareto-scaled to facilitate comparisons. Statistical significance was determined by

Student's *t*-test, which was defined as p-value < 0.05 after adjustment by false discovery rate (FDR).

2.6. Identification Confirmation and Quantification of Volatile Compounds

A compound of interest that had a rose-scented aroma was identified from the carrot pomace fermentation. From the initial screening, this compound was tentatively identified as phenylethyl alcohol. To confirm its identity, homogenised sample (2 mL) was aliquoted along with the internal standard 12-bromo-dodecanol (5 µL, 10 mM solution) and added sodium chloride (~100 mg). Tetrahydrofuran (1 mL) was then added and vigorously mixed for 2 min using a vortex mixer. Samples were sonicated for 30 min before centrifuging (3000× *g*, 10 min). The organic phase was aspirated into GC-MS vials and kept at 4 °C in a cooling tray pending GC-MS analysis. The same procedure was followed to quantify the content of phenylethyl alcohol in spent synthetic culture media samples.

2.7. Gas Chromatography–Mass Spectrometry Conditions

Extracted volatile compounds were quantified using a GC-7890 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled with an MSD-5975 mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The column and conditions used were as described above.

3. Results

3.1. Substrate Composition

The overall composition of the three agro-industrial by-products used in this work is summarised in Table 1 and was obtained from Grannuci, 2018 [14]. All three pomaces presented high moisture contents (>75%) and acidic pHs. The reducing sugar content varied from 11% in carrot pomace to over 30% in apple pomace, whereas plant fibre content ranged from about 30% in apple pomace to more than 50% in carrot pomace (Table 1). All pomaces presented a very low nitrogen content (all below 1.2%), with apple pomace having the lowest and carrot the highest (Table 1). The mineral content of the pomaces measured as ash made up to less than 7% DM, with apple presenting the lowest values and carrot the highest (Table 1). Therefore, these three pomaces can be broadly described as having a high carbon content (fibre + sugars) and low nitrogen content (>50:1). It is important to take into consideration, however, that sucrose, a non-reducing sugar, is the major sugar found in carrot pomace, which was not quantified here.

Table 1. Overall chemical composition of the three different fruit and vegetable by-products used for fermentation.

Components (%DM)	Apple Pomace	Orange Pomace	Carrot Pomace
Moisture	79.0	78.0	87.5
Nitrogen	0.6	0.7	1.1
Reducing sugars	37.3	33.0	10.9
Fibre	39.5	34.3	53.4
Ash	1.5	3.2	6.2
pH (units)	3.4	4.0	5.1

3.2. Fermentation Performance

Yeast cell numbers associated with each substrate were used as an indirect assessment of fermentation performance. However, examination by bright-field light microscopy showed that *B. bruxellensis* is tightly associated with the substrate particles during fermentation, and absolute cell numbers were, therefore, skewed by this. Nonetheless, after 72 h, all three fermentations appeared to reach the stationary growth phase (Figure 1).

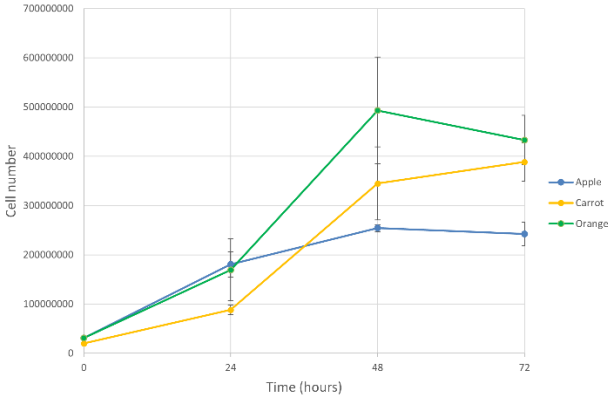

Figure 1. Average numbers of *Brettanomyces bruxellensis* cells counted during submerged solid-substrate fermentation of apple, carrot, and orange pomaces. Error bars show the standard deviation between replicates (n = 5).

During the 72 h fermentation, the concentration of soluble reducing sugars dropped markedly in carrot and orange pomace ferments (Figure 2). Despite apple pomace fermentation showing a significant decrease in reducing sugar content over 72 h, most of the initial available reducing sugars were not consumed by the yeast during that period (Figure 2).

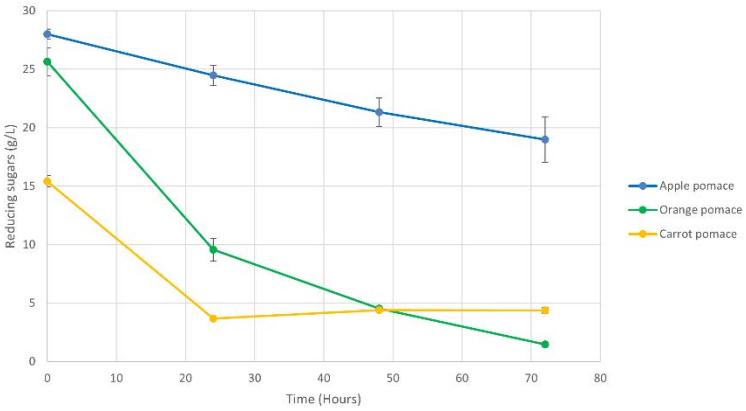

Figure 2. Average soluble reducing sugar concentrations in apple, carrot, and orange pomaces during fermentation with *Brettanomyces bruxellensis*. Error bars show standard deviation between replicates (n = 5).

3.3. Profiling of Volatile Compounds

Over 800 different volatile metabolites were detected following the fermentation of apple pomace (189 compounds), carrot pomace (327 compounds), and orange pomace (332 compounds). However, only about half of the metabolites increased in abundance or were produced *de novo* after fermentation: apple pomace (106 compounds), carrot pomace (160 compounds), and orange pomace (142 compounds) (Supplementary File S1). Most metabolites produced *de novo* during fermentation were observed in carrot pomace substrate (Table 2).

Commercially relevant compounds produced in the three fermentations that had match factors of over 90% to the NIST2014 MS library are short-listed in Table 2. A particularly important fragrance compound, phenylethyl alcohol, had its identity confirmed based on its retention time and mass spectrum compared with a pure chemical standard. Phenylethyl alcohol (rose fragrance) was only detected in the headspace of fermented carrot pomace. The yield was determined from the wet weight of carrot pomace used for the fermentation and was scaled up to reflect how much of the metabolite would theoretically be produced from one kilogram of fermented substrate or one litre of synthetic media (Table 3).

Table 2. Organoleptic qualities of potentially industrially relevant volatile metabolites produced during submerged fermentations of three fruit and vegetable by-products using *Brettanomyces bruxellensis*.

Compound	Substrate	Fold Change *	Descriptor
2-Methylbutan-1-ol	Carrot	887.8	Black truffle
3-Methylbutan-1-ol-	Carrot	797.9	Fusel, banana
	Orange	230.8	
Acetic acid	Apple	6.1	Vinegar
Phenylmethanol	Apple	5.0	Sweet, floral, balsamic
2-Methylbutanoic acid	Carrot	392.2	Acidic, cheesy
	Orange	543.5	
Ethyl 2-methylbutanoate	Orange	256.6	Fruity, green apple
Ethyl decanoate	Apple	6.4	Waxy, sweet, fruity
Methyl decanoate	Carrot	2623.4	Oily, wine-like
Ethyl dodecanoate	Orange	68.8	Sweet, waxy
Ethyl acetate	Apple	5.9	Ethereal, fruity
Ethyl heptanoate	Orange	103.8	Pineapple
Methyl heptanoate	Carrot	370.4	Orris, currant
Ethyl hexanoate	Orange	87.3	Sweet, fruity, pineapple
Methyl hexanoate	Carrot	206.7	Pineapple
3-Methylbutyl acetate	Apple	5.2	Banana, fruity
3-Methylbutyl hexanoate	Carrot	107.6	Apple, pineapple
Methyl pentanoate	Carrot	899.5	Fruity
Ethyl nonanoate	Apple	55.7	Waxy, fruity
Methyl nonanoate	Carrot	351.8	Wine, coconut
(3S)-7-Hydroxy-3,7-dimethyloctanal	Apple	15.4	Floral, lily, green
3-Methylbutyl decanoate	Orange	57.4	Waxy, banana
4-Ethyl-2-methoxyphenol	Apple	91.1	Smoky, spicy, clove, wine taint
	Orange	8358.9	
2-Phenylethyl acetate	Apple	22.4	Floral, honey
	Orange	313.0	
2-Phenylethan-1-ol (phenylethyl alcohol)	Carrot	325.7	Floral, rose
2-Methylpropanoic acid	Orange	295.9	Acidic, sour, cheesy

* Compounds with significantly increased abundance ($p < 0.05$) after fermentation when compared with non-fermented substrates.

Table 3. Phenylethyl alcohol: value, estimated world consumption, and yield on carrot pomace and synthetic media fermented with *Brettanomyces bruxellensis*.

Compound	Value (kg)	Annual Consumption (Tonnes)	Substrate	Yield (g/kg) [3]
Phenylethyl alcohol	USD 500.00 [1]	14,000 [2]	Carrot pomace	2.68 ± 0.280
			Synthetic medium	0.01 ± 0.004
			Synthetic medium plus L-phenylalanine	1.27 ± 0.080

[1] Value per kilogram of pure, natural fragrance chemical (personal communication Jeffrey Buco of Excellentia International). Prices quoted in US dollars and corrected as of September 2017. [2] Estimate for fragrance and flavour industries [19]. [3] Yield as average amount of compound produced per kilogram of wet-weight carrot pomace ($n = 9$) or one litre of liquid synthetic media ($n = 3$).

4. Discussion

Over 400 volatile compounds were produced by submerged solid-substrate fermentation of three different fruit and vegetable pomaces using *B. bruxellensis*. Many of the most interesting compounds from an organoleptic point of view were esters and had fruity, floral, or sweet notes. All three fermentations utilised the free reducing sugars available in the pomaces (Figure 1). However, none were completely exhausted after three days of fermentation. While the cell counting data appeared to support the fermentation reaching the stationary phase, it is possible that yeast growth stopped due to the limitation of some

other nutrients, such as nitrogen and phosphorus, or even due to growth inhibition by toxic metabolic products. For example, in wine-making, this is a common phenomenon when there is inadequate yeast available nitrogen [20,21]. This leads to the cessation of cell division due to insufficient nitrogen, even in the presence of high titres of fermentable sugars. Therefore, the fermentation duration could potentially be extended with nutrient supplementation. Nonetheless, the most abundant simple sugar in carrot pomace is sucrose [14], which is not a reducing sugar. Thus, the total amounts of sugar, consumed and residual, are likely to be underestimated. Moreover, fruit and vegetable pomaces are rich in polysaccharides that can be enzymatically digested by *B. bruxellensis*, such as pectin [15]. Therefore, the contribution of sugars derived from polysaccharide hydrolysis to the residual amount of soluble reducing sugars at the end of the fermentation cannot be ruled out.

One of the most commercially significant metabolites of interest is phenylethyl alcohol, an aroma compound that is commonly found in household cleaning products, deodorisers, soaps, cosmetics, and perfumes as a fragrance ingredient and preservative. Additionally, over one tonne of this compound is consumed in food and beverage products annually. Most are cheaply produced through chemical synthesis using benzene, styrene, or toluene. However, phenylethyl alcohol produced by this method cannot be labelled as "natural".

Natural phenylethyl alcohol is traditionally extracted from rose petals in the form of rose essential oil (~60% phenylethyl alcohol) with an extremely low yield (0.03–0.04%) [22,23]. Several biotechnological attempts have also been made to produce phenylethyl alcohol from the amino acid L-phenylalanine, with titres of over 2 g/L achieved in culture media [23,24]. Although titres achieved through other biotechnological methods are similar to that described herein, carrot pomace may be a competitive option as a feedstock given the high price of L-phenylalanine and other protein and sugar feedstocks. As the yield was 2.7 g/kg wet weight pomace without optimisation, there is significant potential for improvement. Very few other agro-industry by-products have been investigated for the production of phenylethyl alcohol on their own (without nutrient supplementation), and these studies reported considerably lower phenylethyl alcohol yields than the one reported herein [25]. For instance, Conde-Báez and colleagues used *Kluyveromyces marxianus* to ferment different cheese industry wastes without supplementation, and the maximum yield of phenylethyl alcohol obtained was approximately 1 g/L [26]. By comparison, rose petals (the current natural source of phenylethyl alcohol) cost thousands of dollars per tonne, and the extraction process is both energy and labour-intensive [22]. Carrot pomace is an extremely cheap and very abundant substrate, with over 20 thousand tonnes produced annually by the New Zealand juicing industry alone (Farmex New Zealand, personal communication). Accordingly, further optimisation of the carrot pomace fermentation could identify a promising alternative source of natural phenylethyl alcohol.

Compared with solid-state fermentation, submerged solid-substrate fermentation is already much more industrially advanced, and there is existing scale-up technology, e.g., bioreactors and large tank fermenters, including continuous-, batch-, and fed-batch systems [27]. An optimised, submerged solid-substrate fermentation using carrot pomace could be both industrially feasible and financially competitive if yield can be improved.

Other yeasts are already known to produce phenylethyl alcohol, but this is the first report of *B. bruxellensis* being used to produce phenylethyl alcohol for use as a fragrance and flavour additive from the agro-industrial waste product, carrot pomace. This is also the first time carrot pomace has been assessed as a fermentation substrate for the production of phenylethyl alcohol. While this is a preliminary exploratory study, further fermentation optimisation experiments should not only investigate nutrient supplementation of substrate but also the potential for other yeast species to produce phenylethyl alcohol from carrot pomace to determine if yields can be improved by changing the fermenting organism and/or providing nutrient supplementation.

Several other yeasts have been investigated for their ability to produce phenylethyl alcohol during fermentation, such as *Kluyveromyves marxianus*, *Saccharomyces cerevisiae*,

Yarrowia lipolytica, and *Candida albicans* [23,28,29]. These yeasts can produce phenylethyl alcohol by two different methods. The first method is by bioconversion of phenylalanine into phenylethyl alcohol via the Ehrlich pathway. The second method is *de novo* through glycolysis and the Shikimate pathway [30–33]. The yeast *Y. lipolytica* produced a similar titre of pheneylethyl alcohol as *B. bruxellensis* when grown in the same synthetic medium supplemented with phenylalanine (1.98 g/L over 96 h) [29], whereas other yeasts grown on different synthetic media were able to produce between 1.17 and 5.08 g/L phenylethyl alcohol [25]. The optimisation of the fermentation process to increase phenylethyl alcohol yields is an important factor which has not been examined in this current study. Therefore, *B. bruxellensis*' potential for phenylethyl alcohol production is comparable to other yeast species.

Our strain of *B. bruxellensis* produced phenylethyl alcohol more efficiently in a synthetic medium supplemented with phenylalanine (~1.3 g/L in 48 h) (Table 3). Interestingly, very little of this aroma compound was produced in the same medium without phenylalanine supplementation (Table 3). This suggests the preferable route of phenylethyl alcohol biosynthesis by *B. bruxellensis* is probably via phenylalanine bioconversion. Indeed, the carrot pomace used in this fermentation presented approximately 10.8 g of free phenylalanine per kilogram of wet substrate [14], which could explain the high titre of phenylethyl alcohol obtained from its fermentation—making it a genuinely phenylalanine-rich cheap substrate. However, the level of free phenylalanine in orange pomace was even higher (~22.7 g/kg) [14], and no significant production of phenylethyl alcohol was observed (Table 2). Nonetheless, the fermentation of orange pomace by *B. bruxellensis* produced a high level of 2-phenylethyl acetate (floral, honey scent), a closely related compound also derived from phenylalanine (Table 2). Perhaps the lower pH of the orange pomace favoured the production of phenylethyl acetate instead of phenylethyl alcohol. Indeed, a higher pH seems to favour phenylethyl alcohol production by other yeasts [34]. The level of free phenylalanine in the apple pomace, on the other hand, was comparatively low (~3.2 g/kg) [14], which explains the low levels of phenylethyl acetate and the lack of significant phenylethyl alcohol production after its fermentation (Table 2).

5. Conclusions

Three different agro-industrial by-products were screened: apple, orange, and carrot pomace, fermented by *B. bruxellensis,* a non-conventional yeast, in a submerged solid-substrate fermentation. Over 800 volatile metabolites were putatively identified in the headspace of fermented and unfermented pomaces, and over 400 volatile metabolites were produced *de novo* or increased in abundance as a result of the fermentation. One compound of industrial interest, phenylethyl alcohol, was quantified, revealing a yield of 2.7 g/kg (wet weight) of carrot pomace without any supplementation. Carrot pomace seems to be a naturally good source of the amino acid phenylalanine, the main precursor for phenylethyl alcohol.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation8090457/s1, Spreadsheet File S1: Volatile metabolites produced *de novo* or increased in abundance after fermentation. Table S1. Forward and reverse primers used in fungal 5.8S rDNA amplification.

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