

## Effects of Addition of $\alpha$ -Cyclodextrin on the Sensory Quality, Volatile Compounds, and Color Parameters of Fresh Pear Juice

JOSÉ M. LÓPEZ-NICOLÁS,<sup>\*,†</sup> ANTONIO J. ANDREU-SEVILLA,<sup>‡</sup>  
ÁNGEL A. CARBONELL-BARRACHINA,<sup>‡</sup> AND FRANCISCO GARCÍA-CARMONA<sup>†</sup>

<sup>†</sup>Department of Biochemistry and Molecular Biology-A Faculty of Biology, University of Murcia, Campus de Espinardo, 30100-Murcia, Spain, and <sup>‡</sup>Department of AgroFood Technology, Food Safety and Quality Group, Miguel Hernández University, Carretera de Beniel, km 3.2, 03312-Orihuela, Alicante, Spain

Cyclodextrins (CDs) are widely used as browning inhibitors in different fruit juices. However, pear juice quality is affected by many properties, such as odor and aroma, and to date, no paper has reported the effect of the addition of CDs on the flavor profile of a fruit juice. In this study, the aroma profile of pear juice was mainly formed by volatile compounds from four chemical families: esters, aldehydes, alcohols, and hydrocarbons. Even though the addition of  $\alpha$ -CD had a significant effect on both the concentration of individual volatile compounds and their grouping, only the highest concentration, 90 mM, prevented the oxidation of the volatile precursors present in freshly squeezed juice. Moreover, correlation of these results, concerning the color and aroma of pear juice in the presence of CDs, with the consumer preferences has not been reported. A descriptive sensory analysis of pear juices in both the presence and the absence of CDs was carried out, and odor/aroma attributes (fresh, fruity, pear-like, unnatural, etc.), plus global color, odor, aroma, and quality, were quantified using a trained panel of judges. The addition of  $\alpha$ -CD at 90 mM resulted in pear juices with the best color but with low aromatic intensity and low sensory quality. On the other hand, the addition of  $\alpha$ -CD at 15 mM led to a pear juice also with an acceptable color but at the same time with a high intensity of fruity and pear-like odors/aromas, making it the best appreciated juice by the panel.

**KEYWORDS:** cyclodextrin; flavor; sensorial quality; color; volatiles; pear juice

### INTRODUCTION

In order to improve the sensory quality of fruit juices, many studies have paid attention to changes in organoleptic quality and methods of preventing sensory quality losses in the product (1). Several studies have focused on color and aroma as the most important sensory attributes, which can influence consumer preferences (2–4). Thus, the preservation of these organoleptic properties in fruit juice during processing and storage is one of the main objectives of fruit processors. To limit color or aroma degradation or loss during processing and storage, or to increase a characteristic organoleptic property, several papers have reported the advantages of encapsulating volatile ingredients prior to use in foods and beverages (5).

In order to improve the shelf life of foods, several treatments have been applied such as the presence/absence of oxygen, reducing substances, pH, temperature, activation/inactivation of different oxidizing enzymes, or the use of commercial encapsulation practices such as spray-drying, freeze-drying, extrusion, co-acervation, and co-crystallization (6). However, many of these treatments present serious disadvantages for use in the food industry because they can have negative effects on the sensory properties of the products (7). Moreover, some chemical treatments

have been associated with severe allergy-like reactions in certain populations, for which reason the FDA has restricted their use to only a few applications (1).

For these reasons, one of the main goals of the food industry is to develop new additives that improve the color and flavor of foods. In this respect, there has been a renewal of interest in natural additives that improve the sensory properties of foods, and among the most promising of such agents are cyclodextrins (CDs). The hydrophobic cavity of CDs is able to form inclusion complexes with a wide range of organic guest molecules related with the sensory properties of food products which are encapsulated by CDs (8, 9).

In recent years, the color of fruit juices, such as apple, banana, pear, or grape, has been evaluated in the presence of natural and modified CDs, and the effect of these compounds as browning inhibitors has been determined using the CIEL\*a\*b\* color space system (10–14). These studies showed that the fruit juice enzymatic browning could be slowed down (or activated) when increasing concentrations of different CDs were added. However, no attempt to correlate these results with the consumer preferences has been reported. For this reason, it is necessary to study whether the significant differences in total color due to the presence of CDs in fruit juices play an important role in consumer satisfaction and influence the further consumption of fruit juices.

\*To whom correspondence should be addressed. Phone: 34 968 364765. Fax: 34 968 364147. E-mail: josemnl@um.es.

**Table 1.** Volatile Compounds Studied with Their Identification Method, Kovats Indices for the Present Study, and Literature Values and Odor Thresholds

volatile compound	peak	identification <sup>a</sup>	Kovats Index (KI)		threshold (mg L <sup>-1</sup> ) <sup>c</sup>	descriptor
			experimental	literature <sup>b</sup>		
1-butanol	1	std, KI	673	669	0.5	cheesy, light-fruity
propyl acetate	2	std, KI	736	728	0.7	pungent, penetrating, strong-sweet
2-methyl-1-butanol	3	std, KI	764	755	0.3	fruity
hexanal	4	std, KI	803	801	0.0045	green, grass-like, fruity
butyl acetate	5	std, KI	817	813	0.066	apple-like, fresh, fruity
<i>trans</i> -2-hexenal	6	std, KI	851	856	0.017	green, apple-like, grassy
1-hexanol	7	std, KI	873	864	0.5	grass, fruity, light apple
hexyl acetate	8	std, KI	1012	1010	0.002	sweet fruity
nonanal	9	std, KI	1102	1103	0.001	fatty
hexyl 2-methylbutanoate	10	tentatively identified	1208	1220	0.022	pungent
1-decanol	11	std, KI, W	1271	1274		oily, fatty
2,4-decadienal	12	std, KI, W	1317	1316	0.00007	green
$\beta$ -ionone	13	internal standard	1483	1484		
<i>trans</i> - $\alpha$ -bergamotene	14	tentatively identified	1492	1480		
$\alpha$ -farnesene	15	tentatively identified	1506	1500		mild sweet, green herbaceous
coniferol	16	tentatively identified	1739	1729		

<sup>a</sup> "Std, KI, W" means that retention times and Kovats indexes of authentic standards were used for identification besides the general comparison of experimental mass spectra to spectra of authentic standards and Wiley library (W). "KI, W" means that identification was based on Kovats indexes and comparison with Wiley library. "W" compounds tentatively identified (only Wiley library was used for identification). <sup>b</sup> NIST database (27). <sup>c</sup> From references (25, 28, 31).

Moreover, although many investigations have focused on preparing various encapsulated flavors by CDs (15), no paper has reported the effect of the addition of CDs on the flavor profile of a fruit juice. Indeed, this is the first experiment to study the effects of the CD addition on both odor and aroma (components of flavor), using sensory and instrumental protocols. Thus, in this paper, we have studied the effect of the addition of  $\alpha$ -CD on the sensory quality of a pear juice, one of the most frequently consumed fruit juices in Spain (16).

Bearing in mind the above, this work sets four principal objectives: (i) to analyze instrumentally the effect of the addition of  $\alpha$ -CD on the color and volatile compounds of pear juice; (ii) to study the intensities of odor, aroma, and global quality of the pear juice in the presence of different concentrations of  $\alpha$ -CD by a trained sensory panel; (iii) to correlate the instrumental and sensory parameters of this product in the presence of  $\alpha$ -CD; and (iv) according to the obtained results in the previous objectives, to select the optimum concentration of  $\alpha$ -CD to be added as additive to the pear juice.

## MATERIALS AND METHODS

**Chemicals.** The biochemicals were purchased from Fluka (Madrid, Spain).  $\alpha$ -CD was purchased from Sigma-Aldrich (Madrid, Spain) and used as received.

All the aroma standards (1-butanol, propyl acetate, 2-methyl-1-butanol, hexanal, butyl acetate, *trans*-2-hexenal, 1-hexanol, hexyl acetate, 1-decanol, and 2,4-decadienal) used for identification and quantification purposes were food grade (Sigma-Aldrich, Flavours and Fragrances, Milwaukee, WI).

**Equipment and Experimental Procedure. Juice Preparation.** Pears (*Pyrus communis* cv. Conference) were purchased from local supermarkets and stored at 4 °C until needed. They were peeled, cored, and sliced prior to juicing in a Moulinex Y36 blender. The pear juice obtained was immediately collected and mixed in a beaker containing the correspondent volume of distilled water alone or plus  $\alpha$ -CD (15–90 mM). To evaluate the effect of the CDs addition on both instrumental (color and volatile compounds) and sensorial analysis, all of the juices except fresh pear juice (i.e., oxidized, 15, 45, and 90 mM  $\alpha$ -CD) were placed on a magnetic stirrer for 20 min to be oxidized in both the presence and the absence of  $\alpha$ -CD.

Because this was the first paper studying the effects of CDs on the aroma of juices, a simple matrix was required. Different pear cultivars, available at the Spanish market, were tested, and finally, the cultivar

Conference was selected because it presented the simplest possible aroma profile.

**Color Changes Assessment.** To measure the color parameters, a ColorFlex version 1.72 colorimeter (Hunterlab, Reston, USA) certified by ISO 9001 with a D75 light source and the observer at 10° was used at 25 °C.

The color parameters corresponding to the uniform color space, CIEL\*a\*b\* (17), were obtained directly from the apparatus. Within the uniform space, two color coordinates,  $a^*$  and  $b^*$ , as well as a psychometric index of lightness,  $L^*$ , are defined. In this system,  $a^*$  takes positive values for reddish colors and negative values for greenish ones, whereas  $b^*$  takes positive values for yellowish colors and negative values for bluish ones.  $L^*$  is an approximate measurement of luminosity, which is the property according to which each color can be considered as equivalent to a member of the gray scale, between black and white, with values ranging from 0 to 100. The total color difference ( $\Delta E^*$ ), a single value which takes into account the differences between  $L^*$ ,  $a^*$ , and  $b^*$ , of the sample and standard was also studied.

For all experiments, the previously described mixtures containing pear juice were used in the color evolution assays, using the measurements at time 0 as standard. This 0 time corresponded to the first measurement, which was made 1 min after the pears had been juiced and the materials dissolved in the juice. All of the measurements were made at different times during the first 40 min after the materials had been dissolved in the pear juice (i.e., just when the enzymatic browning was beginning).

Three readings were obtained for each replicate to obtain uniform color measurements. Total color difference ( $\Delta E^*$ ) was calculated using the following equation:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

**Extraction of Volatile Compounds.** Liquid–liquid direct extraction method was adopted to extract volatile compounds from the pear juice. This extraction method was previously used by Komthong et al. (27) studying the odors of apple juice during browning and will extract all volatile compounds from pear juice, including those encapsulated in  $\alpha$ -CD complexes. This is one of the simplest extraction methods available in the literature, but its extracts will reflect the juice composition and will not create any artifact. Therefore, this extraction method is adequate for the first studies on the effects of CD addition on the foods' aromatic composition, although when the basic effects are already established, more sophisticated methods, such as solid-phase microextraction (SPME), will be needed. The fresh or oxidized (in both the presence and the absence of different  $\alpha$ -CD concentrations) juice sample (200 mL), prepared as it has been described in the Juice Preparation section and with an internal standard of  $\beta$ -ionone (final concentration 0.50 mg L<sup>-1</sup>), was placed into a 500 mL Erlenmeyer flask. The volatile compounds were extracted with a

**Table 2.** Odor and Aroma Attributes Selected for DSA (28–30)

attribute of odor/aroma	definition
green	associated with odor/aroma of freshly cut green grass (hexanol)
acid	associated with a fresh, sour odor/aroma
fresh	associated with a fresh, new odor/aroma
floral	associated with the odor/aroma of flowers (linalool)
fruity	odor of fruit (e.g., apple, banana, peach (2-/3-methylbutanol)
cooked/caramel	associated with a sweet odor/aroma (e.g., boiled or burnt sugar)
pear-like	odor of Conference pear
unnatural	odor of untypical pear juice, undesirable odor/aroma

dichloromethane, total volume 50 mL. To avoid further oxidation of the juices, 20 mL of saturated  $\text{CaCl}_2$  was added to the juices before starting the extraction. The juice sample with solvents was continuously and gently shaken in a rotary shaker (CertomatR, B. Braun Biotech International, Gaithersburg MD) at very low revolutions, 70 rpm, for 30 min at room temperature. The solvent fraction was then separated from the juice sample using a separating funnel. The solvent extract was dried over 5 g of anhydrous  $\text{Na}_2\text{SO}_4$  (Panreac Química S.A., Barcelona, Spain) and concentrated to about 0.25 mL in a Vigreux column. The volatile compounds in the concentrate were determined by GC–MS.

**GC–MS Analytical Conditions.** The isolation, identification, and quantification of the volatile compounds were performed on a gas chromatograph, Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan), coupled with a Shimadzu mass spectrometer detector GC–MS QP-5050A. The GC–MS system was equipped with a TRACSIL Meta X5 column (Teknokroma S. Coop. C. Ltd., Barcelona, Spain; 30 m × 0.25 mm × 0.25  $\mu\text{m}$  film thickness with silphenylene phase). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL  $\text{min}^{-1}$  in a split ratio of 1:10 in the following program: (a) 40 °C for 5 min; (b) rate of 3.0 °C  $\text{min}^{-1}$  from 40 to 200 °C and held for 1 min; (c) rate of 15 °C  $\text{min}^{-1}$  from 200 to 280 °C and held for 10 min. Injector and detector were held at 250 and 300 °C, respectively. Two microliters of the extracts was always injected.

Mass spectra were obtained by electron ionization (EI) at 70 eV, and a spectrum range of 45 to 450  $m/z$  was used.

**Table 1** shows the authentic chemicals used as standards, the system of identification of each compound and experimental and literature Kovats indices (KI). Most of the compounds were identified by simultaneously using three different analytical methods: (1) KI, (2) GC–MS retention indices (authentic chemicals), and (3) mass spectra (authentic chemicals and Wiley spectral library collection). Identification was considered tentative when based only on mass spectral data. For the quantification of the volatile compounds,  $\beta$ -ionone was added as internal standard (50  $\mu\text{L}$  of 1 g  $\text{L}^{-1}$ ) at the beginning of the extraction process (data included in this study should be considered as semiquantitative because no standard curves were carried out for individual quantified volatile compounds).

**Sensory Evaluation with Trained Panel.** A panel of seven panelists, aged 20 to 42 years (4 female and 3 male), was trained in descriptive evaluation of fruits and juices. Details about panel selection and training can be found in a recent work (18).

Descriptive sensory analysis (DSA) has been successfully used for comparing odor and taste attributes in foods and their products (19, 20). Pear juices were assessed using a flavor profile method (21).

Prior to DSA, panelists discussed the odor and aroma attributes of pear juice during two preliminary orientation sessions, each lasting 90 min, until they had agreed on their use of odor and aroma attributes. During these orientation experiments, panelists evaluated five different coded samples of pear juice from different manufacturers. A total of 19 attributes were identified, and standards were made available for panelists. DSA was performed for the 19 odor and aroma attributes listed in **Table 2**, and color intensity was also considered to allow comparison with previous data.

Measurements were performed in individual booths with controlled illumination (70–90 fc) and temperature ( $23 \pm 2$  °C) (21, 22).

Individual pear juices were scored for the intensity of the studied attributes using a 100 mm long line with line anchors of 0 = no intensity and 100 = very intense.

Panelists relied on their training experience to score products. Samples (coded with a three-digit random number) were presented randomly to each panelist to evaluate (samples were evaluated in triplicate).

**Statistical Analysis.** For each of the above-mentioned analyses, at least three replications were carried out. The  $\pm$  values represent the standard errors of the measurements.

Data from the DSA experiments were subjected to analysis of variance (ANOVA) and the Tukey's least significant difference multicomparison test to determine significant differences among samples.

Statistical analyses were carried out using Statgraphics Plus 5.1 software (Manugistics, Inc., Rockville, MD) and graphics using Sigma Plot 9.0 (SPSS Science, Chicago, IL).

## RESULTS AND DISCUSSION

**Selection of the Optimum Type of Cyclodextrin To Improve the Sensory Properties of Pear Juice.** In recent years, several types of CDs, natural and modified, have been used to complex different guest molecules with high nutritive and sensory values. However, only the three natural types of CDs ( $\alpha$ -,  $\beta$ -, and  $\gamma$ -CDs) have been approved by the FDA for the food use and have GRAS status. Moreover, recently, all three natural CDs have been included in the European lists of additives approved for food use, and the correspondent E-numbers have been assigned for  $\alpha$ -,  $\beta$ -, and  $\gamma$ -CDs (E-457, E-459 and E-458, respectively).

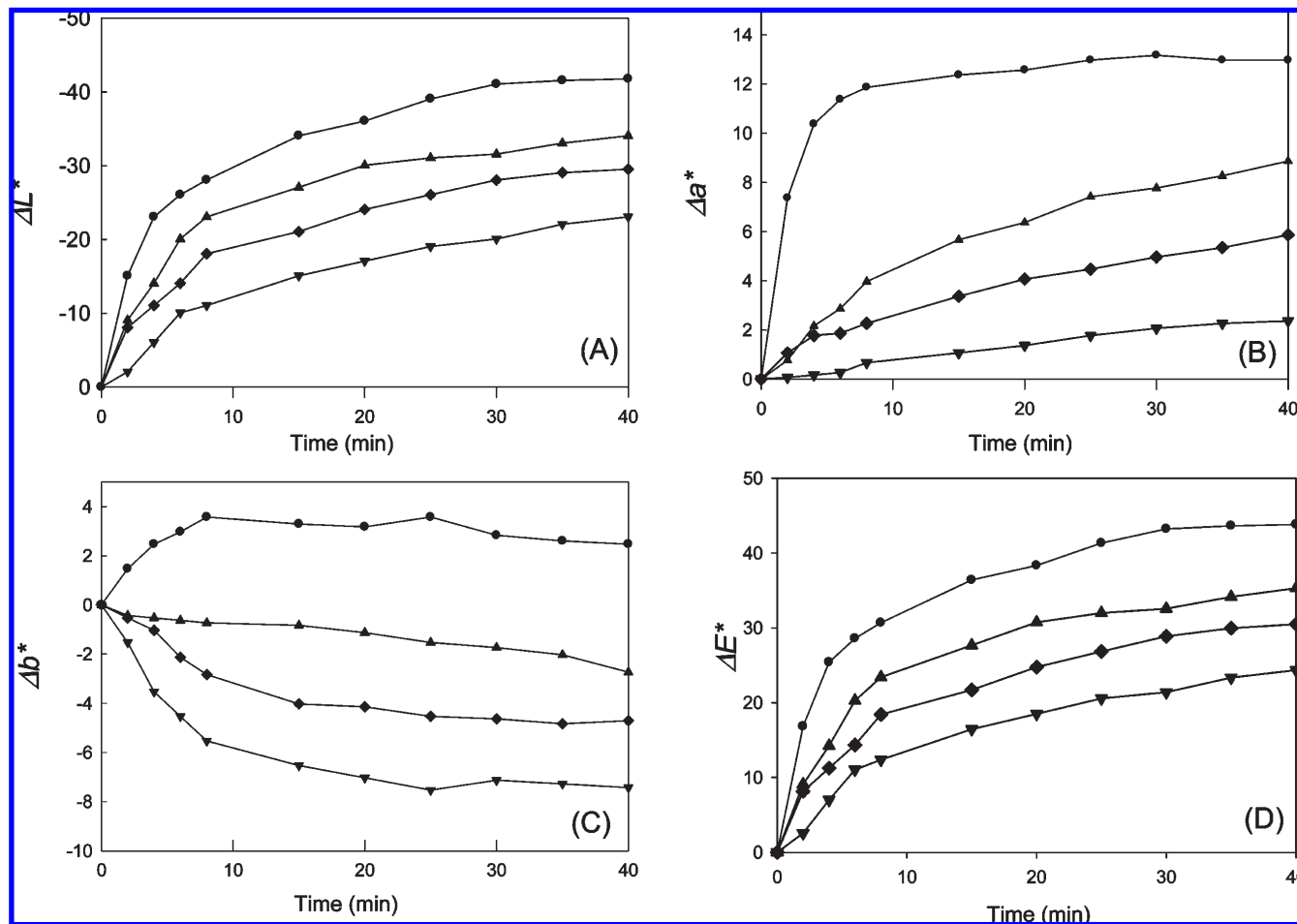
Even though the three natural CDs can be used in the food industry, in this paper, we have used only  $\alpha$ -CD to evaluate the sensory quality of pear juice for the following reasons:

- although  $\beta$ -CD is the most reported type of CD to complex different guest molecules, several papers have demonstrated that its poor solubility in aqueous medium makes it not the most suitable CD for the food industry (8);
- many studies have reported that  $\gamma$ -CD is not profitable due to its high cost, and moreover, it shows low complexation constants for guest/CD interactions (8);
- recent papers have reported that the inner diameter of the hydrophobic cavity of  $\alpha$ -CD (0.47–0.53 nm, corresponding to a structure formed by six molecules of glucose) and the high solubility of this type of CD leads to a more favorable interaction between the CDs and the mixture of phenols and non-phenolic compounds present in pear juice (10). As it will be shown in the next sections, these data are essential to improve the sensory quality of pear juice.

**Effect of the  $\alpha$ -Cyclodextrin Addition on the CIEL\*a\*b\* Color Coordinates of Pear Juice.** Due to the importance of appearance as a quality parameter, because it strongly influences the preferences of consumers, the first instrumental parameter studied in this work was color. Moreover, browning needs to be controlled during the manufacturing of pear juice if its quality is to be preserved because the sensory and nutritional properties may be strongly altered (23).

To define the color of pear juice completely in the absence and presence of increasing concentrations of  $\alpha$ -CD (0–90 mM), scalar ( $L^*$ ,  $a^*$ ,  $b^*$ ) and total color difference ( $\Delta E^*$ ) were measured (**Figure 1**).

In the first 40 min,  $\alpha$ -CD delayed the significant and rapid decrease observed in lightness ( $L^*$ ) in the absence of any chemical agent. These data confirmed the strong reduction of  $L^*$  shown by Lopez-Nicolás and Garcia-Carmona (10). However, in this work, the reduction of the decay observed in the absence of CDs was more intense, perhaps due to the different varieties of pear used in both works (Conference and Barlett). Indeed, different pear



**Figure 1.** Effect of  $\alpha$ -CD concentration on the evolution of  $\Delta L^*$ ,  $\Delta a^*$ ,  $\Delta b^*$ , and  $\Delta E^*$  in pear juice at 25°C in the absence of any CD (●) and in the presence of  $\alpha$ -CD: 15mM (▲), 45 mM (◆), and 90 (▼) mM. Each data point is the mean of three replicates.

cultivars can present different concentration of phenolic compounds, modifying the interaction between these compounds and CDs and, consequently, providing different complexation constants. The main phenolic compounds found in pear juices are chlorogenic acid, epicatechin, caffeic acid, *p*-coumaric acid, and quercetin (12).

In **Figure 1**, it can be observed that, in the absence of  $\alpha$ -CD,  $a^*$  increased sharply during the first 10 min and then kept increasing but at a lower rate; therefore, the pear juice became redder during the reaction time. On the other hand, when increasing concentrations of  $\alpha$ -CD were present in the pear juice, the  $a^*$  was reduced, while the initial green color still remained at the highest  $\alpha$ -CD concentration tested (90 mM). Moreover, when no agent was added to the pear juice,  $b^*$  suffered a strong increase toward yellow colors. However, in the presence of all the concentrations of  $\alpha$ -CD used, this increase in  $b^*$  was significantly slowed.

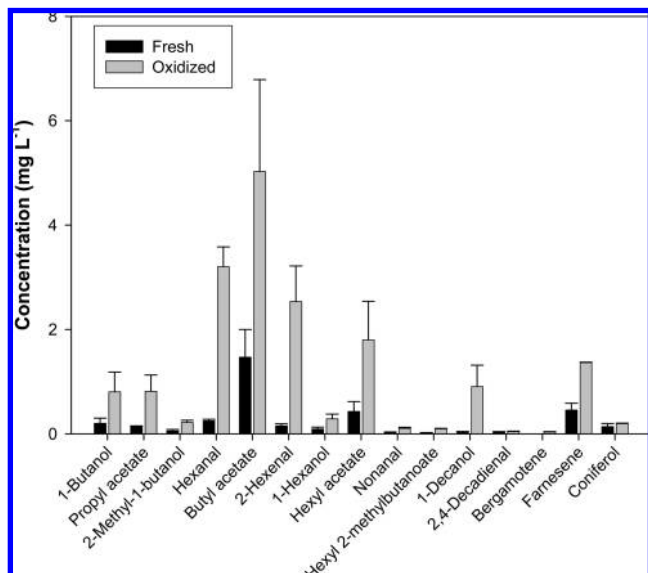
The effect of the addition of  $\alpha$ -CD during processing on the total color difference ( $\Delta E^*$ ) of pear juice was studied (**Figure 1**). As expected, due to a sharp depletion in both lightness ( $L^*$ ) and the blue-yellow coordinate ( $b^*$ ), the addition of  $\alpha$ -CD resulted in lower variations in  $\Delta E^*$  during the 40 min measured compared the values in the control juices with no added  $\alpha$ -CD.

**Effect of the  $\alpha$ -Cyclodextrin Addition on the Total Concentration of Volatile Compounds of Pear Juice.** As indicated in the Introduction, the aroma of pear juice is one of the most important properties influencing consumer preferences. Moreover, the volatile compounds that contribute to fruit aroma are produced through metabolic pathways during ripening, harvest, postharvest,

storage, and processing and are influenced by many factors, including fruit variety and technological treatments, among others (4).

For this reason, it is essential to control the changes in the volatile compounds of pear juice to maintain its sensory quality. Therefore, the next step of the present investigation was to evaluate the effect of oxidation (contact with air) on the total concentration of volatile compounds in the pear juice. During oxidation, the aroma precursors initially present in the pears will react, producing the complex and characteristic aroma of the pear juice. For instance, esters are the most significant contributors to the aroma of pears. Volatile esters are generated by esterification of alcohols and acyl-CoAs derived from both fatty acid and amino acid metabolism (24).

**Figure 2** shows the volatile compounds found in the pear juice before (fresh juice) and after its oxidation (oxidized juice; 20 min after pears were squeezed). In the fresh juice, the main compounds were butyl acetate (main sensory descriptors: apple-like, fresh, fruity),  $\alpha$ -farnesene (mild sweet, green herbaceous), hexyl acetate (sweet fruity), hexanal (green, grass-like, fruity), and 1-butanol (cheesy, light-fruity). However, as was expected, when the juice was left in direct contact with the oxygen from the air, the concentration of all volatile compounds analyzed significantly increased (**Figure 2**), making the total concentration rise from  $3.49 \pm 1.12 \text{ mg L}^{-1}$  in fresh juice to  $17.4 \pm 2.7 \text{ mg L}^{-1}$  in oxidized juice. This strong increase in the concentration of all volatile compounds is fundamental for the sensory quality of pear juice.



**Figure 2.** Concentrations of the main volatile compounds detected in fresh (freshly squeezed) and oxidized (20 min after squeezing) pear juices.

Although a paper published recently by our research group (10) studied the effect of the addition of CDs on the color of pear juice after its oxidation, no work has reported the effect of CDs on the volatile compounds of this product. Thus, the next step was to analyze the changes of the total concentrations of volatile compounds in the presence of increasing concentrations of  $\alpha$ -CD. In Table 3, it can be observed that the addition of  $\alpha$ -CD to the pear juice at concentrations of 15 and 45 mM led to decreases in the total concentrations of the main volatile compounds ( $9.71 \pm 0.12$  and  $9.06 \pm 0.14$  mg L<sup>-1</sup>, respectively) compared to the oxidized juice ( $17.4 \pm 2.7$  mg L<sup>-1</sup>). However, the decrease was much higher when 90 mM  $\alpha$ -CD was added to the medium. Thus, the highest concentration of  $\alpha$ -CD, 90 mM, led to a total concentration of volatile compounds ( $5.48 \pm 0.08$  mg L<sup>-1</sup>) much closer to that of the fresh juice ( $3.49 \pm 1.12$  mg L<sup>-1</sup>) than to that of the oxidized juice ( $17.4 \pm 2.7$  mg L<sup>-1</sup>). These results may be due to the ability of  $\alpha$ -CD at its highest concentration, 90 mM, to sequester many pear compounds responsible for the aroma of fresh pear juice and prevent the oxidation observed in the CD-free juice (control) and juices treated with 15 and 45 mM  $\alpha$ -CD.

The strong decrease of the total concentration of volatile compounds of pear juice in the presence of 90 mM  $\alpha$ -CD compared with the concentration observed in the oxidized product led to an important conclusion (reported for the first time here): the substantial variation in the evolution of the color shown in the previous section and reported as a positive aspect by López-Nicolás and García-Carmona (10) in the presence of 90 mM  $\alpha$ -CD was accompanied by a marked reduction in the total volatile compounds of the  $\alpha$ -CD-treated but oxidized pear juice.

The fact that the addition of high concentrations of  $\alpha$ -CD resulted in marked changes in the two most important properties related with the physicochemical or instrumental quality of the pear juice (CIEL\*a\*b\* coordinates and total concentration of volatile compounds) meant that an in-depth study into the influence of these results on the sensory quality of the juices as perceived by a trained sensory panel is necessary.

**Effect of the  $\alpha$ -Cyclodextrin Addition on the Concentrations of the Main Chemical Families of Pear Juice.** The aroma of fresh and processed fruit products is formed by a complex group of chemical substances (e.g., aldehydes, alcohols, ketones, esters,

lactones, terpenes, etc.). The total concentration of each one of these chemical families in pear juice is generally low ( $\mu\text{g L}^{-1}$ ) and can be affected by a number of agronomic (variety, climatological conditions, ripening stage, etc.) and technological (harvest, post-harvest, storage, and processing conditions) factors (4).

However, no study about the complexation of the main volatile compounds of pear juice by CDs has been reported to date. Besides, there is a clear relationship between concentrations of the above-mentioned chemical families and the final quality of the pear juices. Therefore, the next step in this study was to study the effects of the addition of  $\alpha$ -CD pear juice on the total concentrations of each one of the chemical families under study (esters, aldehydes, alcohols, and hydrocarbons) to evaluate which chemical structures were better complexed by CDs.

In our study, the isolation, the identification, and the quantification of the main volatile compounds of pear juice were carried out by GC-MS. The identified volatiles could be grouped into four chemical families:

- esters (propyl acetate, butyl acetate, hexyl acetate, and hexyl-2-methylbutanoate)
- aldehydes (hexanal, *trans*-2-hexenal, nonanal, and 2,4-decadienal)
- alcohols (1-butanol, 2-methyl-1-butanol, 1-hexanol, 1-decanol, and coniferol)
- hydrocarbons (*trans*- $\alpha$ -bergamotene and  $\alpha$ -farnesene)

All of these compounds have previously been isolated and identified in fresh pears and pear juices (25, 26). For instance, Lara et al. (2003), while studying the biosynthesis of volatile aroma in long-term stored pear fruits, quantified the concentrations of butyl and hexyl acetates as the main straight-chain esters in aroma of “Doyenne du Comice” pears; additionally, these authors also reported emission of ethyl acetate, 1-butanol, and 1-hexanol.

The GC-MS analysis showed the following distribution of the volatile compounds in fresh pear juice: esters (59%), aldehydes (13%), alcohols (15%), and hydrocarbons (13%). However, when the pear juice was oxidized, these percentages were modified and a new pattern was emerged. Thus, while the ester concentration decreased to 44% and the aldehydes suffered a significant increase (34%), alcohols and hydrocarbons practically remained constant at 14 and 8%, respectively.

The addition of increasing concentrations of  $\alpha$ -CD also modified the concentrations of these volatile compounds and the aroma profile of the different chemical families. The addition of low and medium concentrations (15 and 45 mM) of  $\alpha$ -CD did not significantly affect the profile of the chemical families. These concentrations of  $\alpha$ -CD were not able to retain the aroma components of the fresh juice, and the pattern of the volatile compounds was similar to that of the oxidized pear juice. Indeed, the aroma profiles of oxidized and 15 and 45 mM  $\alpha$ -CD-treated juices presented similar patterns, with esters, aldehydes, alcohols, and hydrocarbons representing 40, 34, 14, and 8% (oxidized juice), 42, 27, 20, and 12% (15 mM  $\alpha$ -CD juice), and 40, 36, 11, and 14% (45 mM  $\alpha$ -CD juice); the aldehydes played an important role at about 32%, with the most abundant aldehyde being hexanal (main sensory descriptor: green, grass-like, fruity), with a mean concentration of 2.73 mg L<sup>-1</sup>.

In contrast, the addition of 90 mM  $\alpha$ -CD significantly modified the aroma volatile pattern compared with that of the oxidized pear juice. Indeed, the chemical grouping showed similar aroma profiles for fresh and 90 mM  $\alpha$ -CD-treated juices, with esters, aldehydes, alcohols, and hydrocarbons representing 59, 13, 15 and 13% (fresh juice) and 62, 12, 23, and 3% (90 mM  $\alpha$ -CD juice),

**Table 3.** Volatile Compounds (mg L<sup>-1</sup>) Quantified in Control and  $\alpha$ -Cyclodextrin Treated (15, 45, and 90 mM) Pear Juices

volatile compound	Concentration (mg L <sup>-1</sup> )			
	control	15 mM $\alpha$ -CD	45 mM $\alpha$ -CD	90 mM $\alpha$ -CD
1-butanol	0.80 ± 0.38 a <sup>a</sup>	0.31 ± 0.09 b	0.25 ± 0.08 b	0.29 ± 0.03 b
propyl acetate	0.82 ± 0.31 ab	0.46 ± 0.09 b	0.30 ± 0.11 bc	0.21 ± 0.02 c
2-methyl-1-butanol	0.22 ± 0.04 a	0.29 ± 0.03 a	0.08 ± 0.02 b	0.07 ± 0.03 b
hexanal	3.20 ± 0.38 a	2.26 ± 0.10 c	2.72 ± 0.22 ab	0.35 ± 0.01 d
butyl acetate	5.03 ± 1.76 a	2.34 ± 0.11 b	2.15 ± 0.07 b	2.46 ± 0.08 b
<i>trans</i> -2-hexenal	2.54 ± 0.68 a	0.19 ± 0.06 b	0.22 ± 0.03 b	0.17 ± 0.03 b
1-hexanol	0.29 ± 0.09 b	0.99 ± 0.26 a	0.24 ± 0.07 b	0.07 ± 0.01 c
hexyl acetate	1.80 ± 0.74 a	1.18 ± 0.10 b	1.08 ± 0.15 b	0.66 ± 0.01 c
nonanal	0.11 ± 0.02 ab	0.07 ± 0.02 b	0.18 ± 0.06 a	0.04 ± 0.01 b
hexyl 2-methylbutanoate	0.10 ± 0.01 a	0.08 ± 0.01 a	0.09 ± 0.02 a	0.05 ± 0.01 a
1-decanol	0.91 ± 0.41 a	0.04 ± 0.01 b	0.10 ± 0.05 b	0.70 ± 0.04 b
2,4-decadienal	0.04 ± 0.01 b	0.06 ± 0.02 b	0.11 ± 0.01 a	0.12 ± 0.01 a
<i>trans</i> - $\alpha$ -bergamotene	0.03 ± 0.01 b	0.05 ± 0.02 b	0.12 ± 0.05 a	0.01 ± 0.01 b
$\alpha$ -farnesene	1.36 ± 0.01 a	1.07 ± 0.18 a	1.10 ± 0.29 a	0.15 ± 0.02 b
coniferol	0.20 ± 0.014 ab	0.32 ± 0.09 a	0.32 ± 0.08 a	0.14 ± 0.07 b
<b>total</b>	<b>17.4 ± 2.7 a</b>	<b>9.71 ± 0.12 b</b>	<b>9.06 ± 0.14 b</b>	<b>5.48 ± 0.08 c</b>

<sup>a</sup> Values followed by the same letter, in the same row, were not significantly different ( $p < 0.05$ ), Tukey's multiple-range test.

respectively. Moreover, in both fresh and 90 mM  $\alpha$ -CD-treated juice, the predominant chemical group was esters (mean of both juices 60%) and the most abundant compound was butyl acetate (main sensory descriptors: apple-like, fresh, and fruity) with a mean concentration of 1.97 mg L<sup>-1</sup>.

The results obtained in this investigation showed that, although the presence in the pear juice of  $\alpha$ -CD substantially modified the pattern of the different chemical families of volatile compounds, high  $\alpha$ -CD concentrations are needed to modify the aroma of oxidized pear juice.

Finally, it is important to mention that the addition of 90 mM  $\alpha$ -CD produced the pear juice with the best instrumental color, almost reproducing the aroma profile of fresh pear juice; however, sensory data later demonstrated that this was the juice with the lowest sensory quality. Therefore, instrumental analyses (color, odor, and/or aroma) of juices after the addition of CD are not enough to prove the positive effects of these chemicals on food quality, and sensory sufficient by a trained panel is needed.

**Effect of the  $\alpha$ -Cyclodextrin Addition on the Main Sensory Properties of Pear Juice.** In previous sections, it has been demonstrated that that addition of increasing concentrations of  $\alpha$ -CD led to important variations in two important instrumental parameters related with the quality of pear juice, the CIEL\**a*\**b*\* color coordinates and both the type and concentration of volatile compounds. However, no work has studied if the variations in these instrumental parameters have a direct influence on the main sensory quality properties. For this reason, the next step in this investigation was to study the effects of the  $\alpha$ -CD addition on the sensory parameters of pear juice.

Several important conclusions can be extrapolated from the results obtained for the sensory evaluation of pear juice using a trained panel. First, the improvement in color (reduction of the color intensity from dark to clear) was not always related with an improvement in global odor, global aroma, and global quality (**Figure 3**). A higher intensity of sensory color is related with browning. A highly significant negative correlation ( $R^2 = 0.9951$ ) was found between sensory color and sensory odor, described as the perception of volatile compounds through the nose with the pear juice out of the mouth. In other words, the darker the color of the juice (higher intensity of color), the more intense the sensory odor.

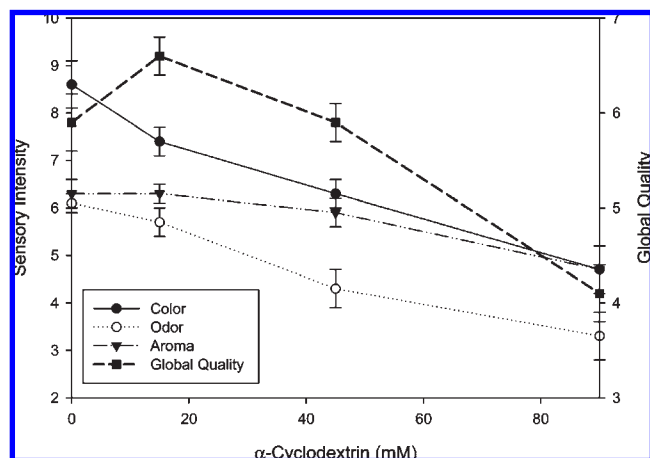
The addition of 15 mM  $\alpha$ -CD to the pear juice only had a slightly negative effect, a nonstatistically significant decrease in the intensity of odor from 6.1 ± 0.2 to 5.7 ± 0.3 (**Table 4**).

However, several positive effects were found, for instance, in the color intensity (decrease from 8.6 ± 0.5 down to 7.4 ± 0.5) and global quality (increase from 5.9 ± 0.3 up to 6.6 ± 0.3). Finally, no effect was observed for the juice aroma.

The first increase in the  $\alpha$ -CD concentration from 15 up to 45 mM had negative effects on (a) odor, whose intensity fell from 5.7 ± 0.3 to 4.3 ± 0.4, and (b) global quality, whose value was reduced from 6.6 ± 0.2 to 5.9 ± 0.3. A further increase in the concentration of  $\alpha$ -CD up to 90 mM resulted in a significant decrease in juice browning, with a mean value for the intensity of sensory color being 4.7 ± 0.5 compared with 7.4 ± 0.3 and 6.3 ± 0.3 for the lower concentrations (15 and 45 mM, respectively) as the only advantage. On the other hand, several unfavorable consequences were evident: significant reductions in odor, aroma, and global quality. Consequently, from a sensory point of view, the concentration of 90 mM of  $\alpha$ -CD cannot be considered useful for industrial purposes (the ideal being an improvement of color but without simultaneously reducing the quality of other sensory properties or attributes, such as odor and aroma).

As indicated in the Introduction, several works have focused on the effect of the addition of different CDs on the CIEL\**a*\**b*\* color parameters of fruit juices in order to evaluate the capacity of CDs to improve their quality. However, the sensory study carried out in this work with a trained panel showed that the sensory property showing the best correlation with the global quality of the pear juice was aroma with a determination coefficient of 0.9209, while lower  $R^2$  values were obtained for odor and color (0.6699 and 0.6076, respectively).

Moreover, the fact that aroma (perception of volatile compounds while the juice is in the mouth) was better correlated with the global quality than odor (perception of volatile compounds while smelling the juice out of the mouth) could be related to the fact that, with the juice in the mouth, some of the complexes among the CD and the volatile compounds might be destroyed, releasing chemicals that are not perceived during direct smelling of the juice out of the mouth. This could also be one of the reasons why the scores given by the trained panel were always higher for aroma than odor (6.3, 6.3, 5.9, and 4.7 for aroma and 6.1, 5.7, 4.3, and 3.3 for odor in 0, 15, 45, and 90 mM  $\alpha$ -CD, respectively). Besides, the perception of the strong sweet taste of  $\alpha$ -CD could also help in increasing the intensity of aroma compared with the intensity of odor. On the other hand, it was also expected that  $\alpha$ -CD modified the composition of headspace volatile components, which can cause acute changes in the odor of  $\alpha$ -CD-treated



**Figure 3.** Intensities of the main sensory properties (color, odor, and aroma) describing the quality of control and  $\alpha$ -cyclodextrin-treated (15, 45, and 90 mM) pear juices.

pear juices. However, further research is needed to verify all of these positive and negative correlations.

A more detailed descriptive analysis of the juices under study (considering the main *attributes* of the sensory properties: odor and aroma) showed that increases in the concentration of  $\alpha$ -CD in the pear juice significantly decreased the intensities of several characteristic and positive odor and/or aroma attributes, such as those of green, fresh, floral, fruity, and pear (Table 4). On the other hand, an increase in the intensity of unnatural compounds was detected as the  $\alpha$ -CD concentration increased. The intense sweet taste of  $\alpha$ -CD could mask the perception of several attributes and was responsible, at least in part, for the decreases observed in the intensities of the fruity and pear notes in juices with the lowest  $\alpha$ -CD concentration (15 mM), compared with the oxidized pear juice.

In summary, the addition of  $\alpha$ -CD at the highest concentration, 90 mM, to the pear juice led to the deterioration of its odor and aroma attributes or notes, due to increases in unnatural notes and decreases in natural and pleasant notes, such as fresh, fruity, and pear-like. On the other hand, the addition of  $\alpha$ -CD at a concentration of 15 mM had a significant positive effect on the sensory quality of pear juices, improving significantly the juice color but without any reduction in odor and/or aroma.

**Relationships among the Instrumental and Sensory Quality Parameters of Pear Juice.** A general decrease in the intensities of most of the odor and aroma attributes (green, acid, fresh, fruity, and pear-like) was found (Table 4). Similar trends were observed in the instrumental analysis of the aroma profiles of pear juice for propyl acetate, 2-methyl-1-butanol, hexanal, 1-hexanol, hexyl acetate,  $\alpha$ -farnesene, and coniferol. Of these volatile compounds, propyl acetate (pungent, penetrating, strong sweet), 1-hexanol (grass, fruity, light apple), and hexyl acetate (sweet fruity) steadily decreased at all the concentrations of  $\alpha$ -CD. Other chemicals, such as 2-methyl-1-butanol (fruity), decreased, as the concentration of  $\alpha$ -CD increased from 15 to 45 mM and remained constant for 90 mM. Finally, hexanal (green, grass-like, fruity),  $\alpha$ -farnesene (mild sweet, green herbaceous),  $\alpha$ -farnesene (mild sweet, green herbaceous), and coniferol remained almost constant for the first two concentrations of  $\alpha$ -CD (15 and 45 mM) and only decreased at the highest  $\alpha$ -CD concentration, 90 mM.

The sensory panel was unable to recognize significant changes (remained almost constant) in floral and sweet odors and aromas. Several volatile compounds were not significantly affected by the

**Table 4.** Descriptive Sensory Analyses of Control and  $\alpha$ -Cyclodextrin-Treated (15, 45, and 90 mM) Pear Juices

attribute	ANOVA Test			Treatments Concentration $\alpha$ -CD (mM)		
	assessor	treatment	control	15	45	90
<b>Odor</b>						
green	NS <sup>a</sup>	**	5.6 a <sup>b</sup>	4.0 b	2.4 c	2.9 c
acid	NS	*	4.6 a	3.1 b	2.3 b	2.4 b
fresh	NS	**	4.9 a	3.7 ab	2.9 b	1.6 c
floral	NS	*	3.0 a	3.1 a	3.4	2.4 b
fruity	NS	**	4.7 a	4.1 a	4.0 a	2.6 b
cooked/caramel	NS	NS	1.7	1.6	2.4	1.6
pear	NS	**	6.3 a	5.6 a	4.4 b	3.7 b
unnatural	NS	*	0.9 c	1.0 c	1.9 b	3.9 a
<b>aroma</b>						
green	NS	*	3.9 a	3.9 a	2.6 b	2.0 b
acid	NS	*	3.6 a	3.3 a	2.7 a	1.3 b
fresh	NS	*	4.4 a	4.6 a	4.0 a	2.9 b
floral	NS	*	3.3 a	4.3 a	3.9 a	2.6 b
fruity	NS	**	6.1	4.4 b	4.6 b	3.3 c
cooked/caramel	NS	NS	3.6	4.3	3.4	4.9
pear	NS	**	6.6 a	5.6 ab	5.0 b	4.1 c
unnatural	NS	**	1.6 b	1.4 b	2.6 b	3.6 a
<b>global</b>						
color	NS	**	8.6 a	7.4 b	6.3 b	4.7 c
odor	NS	**	6.1 a	5.7 a	4.3 b	3.3 c
aroma	NS	**	6.3 a	6.3 a	5.9 a	4.7 b
quality	NS	**	5.9 b	6.6 a	5.9 b	4.1 c

<sup>a</sup> NS = not significant F ratio ( $p < 0.05$ ); \*, \*\*, and \*\*\*, significant at  $p < 0.05$ , 0.01, and 0.001, respectively. <sup>b</sup> Values followed by the same letter, within the same variation source, were not significantly different ( $p < 0.05$ ), Tukey's multiple-range test.

addition of  $\alpha$ -CD to pear juices or by the concentration of this particular CD; these volatiles included 1-butanol, butyl acetate, hexyl-2-methylbutanoate, 2,4-decadienal, and *trans*- $\alpha$ -bergamotene. This situation was of particular interest in the case of butyl acetate, the predominant compound in all pear juices, and of positive sensory descriptors such as apple-like, fresh, and fruity.

On the other hand, the unnatural odor and aroma steadily increased with the increase in  $\alpha$ -CD concentration. The only chemical compound that presented this type of pattern in the instrumental analysis of pear juice was 1-decanol; this chemical has some sensory descriptors which are inappropriate for pear juice (for instance, oily and fatty) and could be responsible, at least in part, for the increase in the intensity of the unnatural notes detected by the trained sensory panel.

The changes in odors and aromas of pear juice showed the negative effects of the highest  $\alpha$ -CD concentration (90 mM); this concentration is, therefore, rejected for any application in the pear juice industry. Our final recommendation is to add  $\alpha$ -CD at a concentration of 15 mM to pear juice because it will significantly increase the global quality of pear juice and reduce its browning without producing significant decreases in aroma.

However, this is the first experiment investigating the effects of  $\alpha$ -CDs on volatiles of pear juices, and further research is needed to clearly establish the relationships among instrumental and sensory quality parameters of pear juices.

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