

Article

Application of Microwaves as an Advanced Technique for the Development of Sherry Vinegar Macerated with Pineapple

Samuel Párraga, Enrique Durán-Guerrero *  and Remedios Castro 

Analytical Chemistry Department, Faculty of Sciences-IVAGRO, University of Cadiz, Agrifood Campus of International Excellence (ceiA3), Polígono Río San Pedro s/n, Puerto Real, 11510 Cadiz, Spain; samuelpr2806@hotmail.es (S.P.); remedios.castro@uca.es (R.C.)

* Correspondence: enrique.duranguerrero@uca.es; Tel.: +34-956-016456

Abstract: This work proposes the elaboration of a product based on the maceration of Sherry Vinegar together with pineapple in order to extract certain volatile compounds that can be found in pineapples, giving a final product with new organoleptic properties and increased polyphenolic content. Maceration trials were carried out with the application of microwaves and ultrasound, which reduced the maceration time from the traditional three-day solid-liquid maceration to just a few minutes. In addition, through maceration, the total polyphenol index increased significantly with respect to unmacerated vinegar, and the volatile profile of the vinegars was significantly modified. The tasting scores placed the pineapple macerated vinegar sample obtained by traditional maceration in the first place with respect to pineapple aroma; however, the microwave extraction samples were better rated in terms of overall quality. It can be concluded that the application of extracting energies, such as microwaves, can be a viable alternative for the production of sherry vinegar macerated with pineapple.

Keywords: sherry vinegar; pineapple; microwaves; ultrasound; volatile compounds; sensory analysis



Citation: Párraga, S.; Durán-Guerrero, E.; Castro, R. Application of Microwaves as an Advanced Technique for the Development of Sherry Vinegar Macerated with Pineapple. *Beverages* **2021**, *7*, 18. <https://doi.org/10.3390/beverages7020018>

Academic Editors: Giacomo Luigi Petretto, Alberto Mannu and Quan V. Vuong

Received: 15 February 2021

Accepted: 7 April 2021

Published: 8 April 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

There is a current trend in the food and beverage market to diversify categories, with the aim of obtaining new products with properties that contribute to preventing diseases and that have new sensory qualities in comparison with conventional products [1]. Consumers are increasingly seeking to incorporate foods and beverages into their diet that, in addition to being different from the conventional ones, include attractive health promoting compounds [2].

In recent years, pineapple (the fruit of *Ananas comosus*, a plant grown in tropical areas) has become one of the most popular exotic fruits in demand. Because of its attractive sweet taste and tropical aromas, pineapple is widely consumed both as a fresh and a canned product, as well as in processed juices or as a seasoning ingredient in exotic dishes [3]. Pineapple varieties are numerous, but only a few of the main types are widely sold to the general public, such is the case of Perolera or Cayena Lisa. It is rich in caloric and nutritional content, thanks to, on the one hand, its high concentration of carbohydrates, and, on the other, minerals (calcium, potassium, phosphorus, magnesium, sodium, copper and iodine) and vitamins, especially ascorbic acid, niacin, thiamine and riboflavin [4].

Of all the oenological products, wine vinegar in general, and sherry vinegar in particular, is a traditional one, with an extensive market that is loyal to its taste attributes, but at the same time, holds great potential for the creation of new products. Until now, a number of studies have been carried out aiming at the elaboration of new products derived from sherry vinegar, such as beverages obtained from fruit juice and vinegar [1], vinegars with fiber content [5] or vinegars macerated with different fruits [2,6]. The results have been very satisfactory in terms of product enrichment with compounds that are beneficial to health as well as having new and appreciated sensory qualities.

When it comes to the development of new products using fruits, volatile compounds play an important role. On the one hand, aroma is diversified by maceration with fruits thanks to the addition of new volatile compounds derived from these fruits and, on the other hand, it is improved due to the harmonization of other volatile compounds that are already present in the traditional product. Both factors have a positive impact on the final quality of the new products [6]. Moreover, in today's food industry, it is increasingly common to use new energies in extraction procedures. These new energies improve yields and reduce the time required for such operations, while being environmentally friendly.

Two of the most commonly used energies are microwaves and ultrasound. These extracting energies have already been widely used for the extraction of compounds in the production of oenological products such as wines [7–10] or brandies [11,12]. In the field of vinegar production, they have been successfully applied to accelerate wood aging [13], or for maceration with citrus fruits [14,15], with valuable organoleptic results and a very considerable shortening of the time required for production.

Therefore, this study aimed to optimize a microwave technique for the maceration of pineapple with Sherry vinegar, and compare it with an ultrasonic technique and traditional maceration in the extraction of phenolics and volatile compounds from Sherry vinegar and pineapple.

2. Materials and Methods

2.1. Raw Material

Three different pineapple presentations were used: fresh peeled pineapple (90% approx. water content), freeze-dried pineapple (1% approx. water content) and dehydrated pineapple (10% approx. water content). All of them were of the Cayena Lisa variety purchased from the local market. The dehydrated pineapple used was a commercially available product and the freeze-dried pineapple was produced by our team by freeze-drying the same type of fresh peeled pineapple acquired from local markets. For this purpose, VirTis Bench Top K equipment (SP Scientific, Gardiner, NY, USA) was used. The temperature, pressure and time parameters of the process were respectively $-66\text{ }^{\circ}\text{C}$, 7.46 Pa and 5 days.

The vinegar used was a suitable vinegar for the production of Sherry vinegar and was supplied by a local vinegar producer. It had the appropriate organoleptic qualities, with an acidity level of 7 and an alcohol content of 0.5.

2.2. Maceration with Pineapple

The traditional maceration of the different types of pineapple was carried out, based on the conditions established by previous studies [2], in which the maceration of different fruits with vinegar was carried out by employing 200 g/L and 400 g/L of peel, without heating and for three days. In our study, 3-L glass jars were employed, where 1 L of vinegar and the respective dose of pineapple (200 g/L or 400 g/L) were incorporated. The pineapple was cut into approximately 1–2 cm pieces. The maceration temperature was $25\text{ }^{\circ}\text{C}$ and the maceration time was 72 h. For the traditional static maceration, two stirrings were performed per day, one in the early morning and one in the afternoon. For the traditional maceration with agitation, magnetic stirrers were permanently operating at 300 rpm. All trials were conducted in duplicate.

The extractions with microwave energy were carried out with a Mars 6 equipment (CEM Corporation, Barcelona, Spain). The starting conditions were based on previous studies on the maceration of vinegar with citrus fruits in which $65\text{ }^{\circ}\text{C}$, 390 W and 11 min as maceration time were employed [15]. In our research, the power level was set at 350 W, the ramp time at 1 min 30 s and the treatment time ranged from 1 min 30 s to 20 min. The type of pineapple used for these macerations was fresh pineapple at a concentration of 400 g/L. All experiments were carried out in duplicate.

For the extractions with ultrasound energy, a Q1375 Qsonica sonicator, featuring 1375 W and 20 kHz (Qsonica, Newtown, CT, USA), was employed. The methodology

and experimental conditions had already been fine-tuned in previous studies [14,16]. The operating parameters were therefore set as follows: sonication power 550 W/L, pulses 40 s ON/20 s OFF and total sonication time 30 min. In all the experiments the temperature of the sample was controlled through ice baths. The type of pineapple used was fresh pineapple at a concentration of 400 g/L and all experiments were carried out in duplicate.

2.3. Spectrophotometric Analysis

The different spectrophotometric analyses conducted in the study were carried out using UV-Vis equipment (Spectronic Helios, Thermo Electron Corporation, Waltham, MA, USA). For the analysis of the total polyphenol index (TPI), the absorbance at 280 nm of the samples diluted at 1:10 in distilled water was measured. The concentration of total polyphenols was also determined by Folin–Ciocalteu index (FCI) following the methodology described by Singleton [17]: a colorimetric method based on the absorbance measurement of the polyphenols of the sample, employing a reagent of phosphomolybdate and phosphotungstate (Folin's reagent). For that method, a calibration curve employing gallic acid at five levels of concentrations was employed and the absorbance was measured at 750 nm. Folin's reagent and gallic acid were supplied by Merck (Darmstadt, Germany). The Milli-Q water used for the analyses was obtained from a Millipore water purification system (Belford, MA, USA).

2.4. Analysis of Volatile Compounds

A Stir Bar Sorptive Extraction (SBSE) methodology described and optimized by previous studies [18,19] was employed. Briefly, 25 mL of sample together with 5.85 g of NaCl (Scharlab, Barcelona, Spain) and 84 μ L of an internal standard solution (4-methyl-2-pentanol, Sigma-Aldrich, St. Louis, MO, USA) were extracted by the stir bar at room temperature at 1250 rpm for 120 min. Commercial stir bars made of a non-polar material, polydimethylsiloxane (PDMS), with dimensions of 10 mm \times 0.5 mm (length \times film thickness), were supplied by Gerstel (Müllheim a/d Ruhr, Germany).

For the GC-MS analyses, a gas chromatograph with a mass spectrometer detector, Agilent 6890 GC-5973N MS (Agilent Technologies, Palo Alto, CA, USA), fitted with a capillary column DB-Wax model (J&W Scientific, Folsom, CA, USA) of 60 m \times 0.25 mm internal diameter with a 0.25 μ m coating was used. A helium flow rate of 1 mL/min was employed as the carrier gas.

The peaks of the chromatogram were identified by analogy of the mass spectra according to Wiley library (Wiley Registry of Mass Spectral Data, 7th Edition, 2000) and supported by the use of standards in those cases where these were commercially available. For the semi-quantification, the measurement of the relative area of the base peak of each compound in relation to that of the internal standard, 4-methyl-2-pentanol, was employed.

2.5. Sensory Analysis

In order to determine the intensity of the pineapple aroma and the overall quality of the different maceration methods (traditional, microwaves and ultrasound), tasting sessions were carried out to evaluate and establish a ranking according to odor stimulus [20,21]. Two tasting sessions were held in a certified tasting room [22], and using standardized glasses [23]. The samples were presented to the judges accompanied by a randomly assigned identification three-digit code to preserve their traceability. The pineapple-macerated vinegars were tasted by a tasting panel formed by trained laboratory staff members (7 women and 4 men) between 20 and 50 years of age. The intensity of the pineapple aroma and the overall quality (based on aromatic intensity and absence/presence of defects) were evaluated according to a 5-point scale. Since the scope of the research was the obtention of a new product with a good acceptance by consumers, the panel was only focused on the intensity of the pineapple aroma and the overall quality, keeping in mind the point of view of a regular consumer who would evaluate these two main aspects.

2.6. Statistical Study

For the experimental design in the optimization of the microwave maceration conditions, Statgraphics Centurion XVIII (Statpoint Technologies Inc., Warrenton, VA, USA) was employed. Statistica 8.0 (StatSoft Inc., Tulsa, OK, USA) was used for the rest of the statistical study of the samples: Analysis of Variance (ANOVA), Principal Component Analysis (PCA), and post hoc analysis of the comparison of means based on Tukey's test ($p < 0.05$).

3. Results and Discussion

3.1. Optimization of the Product Development by Traditional Maceration

The experiments were started by performing traditional macerations with and without agitation, using different pineapple presentations (Table 1). As can be seen, in the case of freeze-dried pineapple, the concentration of pineapple used was considerably lower than that used in the other experiments, although the lowest amount of fresh pineapple used was 200 g/L. This strategy was based on the water loss that takes place in lyophilization processes. Since the freeze-drying process was performed in the laboratory, the initial amount of pineapple employed was known, and an amount of freeze-dried material corresponding to the initial 200 g/L of fresh pineapple was employed. In the case of dehydrated pineapple, the dehydration process was not carried out, but the product was acquired already prepared from the market. Therefore, the initial amount of fresh pineapple employed to obtain the dehydrated material was unknown. Therefore, the same amount as if it was fresh was employed, but taking into account that, actually, the initial amount of pineapple employed to obtain this dose was higher than 200 g/L. In all cases, a maceration time of 72 h was used [14].

Table 1. Experiments carried out (in duplicate) using traditional maceration.

No.	Pineapple Presentation	Concentration Ratio (g/L)	Stirring
1	Fresh	200	Shaking
2	Fresh	200	300 rpm
3	Fresh	400	Shaking
4	Fresh	400	300 rpm
5	Dehydrated	200	Shaking
6	Freeze-dried	26.5	Shaking

After separating the solid parts from the pineapple, different yields of macerated vinegar (product) were obtained depending on the pineapple presentation used. In the case of fresh pineapple, 100% of the product volume was recovered. For dehydrated pineapple, the yield was 45% and for freeze-dried pineapple, it was 95%. The dehydrated pineapple absorbed 55% of the product, which after the separation of the solid parts was discarded together with the residue.

Samples from all trials were tasted to try and discriminate between the types of traditional maceration. The samples that received the highest score with respect to pineapple aroma intensity on a 1 to 5 scale were those elaborated with 200 g/L dehydrated pineapple (4.8 points out of 5), followed by 400 g/L fresh pineapple without magnetic agitation (4.1 points out of 5). This was a logical result, taking into account that, due to the dehydration process, the amount of dehydrated pineapple employed actually came from a higher amount of fresh pineapple. Of these two, the one with the highest pineapple aroma score was the dehydrated pineapple sample; however, it should be taken into account that the price of the dehydrated pineapple was 4 times that of the fresh pineapple and that the losses in the macerated product implied a drop of 55% in the final yield. The price of the raw material is a very important aspect to be considered when dealing with the obtention of new products and should not be underestimated.

When analyzing the pros and cons of the two best trials, it was concluded that the samples corresponding to the traditional maceration with fresh pineapple were the best

option compared to that of dehydrated pineapple vinegar. The price of the raw material was not the only variable considered, but taking into account that other cheaper options also presented a high value of pineapple character, the decision was made by taking all these aspects into account, together with the yield of the process. Therefore, the optimal conditions for traditional maceration were established as 400 g of fresh pineapple/L of vinegar, two daily stirrings, and a maceration time of 72 h.

3.2. Optimization of Microwave Maceration

Once the target product had been developed by traditional maceration, efforts were made to optimize the process when microwave energies were applied with the objective of elaborating a product that was similar or even better than the one obtained by traditional maceration, but in a shorter maceration time. For this purpose, a central composite design of experiments consisting of a response surface ($2^2 + 1$ central point, 10 experiments, in duplicate) was established. The dose of fresh pineapple in all cases was 400 g/L, and the microwave power was 350 W. The parameters to be optimized were time (values limits: 3–10 min) and temperature (values limits: 30–60 °C). Total polyphenol index (TPI) and Folin–Ciocalteu index (FCI) were set as the response variables to be optimized because the objective was to obtain a product with the highest healthy character, related to the phenolic content. Table 2 presents the obtained parameters from the model.

Table 2. Obtained parameters from the response surface model for Total Polyphenol Index (TPI) and Folin–Ciocalteu Index (FCI).

ANOVA for TPI			ANOVA for FCI		
Source	F-Ratio	p-Value	Source	F-Ratio	p-Value
A: temperature	8.58	0.0117	A: temperature	22.53	0.0413
B: time	4.35	0.0572	B: time	20.47	0.0555
AA	3.39	0.0886	AA	0.11	0.7691
AB	0.27	0.6111	AB	0.02	0.9120
BB	0.43	0.5231	BB	0.32	0.6263
Blocks	0.56	0.4688	Blocks	0.07	0.8163
Lack of fit	2.58	0.1176	Lack of fit	1.64	0.4396
R ²	56.9172%		R ²	62.8244%	
Adjusted R ²	37.0329%		Adjusted R ²	45.6664%	
PRESS	10.6002		PRESS	23261.6	
Predicted R ²	0.0%		Predicted R ²	10.2544%	
Standard error of est.	0.590299		Standard error of est.	27.2252	
Mean absolute error	0.419217		Mean absolute error	18.3628	
Durbin-Watson statistic	1.86518 (<i>p</i> = 0.3105)		Durbin-Watson statistic	2.11939 (<i>p</i> = 0.5191)	
Residual autocorrelation	−0.0148876		Residual autocorrelation	−0.154277	
Coefficients for TPI		Estimate	Coefficients for FCI		Estimate
Constant		12.419	Constant		233.15
A: temperature		0.101465	A: temperature		0.44043
B: time		0.0452361	B: time		12.3087
AA		0.00159721	AA		0.0108245
AB		−0.00207143	AB		−0.0184524
BB		0.010459	BB		−0.337534

Based on the evaluation of the statistic results, it was determined that temperature was the variable with the greatest influence on the concentration of polyphenols (Figure 1), with a direct correlation. It was also observed, that the samples that had been obtained at higher temperatures presented higher TPI and FCI values. Therefore, high temperature values (60 °C) were used.

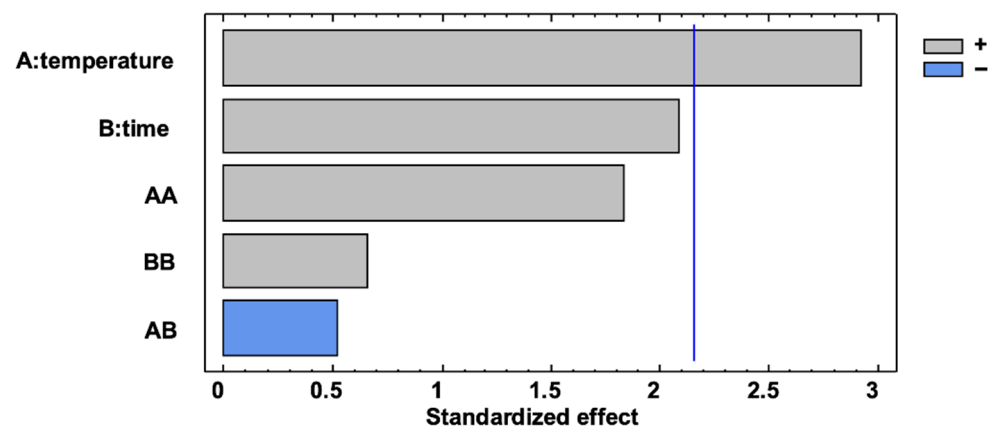


Figure 1. Pareto chart of the optimization of the microwave extractions (TPI).

Subsequently, in order to corroborate the effect of the time variable, several additional experiments were carried out (in duplicate) while keeping the temperature steady at a high level (60 °C) and varying the extraction time as follows: 10, 12.5, 15 and 20 min. The TPI and FCI values obtained are displayed in Table 3. For each one of these responses, significant differences were presented with different letters in the same column.

Table 3. Mean values (mean standard deviation, $n = 2$) for TPI (Total Polyphenolic Index) and FCI (Folin–Ciocalteu Index, ppm gallic acid) measurements from the additional microwave extraction experiments (400 g/L pineapple, 350 W, 60 °C).

No.	Extraction Time (s)	TPI (Mean \pm SD)	FCI (Mean \pm SD)
1	10	11.57 \pm 0.09 a	367.77 \pm 21.70 a
2	12.5	13.03 \pm 0.04 b	403.20 \pm 12.12 ab
3	15	13.45 \pm 0.07 c	397.89 \pm 8.35 a
4	20	14.22 \pm 0.09 d	439.58 \pm 13.04 b

Different letters in each column indicate significant differences according to Tukey's test ($p < 0.05$).

As can be seen, the highest TPI and FCI values were obtained when the extraction times were longer. Therefore, although initially the time variable appeared as non-significant in the experimental design for the interval 3–10 min, it seems that longer extraction times favor the increment of the polyphenolic content in the macerated vinegar samples.

On the other hand, we should note that the sensory analysis of the samples would focus on pineapple aroma intensity (data not shown). The results from such analyses revealed that longer maceration times did not significantly modify the vinegars obtained in relation to pineapple aroma, even when they presented greater polyphenolic contents.

Therefore, based on the results obtained for PTI, FCI, pineapple aroma and extraction time (all of them, important variables to be taken into account for the final decision), a pineapple concentration of 400 g/L, a power of 350 W, a temperature of 60 °C and maceration times of 10 and 20 min were adopted as the conditions to be employed in the subsequent microwave studies.

3.3. Comparison of the Extraction Methods

A comparison of the different optimized extraction methods (traditional maceration, microwave maceration) against ultrasound maceration carried out according to the conditions optimized by other authors was conducted [14]. The FCI obtained by the different methodologies was measured and presented in Figure 2.

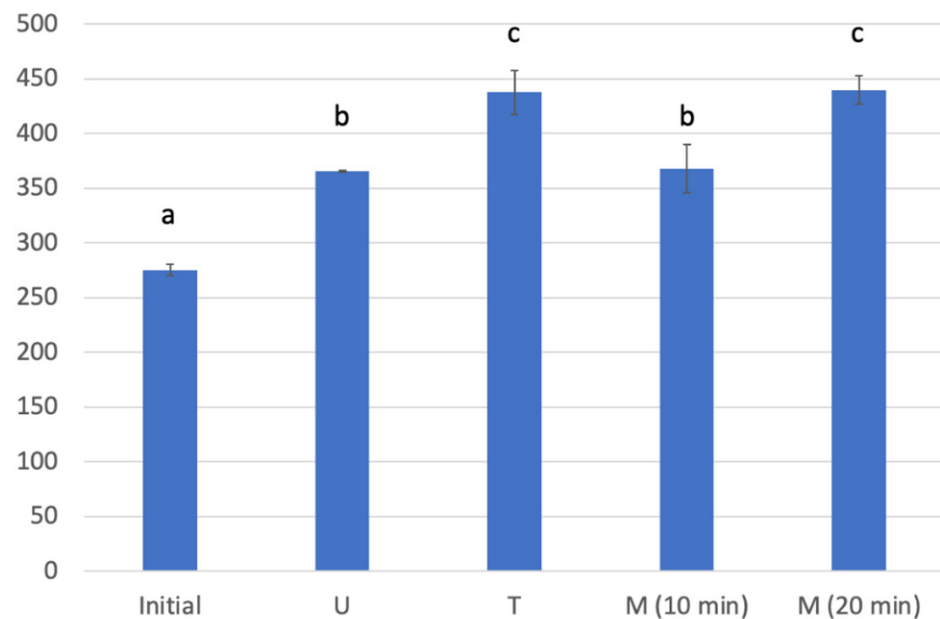


Figure 2. FCI (ppm gallic acid) obtained by the different extraction methods used. U: ultrasound; T: traditional; M: microwave 10–20 min extraction. Initial: Unmacerated vinegar sample. Different letters indicate significant differences according to Tukey's test ($p < 0.05$).

As can be observed, all the extraction techniques significantly increased the FCI with respect to the initial unmacerated sample, where the traditional maceration together with the microwave extraction for 20 min was the highest, followed by the extraction with microwaves for 10 min and the extraction with ultrasound. Other authors have corroborated the fact that extraction techniques favor the transfer of polyphenolic compounds to oenological samples [24,25].

On the other hand, the samples studied were subjected to sensory evaluation and arranged in order from lowest to highest pineapple aroma intensity and overall quality. The ranking results are shown in Table 4.

Table 4. Results obtained from the ordering of the samples based on pineapple aroma and overall quality (aromatic intensity, absence/presence of defects). U: ultrasound; T: traditional; M: microwave 10–20 min extraction. Initial: unmacerated vinegar sample.

Criterion	Ranking
Pineapple aroma	Initial < U < M (20 min) < M (10 min) < T
Overall quality	U < Initial < M (20 min) < T < M (10 min)

As can be seen, all of the samples subjected to maceration with pineapple were ranked higher than the sample that had not been macerated. In addition, the traditional maceration sample was at the top, followed by the 10-min microwave maceration sample. However, in relation to the overall quality, it is interesting to mention that the sample to which ultrasound was applied presented defects in its aromatic profile, which caused the judges to rank it below the rest of the samples, including the initial unmacerated sample. In a previous study [15], the use of dynamic ultrasonication for the maceration of citrus fruits with vinegar also provided the product with olfactory flaws, but the employment of static ultrasonication was successfully employed for the maceration of orange and lemon peels with vinegar [14,15]. However, static ultrasound was ranked after traditional and microwave maceration when these three techniques were compared [15]. Our results with pineapple corroborate this preference, because in this case, the judges positioned

the vinegar sample that had been microwaved for 10 min in first place, ahead of the traditionally macerated sample.

These tests demonstrated that the microwave extraction samples had similar organoleptic characteristics to those obtained through traditional maceration and could therefore be a valid alternative for the successful elaboration of a quality product.

3.4. Analysis of Volatile Compounds

The pineapple macerated vinegar samples that had been obtained using the different extraction methods were subjected to analysis of volatile compounds using the Stir Bar Sorptive Extraction technique coupled to Gas Chromatography and Mass Spectrometry detection (SBSE-GC-MS). A total of 32 volatile compounds were identified as shown in Table 5. The approximate number of chromatographic peaks present in the chromatograms was around 100, but due to the lack of commercial standards, only 32 were selected for the study. This selection was based on the previous experience of the researchers and it was focused on those compounds with a high percentage of matching against the library data (>85%).

Table 5. Mean values of the relative areas ($n = 4$) of the volatile compounds studied and ANOVA results.

Volatile Compound	Initial	Microwaves	Ultrasound	Traditional	F	<i>p</i> -Value
Ethyl acetate	9.166 b	5.445 ab	3.958 a	6.373 ab	6.68	0.0488 *
Isobutyl acetate	1.706 b	1.166 ab	0.709 a	1.344 ab	8.84	0.0307 *
Ethyl 2-methylbutyrate	ND a	0.337 b	0.022 a	0.052 a	52.69	0.0011 *
Ethyl isovalerate	0.509 b	0.335 ab	0.185 a	0.395 ab	10.29	0.0236 *
2-Methyl-1-propanol	ND a	0.011 ab	0.034 c	0.021 bc	17.17	0.0094 *
Isoamyl acetate	13.489 b	10.227 ab	5.554 a	10.935 ab	9.83	0.0256 *
2,6-Dimethyl-4-heptanone	ND	0.032	0.027	0.069	2.40	0.2076
Methyl hexanoate	ND a	0.153 a	0.389 a	1.458 b	38.58	0.0020 *
Ethyl hexanoate	0.023 a	1.440 b	0.098 a	0.394 a	30.32	0.0032 *
Acetoin	0.032 b	0.013 a	0.021 ab	0.021 ab	9.74	0.0260 *
3-Hexen-1-ol acetate	0.005	0.003	0.002	0.004	3.44	0.1316
Ethyl heptanoate	ND a	0.019 c	0.001 ab	0.009 b	30.37	0.0032 *
2-Butyl acetate	ND a	0.016 b	0.014 b	0.018 b	92.52	0.0003 *
Methyl octanoate	ND a	0.065 ab	0.060 ab	0.365 b	9.30	0.0281 *
Furfural	0.009 b	0.003 a	0.003 a	0.003 a	53.29	0.0011 *
Linalool oxide	ND a	0.002 b	0.002 b	0.002 b	40.60	0.0018 *
2,3-Butanediol diacetate	0.026	0.022	0.018	0.022	6.24	0.0545
Benzaldehyde	0.069 b	0.043 a	0.048 a	0.053 a	22.16	0.0059 *
Isobutyric acid	0.096	0.035	0.046	0.053	5.00	0.0768
Pentanoic acid	ND a	0.004 ab	0.006 b	0.005 ab	8.44	0.0332 *
3-Methylbutanoic acid	2.024 b	0.854 a	0.836 a	0.897 a	6.97	0.0456 *
Benzyl acetate	0.023 b	0.015 a	0.014 a	0.024 b	28.72	0.0036 *
Methylbenzeneacetic acid	0.003	0.003	0.003	0.005	6.11	0.0563
Methyl salicylate	0.018 c	0.011 b	0.008 a	0.011 b	73.33	0.0005 *
Ethyl phenyl acetate	0.322 c	0.237 ab	0.179 a	0.239 b	31.68	0.0030 *
2-Phenetyl acetate	9.559 c	7.249 b	5.373 a	7.118 b	47.70	0.0013 *
Hexanoic acid	0.143	0.125	0.076	0.100	2.33	0.2154
Benzenemethanol	1.070 b	0.707 a	0.659 a	0.785 ab	12.33	0.0172 *
<i>p</i> -Ethylguaiaicol	0.093 b	0.063 a	0.058 a	0.064 a	88.13	0.0004 *
Octanoic acid	0.871 b	0.595 a	0.416 a	0.498 a	20.71	0.0067 *
4-Ethylphenol	0.151 b	0.089 a	0.078 a	0.090 a	43.97	0.0016 *
Decanoic acid	0.228 a	0.456 b	0.109 a	0.104 a	36.31	0.0023 *

* Different letters within each row indicate significant differences according to Tukey's test ($p < 0.05$). ND: Not detected.

As can be seen, a greater number of compounds were identified in the macerated samples (32 different compounds) compared to their presence in the initial vinegar that had not been macerated and where only 23 compounds were identified. The increase in the number of volatile compounds detected in Sherry vinegar after maceration with fruits had

been previously reported by other authors [6,14,15]. Some of the compounds found in the samples macerated with pineapple are directly related to the volatile compounds profile of pineapple. Among these, we should mention methyl octanoate, decanoic acid, ethyl hexanoate, 3-methyl-1-butanol, and ethyl acetate [3]. Some compounds, such as methyl octanoate, ethyl heptanoate, 2-butyl acetate, linalool oxide, pentanoic acid, 2,6-dimethyl-4-heptanone, hexanoic acid, ethyl 2-methylbutyrate, and 2-methyl-1-propanol only appeared in the samples that had been macerated with pineapple, which seems to indicate that these compounds were exclusively contributed by the added pineapple. Previous studies [26] have mentioned that esters, lactones, furanoids and sulfur compounds act as very significant components in pineapple aroma. Esters such as ethyl 2-methylbutanoate, methyl hexanoate and ethyl hexanoate provide fruity notes from fresh pineapple as well as from other fruits [27]. Using odor threshold values and concentration data, other authors have concluded that some of the most important contributors to the aroma of fresh pineapple are: methyl 2-methylbutanoate, ethyl 2-methylbutanoate, ethyl acetate, ethyl hexanoate, ethyl butanoate, ethyl 2-methylpropanoate, methyl hexanoate, and methyl butanoate [28], compounds that have been detected in our study.

The data obtained from the study of the volatile profile of the samples were subjected to ANOVA in order to identify any relevant differences between the different extraction techniques (Table 5). The results from the ANOVA confirmed that most of the compounds had been influenced by the treatment, with the exception of 2,6-dimethyl-4-heptanone, 3-hexen-1-ol acetate, 2,3-butanediol diacetate, iso-butyric acid, methylbenzeneacetic acid and hexanoic acid. Similar results were obtained by other authors, who reported significant differences in the majority of the volatile compounds of vinegars macerated with fruits, taking into account the maceration procedure [15]. As expected, the characteristic pineapple aroma compounds increased with maceration. The samples subjected to microwave maceration presented the largest amounts of compounds such as ethyl 2-methylbutyrate, ethyl hexanoate or ethyl heptanoate, among others. These compounds were also found in almost the same quantities in the traditionally macerated vinegar and, to a lesser extent, in the ultrasound macerated samples. In the traditionally macerated vinegar, methyl hexanoate or methyl octanoate, among others, were the most prominent compounds. On the other hand, 2-methyl-1-propanol and pentanoic acid were the most abundant compounds found in the ultrasonic extraction samples, followed by the traditional and microwave ones. Taking into account the influence of the maceration technique on the volatile profile, in general terms, it seems that microwaves and traditional macerations provoked a significant increase of a higher number of compounds, compared to ultrasound extraction (Table 4), which is in agreement with previous research [15]. This fact could be also related to the sensory results, which ranked the traditional and microwave macerated vinegars in the first positions, regarding pineapple aroma and overall quality.

In some cases, the content level of some particular volatile compounds was lower in the samples that had been macerated with pineapple. This is the case for compounds such as ethyl acetate, isoamyl acetate, benzaldehyde, isobutyric acid, 3-methylbutanoic acid, octanoic acid, and others. This phenomenon was more noticeable in the ultrasonic extraction samples, followed by the microwave ones. In this regard, some authors have reported a possible degradation of volatile compounds in white wine samples when subjected to ultrasound treatment [29].

Finally, a multivariate principal component analysis was performed. This analysis included all the traditional maceration samples used for the optimization (T), all the microwave samples with extraction times equal to or longer than 10 min (M), the samples obtained by ultrasound extraction (U) and the unmacerated (initial) vinegar (Figure 3).

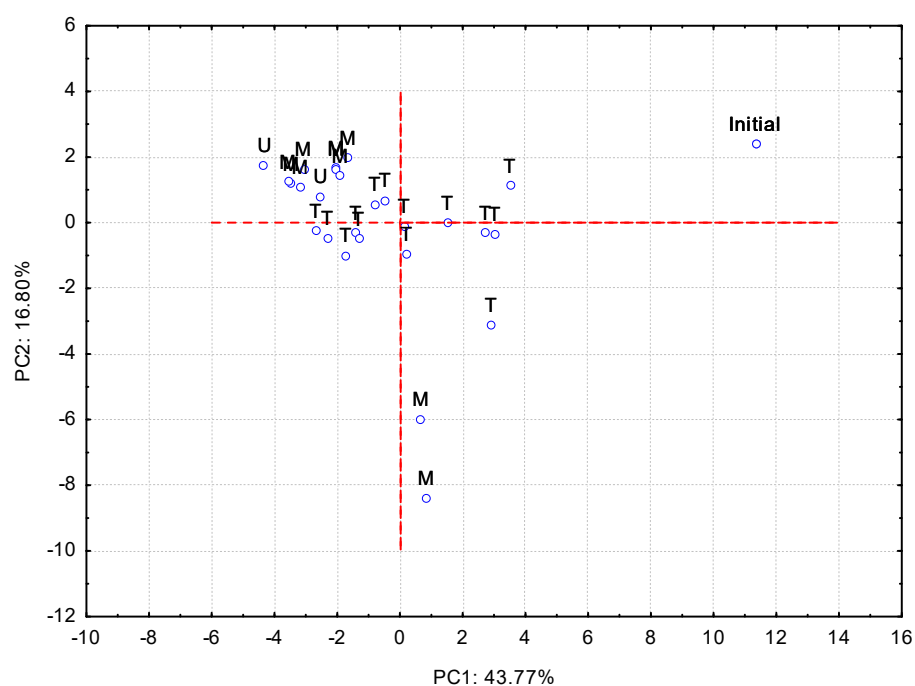


Figure 3. Principal component analysis of the samples subjected to the different maceration procedures. U: ultrasound; T: traditional; M: microwave. Initial: unmacerated vinegar.

Five of the principal components obtained had an eigenvalue >1 and explained 99.94% of the variation between samples. Component 1 (PC1) explained 43.77% while component 2 (PC2) explained 16.80% of the variability. PC1 was able to separate the samples that were subjected to maceration with pineapple from the initial sample, that had not been macerated. As can be seen in Table 6, the 5 compounds that were most relevant to this component were: methyl salicylate, ethyl phenyl acetate, 2-phenethyl acetate, benzenemethanol, and 4-ethylphenol, of which 2-phenethyl acetate is closely related to pineapple aroma [30]. Therefore, as expected, it was confirmed that maceration with pineapple greatly modifies the volatile profile of the resulting vinegar, with some pineapple-derived compounds as clear markers of the macerated samples.

Table 6. Contribution of volatile compounds studied to the first two PC (values > 0.02).

Volatile Compound	PC1	Volatile Compound	PC2
Methyl salicylate	0.063	Ethyl heptanoate	0.164
Ethyl phenyl acetate	0.062	Ethyl hexanoate	0.159
Benzenemethanol	0.060	Ethyl 2-methylbutyrate	0.148
2-Phenethyl acetate	0.058	Decanoic acid	0.080
4-Ethylphenol	0.057	3-Methyl-1-butanol	0.032
<i>p</i> -Ethylguaiaicol	0.051	Linalool oxide	0.028
3-Methylbutanoic acid	0.051	Acetoin	0.026
2,3-Butanediol diacetate	0.049	Isobutyric acid	0.025
Octanoic acid	0.049	Isoamyl acetate	0.021
Ethyl isovalerate	0.048		
Isobutyl acetate	0.045		
3-Hexen-1-ol acetate	0.044		
Isoamyl acetate	0.043		
Ethyl acetate	0.043		
Furfural	0.038		
Isobutyric acid	0.037		
Hexanoic acid	0.037		
Benzaldehyde	0.031		
Benzyl acetate	0.021		

4. Conclusions

The present work intends to present the production methodology for a novel product elaborated from Sherry vinegar and pineapple. Different pineapple presentations have been macerated with Sherry vinegar and several techniques (traditional, ultrasound, microwave) have been applied to the extraction of volatile compounds in order to provide the final product with new organoleptic properties. Furthermore, it has been confirmed that through maceration with pineapple, the total polyphenol index increased significantly with respect to that of vinegar that had not been macerated with pineapple. The vinegar that had been macerated with pineapple by traditional methods was ranked first in terms of pineapple aroma; however, the samples from microwave extractions trials qualified in a better position in terms of overall quality. The use of microwaves could, therefore, be a feasible alternative to the traditional maceration of vinegar with pineapple. In fact, by applying microwaves, 72 h required by traditional maceration methods was shortened to just between 10 and 20 min while very similar results were achieved.

Author Contributions: Conceptualization, E.D.-G.; methodology, E.D.-G. and R.C.; software, R.C. and E.D.-G.; validation, E.D.-G.; formal analysis, S.P.; investigation, S.P.; resources, R.C.; data curation, S.P.; writing—original draft preparation, E.D.-G.; writing—review and editing, E.D.-G. and R.C.; visualization, R.C.; supervision, E.D.-G.; project administration, E.D.-G.; funding acquisition, R.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: The authors would like to thank Productos Majuelo S.L. for their supply of the vinegar specimens used in this study.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Cejudo-Bastante, M.J.; Doderero, M.C.R.; Guerrero, E.D.; Mejías, R.C.; Marín, R.N.; Barroso, C.G. Development and optimisation by means of sensory analysis of new beverages based on different fruit juices and sherry wine vinegar. *J. Sci. Food Agric.* **2012**, *93*, 741–748. [[CrossRef](#)]
2. Bastante, M.J.C.; Guerrero, E.D.; Mejías, R.C.; Marín, R.N.; Doderero, M.C.R.; Barroso, C.G. Study of the Polyphenolic Composition and Antioxidant Activity of New Sherry Vinegar-Derived Products by Maceration with Fruits. *J. Agric. Food Chem.* **2010**, *58*, 11814–11820. [[CrossRef](#)] [[PubMed](#)]
3. Zheng, L.-Y.; Sun, G.-M.; Liu, Y.-G.; Lv, L.-L.; Yang, W.-X.; Zhao, W.-F.; Wei, C.-B. Aroma Volatile Compounds from Two Fresh Pineapple Varieties in China. *Int. J. Mol. Sci.* **2012**, *13*, 7383–7392. [[CrossRef](#)] [[PubMed](#)]
4. Ali, M.M.; Hashim, N.; Aziz, S.A.; Lasekan, O. Pineapple (*Ananas comosus*): A comprehensive review of nutritional values, volatile compounds, health benefits, and potential food products. *Food Res. Int.* **2020**, *137*, 109675. [[CrossRef](#)]
5. Marrufo-Curtido, A.; Cejudo-Bastante, M.J.; Rodríguez-Doderero, M.C.; Natera-Marín, R.; Castro-Mejías, R.; García-Barroso, C.; Durán-Guerrero, E. Novel vinegar-derived product enriched with dietary fiber: Effect on polyphenolic profile, volatile composition and sensory analysis. *J. Food Sci. Technol.* **2015**, *52*, 7608–7624. [[CrossRef](#)] [[PubMed](#)]
6. Cejudo-Bastante, M.J.; Durán, E.; Castro, R.; Rodríguez-Doderero, M.C.; Natera, R.; García-Barroso, C. Study of the volatile composition and sensory characteristics of new Sherry vinegar-derived products by maceration with fruits. *LWT* **2013**, *50*, 469–479. [[CrossRef](#)]
7. Bautista-Ortín, A.B.; Jiménez-Martínez, M.D.; Jurado, R.; Iniesta, J.A.; Terrades, S.; Andrés, A.; Gómez-Plaza, E. Application of high-power ultrasound during red wine vinification. *Int. J. Food Sci. Technol.* **2017**, *52*, 1314–1323. [[CrossRef](#)]
8. Tao, Y.; Zhang, Z.; Sun, D.-W. Experimental and modeling studies of ultrasound-assisted release of phenolics from oak chips into model wine. *Ultrason. Sonochem.* **2014**, *21*, 1839–1848. [[CrossRef](#)]
9. Carew, A.L.; Gill, W.; Close, D.C.; Damberg, R.G. Microwave Maceration with Early Pressing Improves Phenolics and Fermentation Kinetics in Pinot noir. *Am. J. Enol. Vitic.* **2014**, *65*, 401–406. [[CrossRef](#)]
10. Casassa, L.F.; Sari, S.E.; Bolcato, E.A.; Fanzone, M.L. Microwave-Assisted Extraction Applied to Merlot Grapes with Contrasting Maturity Levels: Effects on Phenolic Chemistry and Wine Color. *Fermentation* **2019**, *5*, 15. [[CrossRef](#)]
11. Delgado-González, M.; Sánchez-Guillén, M.; García-Moreno, M.; Rodríguez-Doderero, M.; García-Barroso, C.; Sánchez, D.G. Study of a laboratory-scaled new method for the accelerated continuous ageing of wine spirits by applying ultrasound energy. *Ultrason. Sonochem.* **2017**, *36*, 226–235. [[CrossRef](#)] [[PubMed](#)]
12. Caldeira, I.; Pereira, R.; Clímaco, M.; Belchior, A.; De Sousa, R.B. Improved method for extraction of aroma compounds in aged brandies and aqueous alcoholic wood extracts using ultrasound. *Anal. Chim. Acta* **2004**, *513*, 125–134. [[CrossRef](#)]

13. Jiménez-Sánchez, M.; Durán-Guerrero, E.; Rodríguez-Dodero, M.C.; Barroso, C.G.; Castro, R. Use of ultrasound at a pilot scale to accelerate the ageing of sherry vinegar. *Ultrason. Sonochem.* **2020**, *69*, 105244. [[CrossRef](#)] [[PubMed](#)]
14. Bruna-Maynou, F.J.; Castro, R.; Rodríguez-Dodero, M.C.; Barroso, C.G.; Durán-Guerrero, E. Flavored Sherry vinegar with citric notes: Characterization and effect of ultrasound in the maceration of orange peels. *Food Res. Int.* **2020**, *133*, 109165. [[CrossRef](#)] [[PubMed](#)]
15. Chanivet, M.; Durán-Guerrero, E.; Rodríguez-Dodero, M.D.C.; Barroso, C.G.; Castro, R. Application of accelerating energies to the maceration of sherry vinegar with citrus fruits. *J. Sci. Food Agric.* **2021**, *101*, 2235–2246. [[CrossRef](#)] [[PubMed](#)]
16. Schwarz, M.; Rodríguez, M.C.; Sanchez, M.; Guillén, D.A.; Barroso, C.G. Development of an accelerated aging method for Brandy. *LWT* **2014**, *59*, 108–114. [[CrossRef](#)]
17. Singleton, V.L.; Rossi, J.A. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158. [[CrossRef](#)]
18. Duran-Guerrero, E.; Marín, R.N.; Mejías, R.C.; Barroso, C.G. Optimisation of stir bar sorptive extraction applied to the determination of volatile compounds in vinegars. *J. Chromatogr. A* **2006**, *1104*, 47–53. [[CrossRef](#)]
19. Duran-Guerrero, E.; Marín, R.N.; Mejías, R.C.; Barroso, C.G. Stir bar sorptive extraction of volatile compounds in vinegar: Validation study and comparison with solid phase microextraction. *J. Chromatogr. A* **2007**, *1167*, 18–26. [[CrossRef](#)]
20. International Organization for Standardization (ISO). *Sensory Analysis. Guidelines for the Use of Quantitative Response Scales*; ISO: Geneva, Switzerland, 2003; p. 4121.
21. International Organization for Standardization (ISO). *Sensory Analysis. Methodology. Ranking*; ISO: Geneva, Switzerland, 2006; p. 8587.
22. International Organization for Standardization (ISO). *Sensory Analysis. General Guidance for the Design of Test Rooms*; ISO: Geneva, Switzerland, 2007; p. 8589.
23. International Organization for Standardization (ISO). *Sensory Analysis. Apparatus. Wine-Tasting Glass*; ISO: Geneva, Switzerland, 1977; p. 3591.
24. Sommer, S.; Cohen, S.D. Comparison of Different Extraction Methods to Predict Anthocyanin Concentration and Color Characteristics of Red Wines. *Fermentation* **2018**, *4*, 39. [[CrossRef](#)]
25. Wojdyło, A.; Samoticha, J.; Chmielewska, J. Effect of different pre-treatment maceration techniques on the content of phenolic compounds and color of Dornfelder wines elaborated in cold climate. *Food Chem.* **2021**, *339*, 127888. [[CrossRef](#)] [[PubMed](#)]
26. Teai, T.; Claude-Lafontaine, A.; Schippa, C.; Cozzolino, F. Volatile Compounds in Fresh Pulp of Pineapple (*Ananas comosus* [L.] Merr.) from French Polynesia. *J. Essent. Oil Res.* **2001**, *13*, 314–318. [[CrossRef](#)]
27. Tokitomo, Y.; Steinhaus, M.; Büttner, A.; Schieberle, P. Odor-Active Constituents in Fresh Pineapple (*Ananas comosus* [L.] Merr.) by Quantitative and Sensory Evaluation. *Biosci. Biotechnol. Biochem.* **2005**, *69*, 1323–1330. [[CrossRef](#)]
28. Takeoka, G.R.; Buttery, R.G.; Teranishi, R.; Flath, R.A.; Guentert, M. Identification of additional pineapple volatiles. *J. Agric. Food Chem.* **1991**, *39*, 1848–1851. [[CrossRef](#)]
29. Lukić, K.; Brnčić, M.; Čurko, N.; Tomašević, M.; Tušek, A.J.; Ganić, K.K. Quality characteristics of white wine: The short- and long-term impact of high power ultrasound processing. *Ultrason. Sonochem.* **2020**, *68*, 105194. [[CrossRef](#)] [[PubMed](#)]
30. Pino, J.A.; Queris, O. Analysis of volatile compounds of pineapple wine using solid-phase microextraction techniques. *Food Chem.* **2010**, *122*, 1241–1246. [[CrossRef](#)]