Evaluation of the volatile organic compounds associated to Rocha pear's maturation

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Abstract

Rocha pear is one of the most important fruits for Portuguese economy and its storage and conservation throughout the year are crucial factors. However, and despite the different technologies available, there are still problems in preserving the quality of the fruits, with physiological accidents such as superficial scald and internal browning occurring.

The objective of this work was the physico-chemical characterization of Rocha pear and the development of a gas chromatography analysis method coupled with mass spectrometry, to identify the volatile organic compounds VOCs released by the fruits during the ripening stage and throughout storage.

Two different methods were used, one for the detection of ethylene (SIM) and the other for the remaining compounds (full scan). In the gaseous atmosphere of the storage chambers, more than 40 VOCs were detected, including 1-hexanol, hexyl acetate, ethanol, 1-butanol, butyl acetate and acetaldehyde with greater relative abundances. The compound produced by pears in greater quantity was ethanol. In all the tested storage conditions (Normal Atmosphere (NA), Controlled Atmosphere (AC), AC + 1-MCP, and Ultra Low Oxygen (ULO)), there was a higher production of alcohols than esters. The same was verified in the samples tested under the condition of AN with shelf life of 1, 5 and 14 days. 1-hexanol proved to be the most significant compound and is always present in addition to ethanol. This study will allow a better understanding of the pear pear metabolism and possibly the optimization of its storage in the future.

Keywords: Rocha pear, ethylene, volatiles (VOCs), GC-MS, storage atmospheres, 1-MCP

1. Introduction

Rocha pear (*Pyrus communis L*) is a DOP portuguese cultivar characteristic from the western region.

It is the fourth most consumed fruit in Portugal and one of the most exported portuguese agricultural products [1]. Given its importance to the portuguese economy, it is of great interest to storage pears for as long as possible, maintaining their organoleptic properties.

New storage technologies have been developed throughout recent years. The modification of atmospheric conditions, changing O_2 and CO_2 concentrations, has been used in addition to traditional cold storage (around -0,5 ^oC). CA (2,5% of O_2 and 0,5% of CO_2) can preserve pears for up to eight months and ULO atmosphere (0,5% of O_2 and 0,5% of CO_2) can delay even more the maturation process [2][3]. However, there are still problems in successfully maintaining fruit quality and avoiding the appearance of physiological disorders.

The main physiological disorders are superficial scald and internal browning. They are triggered by the exposure of fruits to storage conditions and conditioned by their pre-harvest treatments and circumstances [4].

Suppliers have also used 1-methylcyclopropene (1-MCP) (applied with 4% O_2), that easily establishes irreversible bonds with the fruit's ethylene receptors and prevents superficial scald, internal browning and delays ripening [4][5]. Nevertheless, it has the negative effect of inhibiting fruit ripening (remaining in the *evergreen* state) and sometimes giving them a bitter taste, even after exposure to air (NA) and room temperature [4][6].

Aroma and flavor play an important role in the quality of fruit and are due to a complex mixture of many volatile organic compounds. Production of VOCs are influenced not only by genetics and the maturation state at harvest time, but also by the environment of the fruit, cultivation practices, post-harvest handling and storage conditions [7][8]. There is a decrease in the production of VOCs in fruits stored in CA and ULO conditions, and treated with 1-MCP, after returning to NA and room temperature [9][10].

The volatile profile of pears includes esters, alcohols, aldehydes, ketones and hydrocarbons. Esters are the most important compounds, since they are the predominant class of VOCs and the ones that give the fruits their predominant fruity flavor and aroma associated with ripe fruit [7][11][12]. Hexyl acetate is the most important ester and offers pears the fruity aroma and characteristic smell. Together with butyl acetate, responsible for the sweet fruity aroma, they can represent about 70% of all aromatic volatiles [13]. Alcohols and aldehydes also contribute to the fruit's characteristic flavor and aroma and are precursors for the synthesis of esters [11]. On the other hand, the production of ethanol and acetaldehyde is normally associated with anaerobic

processes, caused by controlled atmospheres (low O_2), being one of the causes of the occurrence of internal browning [14].

Ethylene is responsible for ripening and its regulation. Through its determination, it's possible to know the ripening state of the pears inside the chambers. CA conditions limit the action of ethylene and aerobic respiration, resulting in a decrease in metabolic activity and prolonged storage. The reduction of ethylene action prevents the normal production of volatiles.

As such, investigation on the pear VOCs is performed using gas chromatography-mass spectrometry (GC-MS), having the need to firstly optimize the analytical method conditions. Once optimized different chromatograms were obtained for pears under different conditions.

2. Experimental procedures

2.1. Samples

In this study, pears of the Rocha variety from the 2020/2021 campaign were used as samples. They came from three different orchards in the West region, two from Cadaval and one from Bombarral. The fruits were harvested in August 2020 and placed in RochaCenter storage chambers. All storage chambers were at -0,5 ºC and inside the chambers were containers with different atmospheric conditions and relative humidity of 90-95%. For this work, pears were studied under NA, AC, ULO conditions and with 1-MCP (312 ppb + AC). Each container, with about 0.5 m^3, had two boxes from each orchard (± 30 kg/pear/orchard) inside.

2.2. Reagents

The reagents used were: distilled water (Blandis), iodine (99+%, pure, Acros Organics) and potassium iodide (98%, extra pure, Acros Organics) to determine starch regression rate, NaOH (Pellets, Fisher Chemistry) for the determination of total titratable acidity, gallic acid (Acros Organics), sodium carbonate (extra pure 99,95%, Acros Organics) and Folin-Ciocalteu phenolic reagent (Fisher Scientific) for the quantification of phenolic compounds, methanol (analytical reagent grade 99,9%, Fisher Chemical), HCl (analytical grade reagent, 37%, Fisher Chemical) to make the extracting solution, ascorbic acid (analytical reagent grade, 99,7%, Fisher Chemical) and DPPH (95%, Alfa Aesa, Thermo Fisher Scientific) to determine the antioxidant capacity, Hexane (Carlo Erba Reagents) for the quantification of α-farnesene and conjugated trienes.

2.3. Physico-Chemical Characterization

For these analysis, three samples were used, each consisting of 20 fruits, from each of the three orchards.

To measure fruits' colour (L^*, C^*, ºhue), a colorimeter was used (Chroma Meter CR 400, KONICA MINOLTA). Average weight (g) was measured in a precision analytical scale (Fisherbrand, Precision Series, Fisher Scientific, modelo FPRS4202/E). Fruit size (diameter in mm) was measured with a digital pachymeter (Calibre Digital, POWERFIX, Profi, model HG00962A). A penetrometer (Turoni, Fruit Pressure Tester, model FT 327) was used to determine pulp firmness (kg/0,5 cm^2). Total soluble solids (%Brix) were measured with a digital refractometer (Pocket refractometer, ATAGO, S710133). The starch regression rate was determined using an iodine solution (10g iodine/40g potassium iodide/1L water).

Fresh juice samples were obtained with a commercial blender, and 25 mL of each sample were used to measure total titrable acidity (TTA). A NaOH 0,1M solution was also used to titrate the samples. The juice pH was measured (potentiometer, Seven compact duo S213, Mettler Toledo), while drops of the basic solution were added until pH=8,15. TTA was expressed in malic acid (g/L).

Samples (10g fresh weight) were extracted with 10 mL of methanol:HCl:H_2O (79,5: 0,5: 20, v/v/v). Extractions were carried out for 2 h in a magnetic stirrer. Samples were filtered and supernatants were tested for phenolic compounds and antioxidant capacity. Total phenolic content (PC) determinations were assayed by the *Folin-Ciocalteau* method spectrophotometrically (Genesys 10uv Scanning, Thermo Scientific) at 765 nm.[15] Standard solutions of gallic acid (0.05, 0.2, 0.4, 0.6 and 0.8 g/L) and small quantities of the fruit extracts (50 µL) were mixed with *Folin-Ciocalteau* reagent (250 µL) and deionized water (4,2 mL). Sodium carbonate (20%, w/v) was added to the mixture (500 µL) and then they were let to incubate for 2 h at 20 ºC. PC was expressed in mg of gallic acid equivalents per g of fresh weight, by making use of the calibration curve. A solution of 0,048 g/L of DPPH in methanol was prepared (30 mL) to determine antioxidant capacity (AC) spectrophotometrically at 515 nm. Quantities of 50 µL of standard solutions of ascorbic acid (0.01, 0.05, 0.1, 0.15 and 0.2 g/L) and samples were mixed with 950 µL of the DPPH solution for 15 minutes in a magnetic stirrer.

For each sample, ten small discs (d=1,5 cm) of epidermis were mixed with 5 mL of hexane and left in a magnetic stirrer for 10 min. The extracts were filtered, and 5 mL of hexane were added to each one. Finally, they were analyzed spectrophotometrically at 232 nm for determination of α-farnesene and 281 nm for conjugated trienes (CT). Results were obtained using molar absorptivity values of $\varepsilon_{\#\$\#} = 24{,}740$ for α-farnesene and $\varepsilon_{\#+,} = 25{,}000$ for CT. Results came in µg/g (fresh weight).

2.4. Determination of VOCs: Optimization of the method

Samples were analyzed on a GC (TRACE 1300 Gas Chromatograph, ThermoFisher Scientific) coupled to an MS (ISQ QD Single Quadrupole Mass Spectrometer, ThermoFisher Scientific). The chromatographic column used was a TraceGOLDTM TG-WAXMS (ThermoFisher Scientific) (0.5µm x 0.25mm x 60m). The software used was Thermo Xcalibur version 3.1 from Thermo Fisher Scientific.

2.4.1. Ethylene identification

Ethylene is one of the most important volatile compounds for pears, so it was necessary to optimize a method to identify it using GC-MS. A mixture of N_2 and ethylene was used as the standard sample (4% ethylene; v/v) (Air Liquide). A 1 ml gas tight syringe was used to inject the sample in the GC-MS.

Generally, methods for detecting ethylene using a GC-MS are not developed because the presence of N_2 in the analyzer, even at low concentrations, creates an unacceptably high level of background signal in a low mass discrimination instrument, such as the quadrupole, which cannot distinguish ethylene and N_2 based on differences in exact mass (elementary N_2 has a monoisotopic mass of 28,0061 against 28,0313 for ethylene), since they both have the same nominal molecular mass [16]. Thus, it is a great challenge to analyze ethylene with a GC-MS.

Nitrogen, being the main component of air, is inevitably always injected with the sample. Ethylene has, in addition to its molecular ion, which is the peak of greatest abundance in its mass spectrum, the ions m/z 27 and m/z 26 that are formed with a relative abundance of 62.3% and 52.9%, respectively [16]. The high background signal associated with N_2 is reduced when monitoring only the 23-27 m/z range, which represents products of ethylene fragmentation not produced by N_2.

A chromatogram with a single well-defined peak was obtained, at 3,93 min. The mass spectrum corresponding to this peak showed ions m/z 24, 25, 26 and 27 only present in ethylene. Therefore, it can be concluded that this peak corresponded to ethylene.

However, it was necessary to take into account that the samples of the surrounding air of the fruits would not be so concentrated in ethylene, and it would possibly be more difficult to detect this compound.

Thus, the method for detecting ethylene with helium as a mobile phase was defined, 70 eV of ionization energy and as described in table 1. A solvent delay (*Time*) of 1,8 min was defined in order to eliminate excess nitrogen, which was implemented to reduce the wear of the filament.

Table 1 - GC-MS method parameters for detection of ethylene.

GC		MS	
Injector T (ºC)	150	T.line T (ºC)	180
Mode	Split	Ion source T (ºC)	180
Split ratio	33,3	*Time* (min)	1,8
Flow (mL/min)	1	m/z (amu)	23,5 – 27,3
T program	30 ºC (4,5min) 20 ºC/min to 100 ºC (10 min)		

2.4.2. Real sample analysis

The first sample that was analyzed was the air from the storage chamber which was under NA conditions. A pump (AgroFresh) was used to collect the air from the storage chamber, as well as a gas sampling bulb. Either ethylene or any other volatile compound was detected.

Apart from trying to obtain results changing all the instrumental method parameters, different sampling techniques were used also.

Desiccator

Having in mind that storage was still in its initial months (1 month), volatiles were still at low levels, which could be the reason for the difficulty in identifying and optimizing the method. A strategy was defined to concentrate VOCs and encourage their production, placing the fruits in a smaller space, like a desiccator. There, the fruits would be placed without any control of the atmosphere and at room temperature so that they could ripen normal and naturally. Adding more pears to the desiccator or waiting more days for the fruits to ripen was also tried but results didn't change.

Adsorbents

Pears produce VOCs in very small quantities so a possible solution to detect these compounds would be to concentrate them before performing the analysis. Usually, it is necessary to concentrate the compounds properly for this type of analysis, and there is already a technique to do it (HS-SPME). The materials to perform this technique weren't available at the laboratory so different types of adsorbents were used: three different types of activated carbon, norit RB2, norit RB4 (Cabot), fiber (ACC-507-15, 120 g/m^2, 0.5 mm, Kynol) and a zeolite (sieve) molecular 5A in pellets, Sigma Aldrich). As these materials can have different adsorption capacities, they were all used to see if, with the help of any of them, results could be obtained.

Before being used, they were weighed and placed inside the oven (150ºC) for an hour. To analyze the sample, adsorbents were placed in vials and initially heated with a hair dryer to make the desorption of the compounds. It showed no results.

Heating method

Assuming that the samples were not yet concentrated enough to allow them to be detected in the MS, the problem could be in the method used to heat the adsorbents.

Thus, a heating plate and an oven were also used. The heating temperature was also changed but never showing new results. The oven was used in the following experiments.

***Time (min)* parameter**

Pears were always removed from the cold and left at room temperature overnight. Thirty pears were removed from the cold (-0,5ºC) and left on the laboratory for one day to reach equilibrium. The next day the pears were placed in the desiccator, with 106,7µL of an internal standard solution of 1g/L of 3-octanol and an amount of 0,3832g of zeolite.

This time pears were left on *shelf life* and were only placed on the desiccator when it was time to make an analysis. With this sampling method it was intended that the pears would be able to carry out their breathing process normally, being that many days closed in the desiccator without air renewal could have an effect on the results.

Finally, at the end of this experiment, for the analysis of the air of the pears that had been in shelf life for 14 days, the parameter *Time* of MS was changed, which corresponds to the time that elapses since the GC starts the analysis until the moment when the MS system starts to acquire the data. This parameter is used, in particular, to allow the solvent to pass through the column before starting data acquisition in the

MS, so as not to interfere with the analysis. This was set to 2 minutes and was changed to 4,5. This way, results were finally obtained. The problem was thus found in the analysis method, making it possible that results could have been obtained with all the conditions previously tried, such as all adsorbents, heating methods, collection methods or the number of pears placed in the desiccator.

2.5. Detection of volatile organic compounds

The air of the storage chambers was analyzed. An amount of 0,2658g of zeolite was placed in chamber 7, 0,2975g in chamber 8, 0,2695g in chamber 9 and 0,2176g in 10. Zeolite samples were removed from the chambers the next day. The pellets were placed in vials, and then heated in the oven at 170ºC for 5 minutes. The air was removed with a 1 mL syringe through the septum of the vial and injected into the GC-MS, programmed with the instrumental method described in table 1.

Table 2 - GC-MS method (full scan) defined to detect VOCs.

GC		MS	
Injector T (ºC)	150	T. line T (ºC)	180
Modo	Split	Ion source T (ºC)	180
Split ratio	33,3	*Time* (min)	4,5
Flow (mL/min)	1	m/z (amu)	33 - 150
T program	30 ºC (4,5min) → 20 ºC/min to 100 ºC (1 min) → 2 ºC/min to 150 ºC (10 min)		

3. Results and discussion

3.1. Physico-chemical characterization

The evaluation of the quality parameters of Rocha pears from three different orchards was carried out in order to characterize them and the state of ripeness in which they were harvested.

Table 3 - Results obtained for all the physico-chemical characterization analysis.

Samples	Pomar 1	Pomar 2	Pomar 3
Di (mm)	67,7 ± 4,8	60,5 ± 2,0	67,2 ± 2,8
Weight (g)	189,2 ± 39,6	140,3 ± 8,5	175,3 ± 18,0
Firmness (kg/0,5 cm²)	5,08 ± 0,34	4,87 ± 0,62	5,01 ± 0,48
Colour (ºhue)	106,44 ± 2,04	105,21 ± 3,15	107,54 ± 1,89
Starch regression rate	8,6 ± 1,0	7,7 ± 1,3	7,0 ± 0,8
TSS (ºBrix)	11,91 ± 0,74	12,64 ± 0,77	10,48 ± 0,81
pH	4,72 ± 0,24	4,43 ± 0,08	4,42 ± 0,11
TTA (g/L malic ac.)	1,29 ± 0,29	1,63 ± 0,37	1,64 ± 0,24
PC (mg/g gallic ac.)	0,146 ± 0,006	0,148 ± 0,016	0,154 ± 0,002
AC (mg/g ac. ascorbic)	0,103 ± 0,002	0,102 ± 0,010	0,098 ± 0,005
∝-Farnesene (µg/g)	33,4 ± 13,9	28,6 ± 3,6	26,1 ± 20,4
CT (µg/g)	18,3 ± 5,9	12,6 ± 3,1	14,4 ± 12,7

Orchard 1 was the one with the largest average fruit diameter, 67,7 mm, corresponding to the highest average weight, 189,2 g (table 3). Orchards 1 and 3 presented values of size (60/65mm) and weight (123/153g) above the most common for Rocha pear [17]. Fruit diameter, like other characteristics, has a relationship with superficial scald and internal browning, as large-sized fruits are more likely to develop these disorders than smaller ones [18][19].

In the case of Rocha pear, the reference value for pulp firmness at optimum harvest time is between 5,1 and 6,4 kg/0,5cm² [20]. All orchards had an average value lower than the minimum value of the range (Table 3). This means that they were already at a more advanced level of ripeness than the optimum level for harvesting.

The color was very similar to every orchard, the highest being 107,54º ± 1,89 (Table 3) so it can be concluded that these pears had a light green tone.

For the fruit to be considered of superior quality, there are minimal residues and optimal residues that must be present at harvest time, these being 12 and 14% respectively [20]. As can be seen from the data in table 3, the TSS of two orchards are below the mentioned range, with orchard 2 having the highest value (12,64 ± 0, 77 ºBrix).

In view of the success of cold storage, most fruits should have values for the starch regression rate between 5 and 7 at harvest. Fruits with values equal to or greater than 8 will have to enter the commercial circuit as soon as possible. From the results obtained (Table 3), it was concluded that the fruits of the three orchards were already in a more advanced stage of ripeness than they should have been for the optimum moment of harvest.

In order to obtain a characteristic and good quality Rocha pear, the acidity should have values between 2 and 3 g/L of malic acid. The amount of acids and sugars and in particular the balance of these two constituents are responsible for the organoleptic perception of the fruits by the consumer. The results obtained, for the titratable acidity at harvest, were quite similar for the three orchards, all of which are below the minimum value indicated for the fruit at the time of harvest. The highest value of the three orchards was 1,64 ± 0,24 g malic acid / L for orchard 3.

Regarding the antioxidant capacity, the total AC of the fruits and the amount of PC present were evaluated. The three orchards showed very close values, of 0,146 ± 0,006 mg gallic acid/g of fruit for orchard 1, 0,148 ± 0,016 mg of gallic acid/g of fruit for orchard 2 and 0,154 ± 0,002 mg gallic acid/g of fruit for orchard 3 (Figure 32), the last one having the highest phenol content.

The average total AC of the fruits of the orchards was 0,103 ± 0,002 mg of ascorbic acid/g of fruit for orchard 1,

0,102 ± 0,010 for orchard 2 and 0,098 ± 0,005 for orchard 3 (Table 3), which represent quite similar results between orchards. Thus, despite the fact that Orchard 3 had a higher content of PC, it is expected that, if there are physiological problems, the fruits of Orchard 1 are less likely to develop superficial scald and internal browning since the AC would protect better the fruits from oxidative damage [21][22].

The amount of α-farnesene is also related to physiological accidents, in particular superficial scald. Its oxidation results in the production of the CTs. The amount of CTs then also depends on the antioxidants that exist in the epidermis of the fruits. Orchard 1 was the one with the highest average concentration of both α-farnesene (33,4 ± 13,9 μg/g fruit) and CTs (18,3 ± 5,9 μg/g fruit). Orchard 3 had the lowest concentration of α-farnesene (26,1 ± 20,4 μg/g fruit) and Orchard 2 the lowest concentration of CTs (12,6 ± 3,1 μg/g fruit) (Table 3). It could be said then that the most likely to develop and have superficial scald incidents would be the orchard 1.

After analyzing the maturation indices, it is possible to conclude that the fruits of the three orchards were already in a state of maturity superior to the state of optimum maturity for harvest. They present a pulp firmness, TTA, TSS and lower than those recommended and a starch regression index higher than recommended. In fact, this year the harvest took place a little later

Despite being larger fruits and having a more appealing color and flavor, they can count on a shorter storage time, a marked softening and a greater susceptibility to physiological disorders, namely internal brownings, which will lead to a probable loss of quality and commercial value.

3.2. VOCs analysis

To identify the different components of the sample (unknown), in this case VOCs with a mass spectrum, using the full scan mode is essential. On the other hand, the SIM mode is suitable for making a quantitative analysis of the components that are known to be present in the sample, when the mass spectrum of the components to be analyzed is known, such as ethylene.

It was then concluded that it was necessary to develop two different methods. One for the detection of ethylene, and the other for all other VOCs.

3.2.1. Ethylene detection

It was detected only for chamber 9 (AN) a peak whose mass spectrum presented the ions of m/z 24, 25, 26 and 27, representative of the mass spectrum of ethylene. However, the peak in the chromatogram was not as defined as expected. The other chambers showed a similar peak in the chromatogram, but the respective mass spectrum showed only peaks in m/z 26 and 27.

When using the SIM mode to detect a specific compound, and then quantify it, ions that are abundant should be considered, as they are easily detectable even at low concentrations. In the case of choosing an ion with a very low abundance, despite being a single ion of the compound, this compound may not be detected if it is in low concentrations [23]. This may be the case for ethylene m/z 24 and 25 ions, which have a much lower relative abundance compared to m/z 26 and 27 ions. For this reason it can be explained that not all the peaks appear in the samples of the storage chambers, since they have very low concentrations of ethylene. Naturally, chamber 9 is the one with the greatest amount of ethylene, as it is under normal atmosphere conditions and is therefore the one that least delays fruit ripening.

3.2.2. Volatile organic compounds profile

When analyzing the sample of pears placed in the desiccator after 14 days of shelf life with the optimized method, it was possible to identify 40 volatile organic compounds that are shown table 4, with the respective retention time, peak area of the chromatogram, percentage of area and percentage of height, in order to understand the relative abundance of each compound. The probability of identifying the compounds given by the software was also presented. Compounds from all groups were identified as esters, alcohols, aldehydes, ketones and hydrocarbons.

The most abundant compounds were alcohols (43,35%) followed by esters (32,96%), which is not the case for other studies, as esters usually represent the most significant group of compounds produced by pears [13][24][25][26]. However, it is necessary to take into account that the fruits were not in the same conditions. The marked compounds (with *) were not mentioned in the consulted literature.

The compound that appeared with the highest relative abundance and the largest peak area was 1-hexanol (35,15%). Although it was not initially defined as one of the most interesting compounds to analyze, it is a very abundant compound in some types of pear [27][28]. However, it's possible meaning or function has not been explained. In an experiment carried out on apples, it was concluded that the amount of hexanol can be a good indicator of the development of soft scald, since apples with a higher incidence of this physiological accident had higher levels of this compound [29]. It could then be of great interest to check if this compound is also related to the scald in Rocha pear. The most abundant compound after 1-hexanol was hexyl acetate, which may make sense since the presence of hexanol produces an increase in the amount of this compound [29].

Ethanol was also one of the most abundant compounds. This is a product of the anaerobic metabolism that may have started since the pears were closed in the desiccator for many hours (24h) without having the air renewed. This may be related to the incidence of internal browning [14].

One of the most abundant alcohols was also 1-butanol, one of the most important alcohols for pear being responsible for the fruity aroma. This is necessary for the production of butyl acetate [9] which together with hexyl acetate are the most abundant esters in Rocha pear [28].

Table 4 - VOCs identified for Rocha pear, at 14 days of shelf life. Compounds with respective retention time, probability of identification given by the software, area, %area and %hight of the peaks.

	RT	Compound	R. Match	Prob. (%)	Area	%Area	%Height
Eter	4,84	Ethylic ether*	908	92,64	2,86E+07	0,76	0,51
Esters	9,66	Ethyl acetate	933	92,09	2,28E+07	0,61	0,61
	13,82	Butyl acetate	928	91,49	1,13E+08	3,01	4,39
	16,3	Pentyl acetate	904	78,06	1,46E+07	0,39	0,47
	17,56	Butyl butanoate	912	76,88	2,35E+06	0,06	0,06
	17,95	Butyl-2-metyl-butanoate	886	37,4	2,18E+06	0,06	0,04
	18,05	Ethy hexanoate	891	74,16	5,09E+06	0,14	0,15
	19,39	Hexyl acetate	939	88,91	9,06E+08	24,16	25,75
	21,58	Octyl acetate	826	24,9	2,25E+06	0,06	0,06
	23,24	Heptyl acetate	916	76,38	6,14E+06	0,16	0,15
	24,88	Buthyl hexanoate	890	73,17	1,58E+07	0,42	0,36
	24,99	Hexyl butanoate	943	83,39	1,37E+07	0,37	0,32
	25,46	2-metyl-hexyl butanoate	904	73,18	6,31E+06	0,17	0,14
	25,82	Ethyl octanoate	908	83,42	2,01E+07	0,54	0,46
	27,02	Ethyl (E)-2-octenoate	771	66,63	2,88E+06	0,08	0,06
Alcohols	10,82	Ethanol	988	89,9	2,39E+08	6,38	5,72
	15,78	1-Buthanol	932	65,7	1,17E+08	3,11	3,24
	17,37	2-metyl-1-Buthanol	912	46,84	5,24E+06	0,14	0,12
	18,71	1-Penthanol	947	65,99	1,35E+07	0,36	0,38
	22,38	1-Hexanol	915	52,65	1,32E+09	35,15	33,56
	26,77	1-Heptanol	932	52,44	1,09E+07	0,29	0,24
Aldehydes	5,81	Acetaldehyde	970	85,72	1,27E+07	0,34	0,23
	7,42	Propanal	848	63,7	1,58E+06	0,04	0,03
	9,4	Butanal	888	77,46	7,69E+06	0,21	0,16
	11,82	Pentanal	856	45,6	1,50E+07	0,4	0,39
	14,13	Hexanal	907	72,13	2,69E+07	0,72	0,81
	16,82	Heptanal	808	36,72	3,80E+06	0,1	0,08
Hidrocarbons*	5	cis-1-etyl-2-metyl-cyclopropane	860	18,61	3,90E+06	0,1	0,05
	5,53	Heptane	895	51,85	1,38E+07	0,37	0,21
	6,72	(E,Z)-2,4-Hexadiene	938	10,43	1,91E+06	0,05	0,04
	7,22	Octane	817	12,82	1,28E+06	0,03	0,03
	7,61	(Z),(Z)-2,4-Hexadiene	908	19,72	2,24E+06	0,06	0,04
	12,48	(1,2-dimetylpropyl)-ciclopropane	840	41,34	5,26E+06	0,14	0,14
	12,75	2,5-dimetyl-2,4-Hexadiene	936	23,94	1,09E+07	0,29	0,38
	13	1,5-dimetyl-1,4-ciclohexadiene	902	30,67	3,14E+06	0,08	0,08
	15,61	p-Xilene	973	38,48	2,34E+07	0,62	0,72
Ketones	10,03	2-Butanone	934	87,46	3,73E+06	0,1	0,09
	12,18	2,4-dimetyl-3-Pentanone*	944	71,69	1,10E+07	0,29	0,33
	13,37	1-(2-metyl-1-cyclopenten-1-yl)-etanone*	856	42,1	1,77E+07	0,47	0,63
	18,99	3-Octanone*	899	66,95	2,17E+07	0,58	0,63

Thus, the gaseous atmospheres of the chambers placed under the conditions described in table 5 were analyzed.

Table 5 - Storage conditions of chambers number 7, 8, 9 and 10.

Chamber	T (ºC)	HR (%)	O_2 (%)	CO_2 (%)	1-MCP
7	- 0,5	90-95	4	0,5	312 (ppb)
8			0,5	0,5	-
9			20,8	0,03	-
10			2,5	0,5	-

In order to maintain the organoleptic quality of the fruit, it is important to know how the composition of the VOCs produced is influenced by the treatments that are applied [30].

It is necessary to bear in mind that in most studies, pears are removed from storage conditions, placed in jars and left at room temperature until they reach equilibrium [13][28]. In this work, the compounds that were produced inside the chambers were directly analyzed.

The air in the chambers was analyzed on December 9, 2020, corresponding to approximately four months of storage.

Table 6 - VOCs of most significance detected for chamber 7 with respective retention time (RT), probability, area, %area and %height of the chromatogram peaks.

Compound	RT (min)	Prob. (%)	Area	%Area	%Height
Acetaldehyde	5,87	81,23	1,14E+07	1,61	1,13
Ethyl acetate	9,69	90,55	6,21E+06	0,88	0,9
Ethanol	10,86	90,07	2,48E+08	35,06	42,83
Butyl acetate	13,86	86,55	5,26E+06	0,74	1,06
1-butanol	15,81	74,2	1,14E+07	1,61	1,76
Hexyl acetate	19,43	91,31	8,08E+06	1,14	1,09
1-hexanol	22,42	57,31	3,10E+07	4,38	3,79

In 1-MCP conditions the compounds detected with the greatest relative abundance were ethanol, 1-hexanol, 1-butanol, acetaldehyde and hexyl and butyl acetates (Table 6). 1-MCP is an inhibitor of ethylene action. Since ethylene is the compound that promotes fruit ripening, if it is inhibited, the fruits will not ripen, so the production of volatile compounds in these conditions will be much less than normal.

Table 7 - VOCs of most significance detected for chamber 8 with respective retention time (RT), probability, area, %area and %height of the chromatogram peaks.

Compound	RT (min)	Prob. (%)	Area	%Area	%Height
Acetaldehyde	5,88	83,43	3,65E+07	3,32	2,38
Ethyl acetate	9,7	90,78	2,56E+06	0,23	0,23
Ethanol	**10,85**	**89,08**	**7,56E+08**	**68,75**	**71,77**
Propyl acetate	11,7	87,03	1,57E+05	0,01	0,02
Butyl acetate	13,86	92,62	2,52E+06	0,23	0,29
1-Butanol	15,83	69,64	2,29E+07	2,08	1,78
Hexyl acetate	19,25	87,01	4,53E+06	0,41	0,36
1-Hexanol	22,5	60,77	4,35E+07	3,95	3,29

In ULO conditions the compound with the greatest relative abundance was also ethanol (Table 7), having been the largest of those recorded for the different storage chambers. This can once again be explained by some prevalence of anaerobic respiration since this is the chamber with the lowest level of oxygen. However, other volatile compounds were also produced, with 1-hexanol and acetaldehyde being the compounds with greater abundance after ethanol. The relative abundances of esters were not significant, which goes accordingly with what was said earlier.

Studies have found that low levels of oxygen during the cold storage period affect the fruit's metabolism, reducing its yellowing, inhibiting the production of ethylene and some esters and affecting its emission during the subsequent shelf life period [31][32]. For Conference and Alexander Lucas pears it was found that this reduction was less pronounced in ULO than for treatments with 1-MCP, and that it occurs regardless of the state of ripeness at harvest [33].

Table 8 - VOCs of most significance detected for chamber 9 with respective retention time (RT), probability, area, %area and %height of the chromatogram peaks.

Compound	RT (min)	Prob. (%)	Area	%Area	%Height
Acetaldehyde	5,83	81,67	7,28E+06	2,86	1,99
Ethyl acetate	9,68	83,38	9,46E+05	0,37	0,35
Ethanol	10,83	88,93	3,55E+07	13,94	15,46
Butyl acetate	13,87	92,34	6,46E+06	2,54	3,11
1-Butanol	15,56	59,88	2,53E+06	0,99	0,93
Hexyl acetate	19,59	73,49	4,05E+06	1,59	0,68
1-Hexanol	22,58	41,24	4,50E+06	1,76	0,62

In NA conditions the compound that showed the greatest relative abundance after ethanol was butyl acetate and then acetaldehyde, 1-hexanol and hexyl acetate (Table 8). For all chambers the most abundant compound was ethanol (Figure 1), with less relative abundance in chamber 9, as would be expected since it is the chamber with a higher oxygen level, allowing pears to perform aerobic respiration. However, even under AN conditions, pears partly demonstrate anaerobic respiration along with aerobic metabolism [33]. The AN chamber is also the one with the greatest variety and quantity of VOCs in terms of relative abundance.

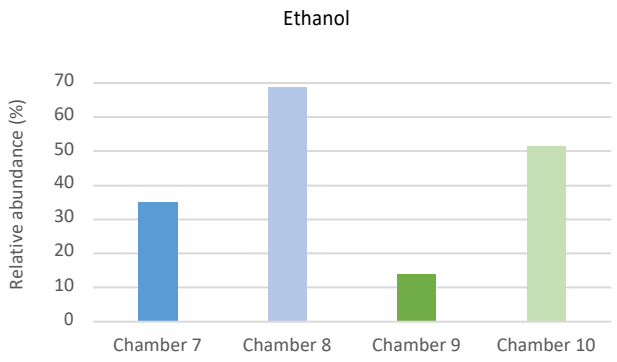

Figure 1 - Relative abundance of ethanol in the different storage chambers.

Table 9 - VOCs of most significance detected for chamber 10 with respective retention time (RT), probability, area, %area and %height of the chromatogram peaks.

Compound	RT (min)	Prob. (%)	Area	%Area	%Height
Acetaldehyde	5,82	83,48	1,08E+07	2,54	1,58
Ethanol	10,81	90,52	2,19E+08	51,32	50,8
Butyl acetate	13,81	92,48	1,66E+06	0,39	0,46
1-Butanol	15,81	56,57	1,20E+07	2,8	2
Hexyl acetate	19,29	70,35	1,92E+06	0,45	0,34
1-Hexanol	22,39	60,13	8,49E+06	1,99	1,5

In CA conditions the compound that presented a relative abundance much higher than the other VOCs was ethanol, followed by 1-butanol, acetaldehyde and 1-hexanol, with the relative abundances of the esters already being considerably lower (Table 9).

There is also a decrease in the production of VOCs in fruits stored in controlled atmosphere, after returning to normal atmosphere and room temperature conditions.[8][9]

As seen in the analysis of the air in the different chambers under study (Figure 2), Rizzolo et. al. (2005) also found that under conditions of AN, CA and different amounts of 1-MCP, the compound present in the atmospheres, in greater concentration, was always ethanol [31].

For all storage conditions it was found that the same compounds were always produced with greater importance. However, they showed different relative abundances between them for each chamber (Figure 2). For chambers 8 and 10 the esters did not show significant relative abundances in comparison with the other compounds of interest that were detected. For chamber 9, esters were the most significant compounds after ethanol and for chamber 7, butyl and hexyl acetate only appeared after alcohols and acetaldehyde, but with more significant percentages (above 1%). It was also found that only for chamber 9, butyl acetate was detected in greater abundance than hexyl acetate.

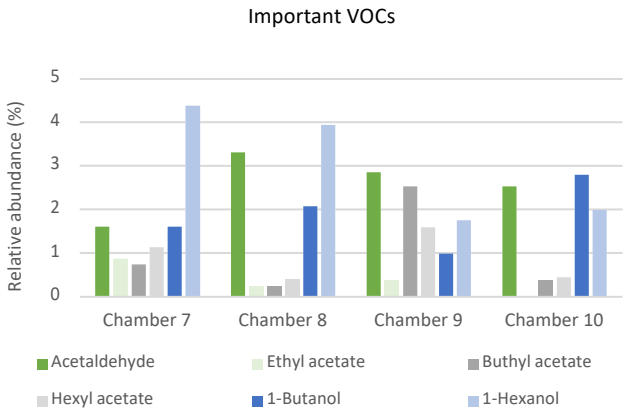

Figure 2 - VOCs of interest detected in each chamber.

4. Conclusions

After doing the physico-chemical characterization it was concluded that the fruits were already in a slightly more advanced stage of ripeness than that defined for the optimum harvest time. In fact, pears were harvested later this year.

A more advanced state of maturity can lead to more problematic conservation and storage, in which more physiological accidents may occur.

It would then be interesting to relate the state of ripeness of the pears to the volatile compounds they produce and in what quantities. Thus, it was necessary to develop an analytical method based on GC-MS to perform the detection, identification and quantification of volatile organic compounds produced by pears.

It was concluded that it would be necessary to define and use two specific methods to carry out the analysis of ethylene and to carry out an analysis of the remaining volatile organic compounds.

It was found that there was a great difficulty in optimizing a method for the detection of ethylene by GC-MS due to the complexity problems of the samples themselves, and since this compound was in low concentrations.

It was possible to define a method in GC-MS to establish the profile of VOCs produced and emitted by the pears. The operating conditions were as follows: injector temperature 150ºC; split mode, split ratio of 33,3. Carrier gas: helium with a flow rate of 1 mL/min. The oven temperature was programmed at 30ºC for 4,5 minutes; increase of 20ºC/min up to 100ºC, 1 minute at 100ºC followed by an increase of 2ºC/min up to 150 ºC and 10 minutes at this temperature. The detection was made using 70 eV of ionization energy at an ion source temperature of 180ºC and a transfer line temperature of 180 ºC. It was also programmed to do a full scan in a range from 33 to 150 m/z.

Once the method was defined, the gaseous atmosphere surrounding the pears placed under different conditions of storage and shelf life could be analyzed, having detected more than 40 VOCs including esters, alcohols, aldehydes, ketones and hydrocarbons.

For pears in shelf life for 14 days, the presence of 1-hexanol, hexyl acetate, ethanol and 1-butanol was observed, with the group of compounds present in greater abundance being alcohols (43%) and the main one 1- hexanol (35%).

It was concluded that in all storage conditions used, (controlled atmosphere, ULO atmosphere or with 1-MCP) the compound that pears produce with the greatest intensity is ethanol. They also produce other alcohols mainly, 1-butanol and 1-hexanol. For pears preserved in the presence of 1-MCP, under AC and ULO conditions, there was a greater production of alcohols than esters. The ULO chamber had the highest relative abundance of ethanol (68%), followed by AC (51%), 1-MCP (35%) and finally AN (14%).

Also, despite the fact that 1-hexanol had not been identified as a compound of greater interest for pear in the literature review, after this study it was found that it was always present and is among the compounds of greatest relative abundance. It was often the most abundant compound after ethanol.

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