chemosensors

MDPI

Article

Digital Colorimetric Approach for Rapid Determination of Acetaldehyde in Pisco Head During Distillation

Beatriz Hatta-Sakoda [1], M. Monica Giusti [1,2], Luis E. Rodriguez-Saona [1,2] and Luis Condezo-Hoyos [3,4,*]

[1] Facultad de Industrias Alimentarias, Universidad Nacional Agraria La Molina, Av. La Molina s/n, Lima 15012, Peru; bhs@lamolina.edu.pe (B.H.-S.); giusti.6@osu.edu (M.M.G.); rodriguez-saona.1@osu.edu (L.E.R.-S.)
[2] Department of Food Science and Technology, The Ohio State University, 2015 Fyffe Rd., Columbus, OH 43210, USA
[3] Innovative Technology, Food and Health Research Group, Facultad de Industrias Alimentarias, Universidad Nacional Agraria La Molina, Av. La Molina s/n, Lima 15012, Peru
[4] Instituto de Investigación de Bioquímica y Biología Molecular, Universidad Nacional Agraria La Molina, Av. La Molina s/n, Lima 15012, Peru
* Correspondence: lcondezo@lamolina.edu.pe

Abstract

A digital colorimetric method (ACETimage), which utilizes aldol condensation, crotonization, and resinification, was developed and validated to quantify acetaldehyde in the head fraction of Pisco distillation. The optimal conditions for the reaction were as follows: the head Pisco samples were placed in headspace vials, 20% *w*/*w* NaOH was added, and the mixture was boiled in water for 2 min. The Color Grab app was used to capture and analyze images of the reactions, with a screen brightness intensity of 0.5, within a maximum post-reaction time of 10 min. The Euclidean distance (ED-RGB) was the color parameter most sensitive to changes, showing a linear correlation with the square of acetaldehyde concentration, with R^2 values ranging from 0.9926 to 0.9976. The limit of detection (LOD) and limit of quantification (LOQ) for the ACETimage method were determined to be 30 and 95.3 mg/L, respectively. A significant correlation was observed between the acetaldehyde content measured using ACETimage and gas chromatography (Spearman's r = 0.9373). Bland–Altman analysis indicated that the differences between the two methods were within the 95% limits of agreement. ACETimage offers a rapid, cost-effective, and user-friendly solution for monitoring acetaldehyde levels during Pisco distillation, enabling easy implementation in production environments, both artisanal and industrial, with minimal sample preparation and limited personnel training.

Keywords: acetaldehyde; pisco; digital colorimetry; image analysis; smartphone

Check for updates

Received: 6 November 2025
Revised: 27 November 2025
Accepted: 12 December 2025
Published: 2 April 2026

Copyright: © 2026 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.

1. Introduction

Pisco is a traditional and emblematic distilled alcoholic beverage, with an alcohol content ranging from 38% to 48% by volume, originating from Peru. It is produced along the coastal regions of Lima, Ica, Arequipa, Moquegua, and Tacna [1]. Pisco is a type of fine brandy, crafted through the distillation of fermented grape must or juice derived from grape varieties such as Quebranta, Negra Criolla, Mollar, Uvina, Albilla, Moscatel, Torontel, and Italy. Pisco is categorized into three types: pure (monovarietal), acholado (a blend of varieties), and green must (distilled from must with interrupted fermentation) [1,2]. The traditional Peruvian production process encompasses several stages, including grape

harvest, destemming, crushing, maceration, racking, pressing, fermentation, distillation, oxidative rest, filtration, and bottling. During the distillation process, conducted in falcas, alembics, and alembics with heaters, the head and tail of the distillate are discarded, retaining only the heart or body for oxidative rest for a minimum of three months prior to marketing [1–3].

Acetaldehyde, a carbonyl compound present in Pisco, poses significant health risks to consumers due to its mutagenic and carcinogenic properties when found in high concentrations. Additionally, it adversely affects the sensory quality of the product by imparting unpleasant aromas, thereby underscoring the importance of its quantification [4,5]. As reported by Hatta-Sakoda, Guevara-Pérez, and Morales-Soriano [1], the distillation process of Pisco involves the separation of three distinct fractions: the head, which is rich in alcohol, acetaldehyde, higher alcohols, and methanol; the body, which matures into Pisco and should contain minimal levels acetaldehyde, higher alcohols, and methanol; and the tail, which is characterized by the presence of higher alcohols and furfural. The Pisco head is removed using various indirect and non-quantitative methods, such as the percentage of wine volume allocated for distillation (0.5–2%), alcohol concentration, or sensory analysis. However, sensory analysis poses health risks due to the carcinogenic and mutagenic properties of acetaldehyde [6,7].

Current methodologies for quantifying acetaldehyde in alcoholic beverages and during spirit distillation encompass spectrophotometric [8,9], spectrofluorometric [10–12], colorimetric sensor array [13], HPLC [14], gas chromatography [15–19], and Fourier Transform Raman Spectroscopy (FT-Raman) [20]. These techniques rely on large benchtop instruments that are unsuitable for processing environments, which limits their use for routine quality control in industrial settings, particularly during Pisco production.

In this study, we employed a colorimetric reaction in the presence of hydroxide ions at elevated temperatures that leads to the formation of a strong yellow color (resinification) based on the aldol condensation/crotonization and resinification of acetaldehyde [21–24]. These reactions have been employed to assess fat rancidity spectrophotometrically [23,24] (Figure 1); however, their application in measuring acetaldehyde content in Pisco has not been investigated. Our objective was to develop and validate a rapid, smartphone-based imaging technique for quantifying acetaldehyde during Pisco distillation, leveraging the yellow color produced by aldol condensation, crotonization, and resinification reactions of acetaldehyde.

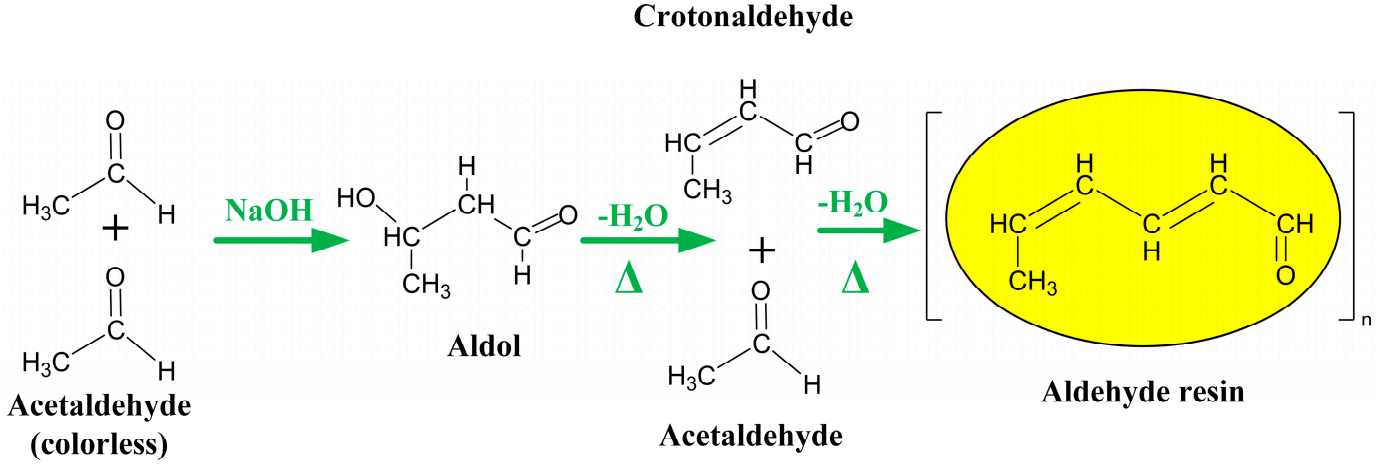


Figure 1. Aldol condensation/crotonization/resinification reaction for the quantification of acetaldehyde using the ACETimage digital colorimetry method. Δ = heat and n = number of times the chemical structure is repeated.

2. Materials and Methods

2.1. Samples and Reagents

The initial fraction of Pisco distillation, known as Pisco head, was collected from Bodega San Nicolás in the Pisco Valley (Ica, Peru). These samples were produced from the traditional fermentation and distillation [1] of two grape varieties: Quebranta (Q) (18 samples) and Italia (I) (10 samples), along with one blend of Q and I (M). Reagents included acetaldehyde (Sigma-Aldrich, Darmstadt, Germany), absolute ethanol (Merck, Darmstadt, Germany), and sodium hydroxide pellets (JT Baker, Radnor, PA, USA). All reagents used are ACS reagent grade. Aqueous solutions were prepared using Milli-Q ultrapure water.

2.2. Quantitation of Acetaldehyde by Gas Chromatography

The acetaldehyde content in the Pisco heads was measured using gas chromatography, based on the method outlined by Menevseoglu et al. (2021) [25], with slight modifications. An Agilent Technologies 7820A gas chromatograph (GC) equipped with a flame ionization detector and a 60 m × 250 µm (i.d.) × 0.25 µm (film thickness) HP-FFAP column (Agilent Technologies, Santa Clara, CA, USA) was used. The sample injection volume was set at 1 µL under split mode at a 15:1 ratio, with a constant flow of 20 mL/min with an injection port temperature of 250 °C. The separation conditions began with an initial oven temperature of 40 °C, held for 4 min, then increased at a rate of 5 °C/min to 100 °C, followed by a ramp up of 10 °C/min to 160 °C, and finally, the temperature was raised to 220 °C at 55 °C/min and held for 2 min. The quantification was performed in triplicate using a calibration curve with acetaldehyde in a 40% *v*/*v* ethanolic solution (0.172–13.94 mg/100 mL, R^2 = 0.9996).

2.3. Development and Validation of a Colorimetric Reaction Coupled to Digital Image Analysis for the Quantification of Acetaldehyde (ACETimage)

2.3.1. Reaction Conditions

The efficacy of the base and heat-catalyzed cyclic aldol condensation and crotonization/resinification reactions (bh-cACCR) for quantifying acetaldehyde was evaluated under four heating systems that included direct heating (DH) using an electric hot plate FZ-201D1CH (Finezza, Lima, Peru), direct heating with reflux (DHR) with hot plate Super-Nuova (Thermo Scientific, Waltham, MA, USA), a reflux water bath (RWB) IVYX (Scientific, Beijing, China), and a dry block heater (DBH) Eppendorf ThermoMixer (Eppendorf, Germany) (Figure S1). The bh-cACCR was performed by combining a 350 mg/L acetaldehyde solution in 40% *v*/*v* ethanol with a 10% *w*/*w* NaOH solution in a 1:1 *v*/*v* ratio. The solutions were heated to boiling using various systems to determine the optimal system, and their absorbance at 320 nm was measured using a 10S Genesys spectrophotometer (Thermo Fisher Scientific, USA). Temperature ranges of 70–90 °C with reaction durations of 15–20 min were investigated. The influence of NaOH concentration (5, 10, 15, 20, and 25% *w*/*w*) on bh-cACCR was examined utilizing a previously selected heating system, temperature, and reaction time.

2.3.2. Image Acquisition and Analysis

The image data acquisition was developed based on the work of Marinho, et al. [26], with certain modifications. The imaging box and sample holder (Figure S2) were fabricated via additive manufacturing using black and white polylactic acid (PLA) filaments (Paulet3D, Lima, Peru), respectively [27–30]. Image acquisition was performed using a Samsung smartphone equipped with an SMP camera providing an illumination intensity of 1032 ± 1 lux. Two color analysis applications (Apps) were evaluated (1) Photometrix and (2) Color Grab. Color analysis with Photometrix was conducted in the RGB color space; the application did not allow for adjusting the screen brightness. Color Grab was performed in

both the RGB and CIELAB color spaces at brightness of 0.5. The App was selected based on color reproducibility (coefficient of variation = RSD) and color intensity (Euclidean distance) by using solutions of 350 and 400 mg/L acetaldehyde.

Color parameters included the Euclidean Distance (ED) (Equation (1)) [31], and IR, IG, IB, IL *, Ia *, and Ib * were calculated using Equation (2) [31]. The selection of color parameters was based on criteria such as color reproducibility, relative standard deviation (RSD), and the slope of the calibration curve, which are directly associated with sensitivity.

$$\mathbf{ED} = \sqrt{(\mathbf{R_s} - \mathbf{R_b})^2 + (\mathbf{G_s} - \mathbf{G_b})^2 + (\mathbf{B_s} - \mathbf{B_b})^2} \quad (1)$$

$$\mathbf{IX} = \mathbf{log}\frac{\mathbf{X_b}}{\mathbf{X_s}} \quad (2)$$

where X corresponds to the color parameter, i.e., R, G, B, L *, a *, and b *, while the subscripts "b" and "s" denote the blank and evaluated samples, respectively.

To evaluate the stability of the reaction, the color parameters were assessed at intervals of 5, 10, and 15 min with the different color analysis apps. After the reaction was completed, the sample was cooled in an ice-water bath maintained at 5–10 °C, and the color was collected when the samples reached room temperature.

2.4. Validation

A calibration curve was constructed in triplicate using various acetaldehyde solution concentrations of 100, 150, 200, 250, and 300 mg/L on different days, employing the color parameter selected in Section 2.3.2. The linearity and linear range of the ACETimage method were assessed using the coefficient of determination (R^2), while their sensitivity was evaluated through the limits of detection (LOD) and quantification (LOQ) [31]. Then, the acetaldehyde content in Pisco head samples was quantified utilizing the gas chromatography reference method and their values were compared to those predicted by the ACETimage procedure. The validation employed Bland–Altman analysis and Passing–Bablok regression analysis [32].

2.5. Statistical Analysis

To select the best heating system, aldol reaction conditions, and image acquisition system, analysis of variance (ANOVA) was performed using Statgraphics 19-X64 (Statgraphics Technologies, Inc., The Plains, VA, USA). Kolmogorov–Smirnov test for the normality and correlation analysis were performed in SPSS 22.0 (IBM Corp., Armonk, NY, USA) and Bland–Altman analysis and Passing–Bablok regression analysis was carried out in MedCalc (MedCalc Software Ltd., Ostend, Belgium).

3. Results and Discussion

3.1. Optimization of the Reaction Conditions for Quantification of Acetaldehyde

In this phase of the investigation, various reaction conditions were analyzed to measure acetaldehyde in the Pisco head fractions. Factors such as temperature, time, and sodium hydroxide concentration were considered, as they had previously been identified as crucial for the reaction by Oliver and Suñé [24]. Figure 2 summarizes the results of using different heating systems to drive the reaction. The dry block heater system (DBH) demonstrated superior performance, yielding absorbance values at 320 nm (A320 nm) that were 2.5 times greater than those obtained through direct heating (DH) and 4.6 times greater than those achieved with direct heating with reflux (DHR) and reflux water bath (RWB). The aldol condensation and crotonization/resinification reactions of acetaldehyde were directly related to the raising of the temperature for all heating systems [24,33]. However, the type of heating

system affected the amount of acetaldehyde available for the reaction by maintaining the acetaldehyde in solution [34]. The A320 nm increased with rising temperature (50–90 °C) and longer reaction times (5–20 min) (Figure 3). Oliver and Suñé [24] reported that elevated temperatures and prolonged reaction times accelerate and intensify the reaction due to the dehydration of acetaldol and the subsequent formation of crotonaldehyde, which, upon polymerization, produces conjugated double-bond structures that result in a yellow coloration. We found that at 90 °C after 10 min of reaction the A320 nm plateau, achieving lower A320 nm compared to that obtained at 80 °C (Figure 3), possibly due to acetaldehyde volatilization and its loss through the caps of the tubes. Finally, the concentrations of NaOH solutions (5–25% *w*/*w*) was assessed, indicating that levels higher that 20% NaOH yielded the highest color intensity (absorbance at 320 nm) in the aldol reaction with no significant difference by using a 25% *w*/*w* NaOH concentration (Figure 4).

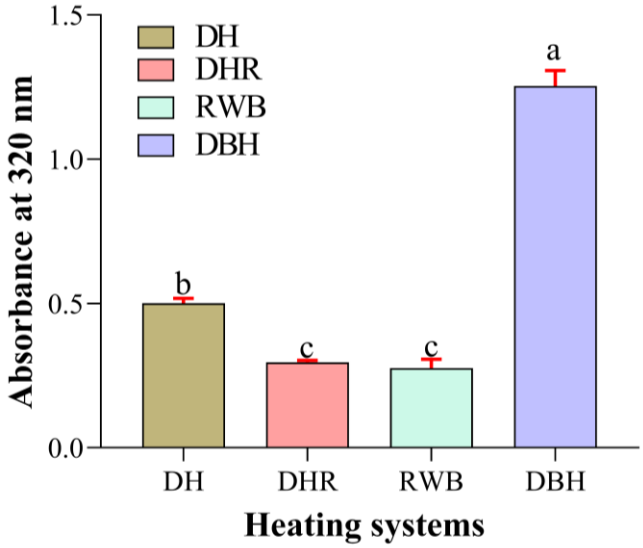


Figure 2. Colorimetric reaction using various heating systems carried out with pure acetaldehyde (350 mg/L) solution in 40% *v*/*v* ethanol and 10% *w*/*w* NaOH aqueous solution in a 1:1 *v*/*v* ratio at the boiling temperature and measured at 320 nm. DH = direct heating, DHR = direct heating with reflux with hot plate, RWB = a reflux water bath, and a DBH = dry block heater. The average absorbance values ± SD (n = 3). Different letters indicate statistically significant differences ($p < 0.05$).

In an effort to reduce reaction time so that it could be used in the Pisco distillation process for determining the head cut, which occurs within a maximum of 30 min, we evaluated the use of boiling water (CBM) to drive the reaction and employed hermetic vials used for headspace analysis to prevent loss of acetaldehyde due to volatilization. This modification was supported by the work by Oliver and Suñé [24] that used boiling temperature for their colorimetric reaction. We found that heating the samples by using boiling water for 2 min yielded higher yellow color intensities and lower coefficient of variability compared to the heating block system for 20 min (CBM = 14.1 ± 3.9 vs. CDBS = 144.4 ± 3.6). The parameters for conducting the aldol condensation, crotonization, and resinification reactions to ascertain the acetaldehyde content in the pisco head were established as follows: utilization of a CBM heating system, maintenance of boiling temperature, a reaction duration of 2 min, and a sodium hydroxide concentration of 20% *w*/*w*.

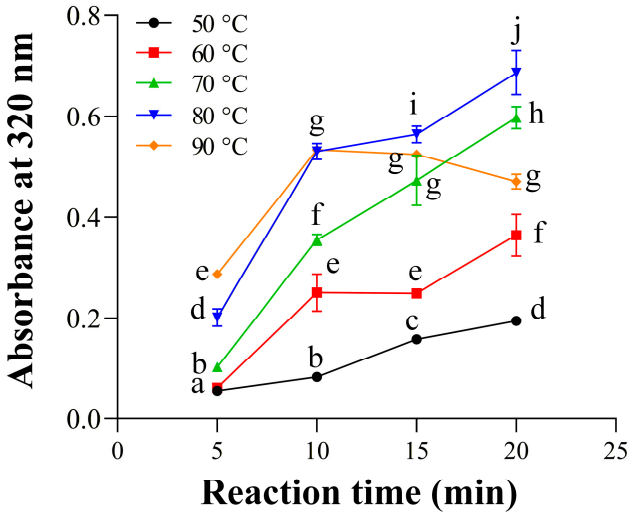


Figure 3. Impact of temperature and reaction time assessed using the dry bath heating system (CDBS) at 50–90 °C for 5–20 min. The colorimetric reaction, bh-cACCR, was quantified by measuring absorbance at 320 nm. The values presented are means, with error bars indicating the standard deviation of three replicates. Distinct letters denote statistically significant differences ($p < 0.05$).

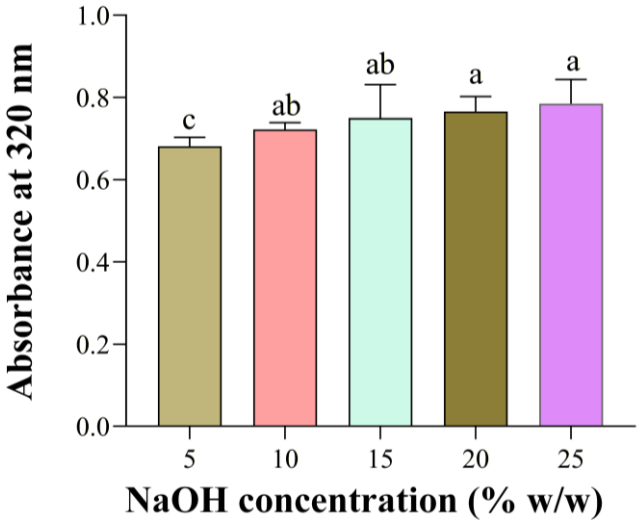


Figure 4. Influence of NaOH concentration on bh-cACCR colorimetric reaction, quantified by measuring absorbance at 320 nm. The values presented are means, with error bars indicating the standard deviation of three replicates. Distinct letters denote statistically significant differences ($p < 0.05$).

3.2. Image Acquisition/Analysis

The Euclidean distance (ED) was employed to quantify color differences by measuring the distance between color coordinates within color spaces such as RGB [35,36]. When comparing results obtained from two free image acquisition and analysis applications, Color Grab and Photometrix, we found that the ED measured by Color Grab was higher than those measured by Photometrix for acetaldehyde concentrations (63.8 vs. 54.7 at 350 mg/L and 85.6 vs. 76.0 at 400 mg/L) and also gave lower relative standard deviation (RSD) (4.2 vs. 4.6 at 350 mg/L and 1.3 vs. 2.3 at 400 mg/L). The ability in the Color Grab application to select options such as autofocus and illumination [35] allowed us to collect images with improved quality compared to Photometrix. In addition, Color Grab facilitates the rapid collection of responses from RGB channels and swiftly determines the associated color name. This capability eases the analyst's job of handling and interpreting the data gathered during each procedure [35,36]. Adjusting the screen brightness in the Color Grab app impacted

the ED-RGC values (Figure 5A), achieving a maximum ED-RGB value (16.7 ± 2.3) when the screen brightness intensity was set to 0.5, and the Euclidean distance diminished at either higher or lower settings. At a brightness intensity of zero, the images appeared dark, thereby reducing ED-RGB. Conversely, values exceeding 0.5 minimized the color differences relative to the control. Increased image brightness elevates the RGB values, thereby reducing the differences between the samples and white, which in turn decreases the ED-RGB values [37].

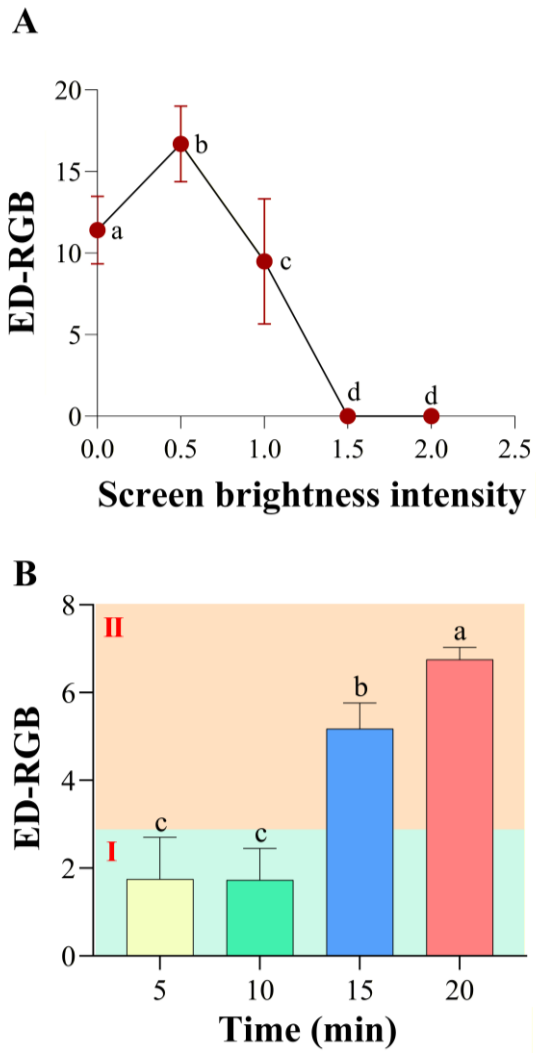


Figure 5. The impact of brightness intensity (**A**) and elapsed time (**B**) on the colorimetric bh-cACCR for acetaldehyde quantification was assessed using Euclidean distance (ED-RGB). The values presented represent the mean ± standard deviation (SD) of three replicates. Distinct letters denote statistically significant differences ($p < 0.05$).

Upon completion of the colorimetric reaction for acetaldehyde determination, the resultant color remained stable for a duration of up to 10 min (Figure 5B, zone I). Consequently, it is imperative to conduct measurements within this time frame to ensure accuracy. Given the time-sensitive nature of this process, measurements should be performed expeditiously. Furthermore, a notable increase in the ED-RGB values was observed over time, attributable to the formation of three primary polymeric reaction products: crotonaldehyde (acetaldehyde dimers), 2,4-hexadienal (acetaldehyde trimers), and 2,4,6-octatrienal (acetaldehyde tetramers) (Figure 5B, zone I). The latter compound is characterized by extended-chain molecular structures with conjugated double bonds, whose resonance is responsible for

generating the yellow color observed in the colorimetric reaction [24,38]. However, the polymerization reaction can progress to form new oligomers, further extending the conjugated double-bond chain and intensifying the color [38,39]. This intensification could explain the increase in ED-RGB for times exceeding 10 min (Figure 5B, zone II). From an analytical standpoint, it is desirable for the procedure to be carried out in the shortest possible time; therefore, we recommend that measurement times do not exceed 10 min after the reaction was completed to achieve best reproducibility.

Various color parameters at differing acetaldehyde concentrations were derived from the RGB and CIELAB color spaces utilizing the Color Grab application. The best responses to acetaldehyde concentration were achieved with b * values, ED-L *a *b *, and ED-RGB (Figure 6). Conversely, other color parameters such as IR, IG, IB, IL *, Ia *, and Ib * exhibited minimal changes when the acetaldehyde concentration was less than 200 mg/L (Figure S3). The good response found with b* values can be attributed to the association of +b * with yellow tones developed during the aldol condensation and crotonization/resinification reactions [23]. The b * values exhibited a similar pattern to ED-L *a *b *, but gave lower RSD values (Figure 5). ED-RGB demonstrated lower RSD values compared to b * values and ED-L *a *b * and was selected as the color parameter to monitor for color development by the ACETimage method (Figure 6). The RGB color space has been employed in various image-based colorimetric analyses, including the quantification of volatile acidity in cachaça [35], furfural in sugarcane distillates [36], and methanol in sugarcane distillate [40]. RGB color space can accurately predict changes in color intensity and handle variations in lighting conditions [41]. In contrast, the application of the CIELab * color space in image-based colorimetric analysis of alcoholic beverages has not been documented.

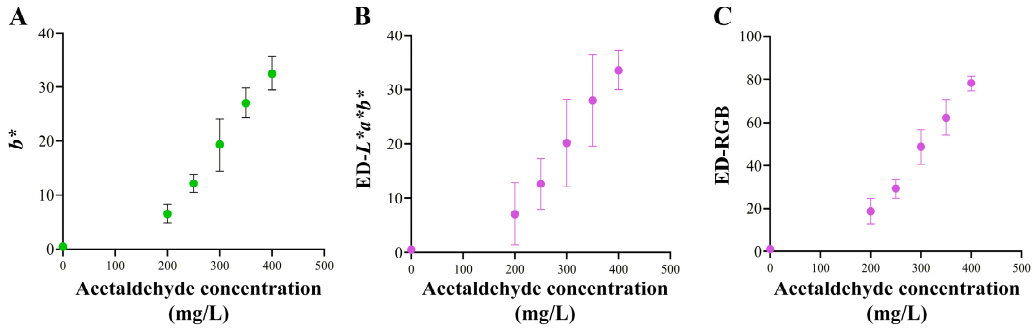


Figure 6. The relationship between color parameters b * (**A**), ED-L *a *b * (**B**), and ED-RGB (**C**) was examined. The values presented are the means, with error bars representing the standard deviation (SD) of three replicates.

3.3. Validation of ACETimage Method

The linearity of the ACETimage method was assessed across a concentration range of 0–300 mg/L acetaldehyde using alcoholic acetaldehyde solutions at concentrations of 100, 150, 200, 250, and 300 mg/L. The ED-RGB color parameter, derived from images of each acetaldehyde solution subjected to bh-cACCR, exhibited a nonlinear correlation with acetaldehyde concentration (Figure 7). The bh-cACCR, which underpins the ACETimage method, can exhibit first- or second-order kinetics with respect to acetaldehyde, contingent upon the concentrations of catalysts such as carbonate ions (CO_3^{2-}), hydroxide (OH^-), and amino acids [39,42]. In this study, a 20% *w*/*w* OH^- catalyst was utilized, accounting for the quadratic relationship between ED-RGB and acetaldehyde concentration. This relationship indicates that the aldol condensation reaction kinetics are second-order for the primary reaction products, including crotonaldehyde, 2,4-hexadienal, and 2,4,6-octatrienal, consistent with the findings of several researchers [21,22,39]. In this study, a 20% *w*/*w* OH^- catalyst

was utilized, accounting for the quadratic relationship between ED-RGB and acetaldehyde concentration. In certain analytical techniques, the relationship between measured signals and analyte concentration is nonlinear, and nonlinear or polynomial models offer a superior fit [43]. In this study, the complexity of reactions involving acetaldehyde contributed to the nonlinear relationship between ED-RGB and acetaldehyde concentration. The experimental data were fitted to a second-order polynomial model with an R^2 value of 0.9972 (Figure 7). The linearity of the ACETimage method was confirmed over the studied concentration range (29.96–300 mg/L), yielding a coefficient of determination R^2 = 0.09927–0.9976 in 4 inter-day experiments. The relative standard deviation (RSD) values for each acetaldehyde solution used to construct the calibration curve ranged from 2.8% to 7.3%.

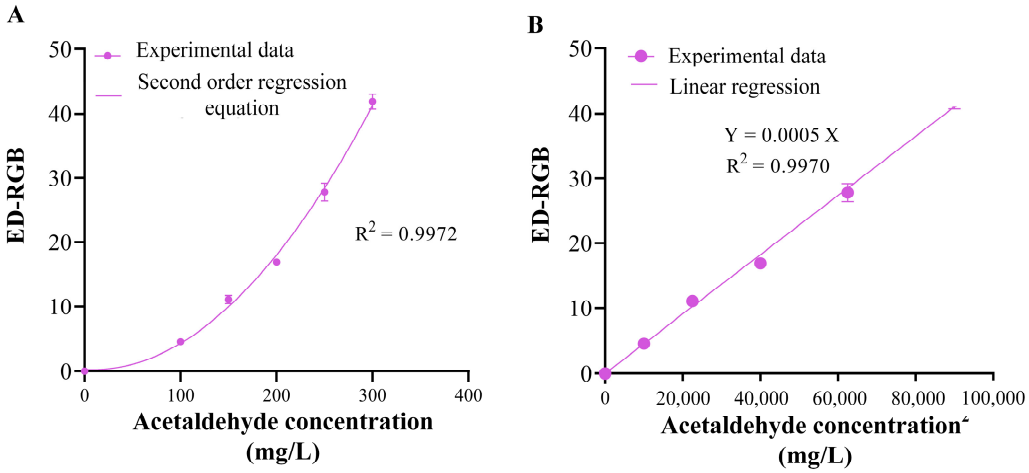


Figure 7. Second-order polynomial relationship between ED-RGB and acetaldehyde concentration (**A**) and linear calibration curve of the ACETimage method for acetaldehyde quantification (**B**). The values are presented as mean ± SD (n = 16).

The LOD and LOQ values of the ACETimage method, derived from the slope of the calibration curve, were 29.96 and 95.32 mg/L, respectively. The LOD of the ACETimage method is below the maximum permissible acetaldehyde limit in Pisco (240 mg/L) [44]. Consequently, it could be applied in the quality control of the Pisco production process to prevent improper incorporation of the Pisco head fraction. Although the acetaldehyde content influences the aroma of Pisco, levels exceeding the permitted limit compromise its toxicological safety. The LOD of the ACETimage method was significantly higher than that of spectrophotometric methods based on the inhibition of the malachite green–sulfite reaction (0.1 mg/L) [8], inhibition of the piperidine–sodium nitroprusside reaction (0.5 mg/L) [45], and reaction with fuchsin (4.2 mg/L) [46]. While spectrophotometric methods generally offer higher sensitivity than the ACETimage method, they necessitate the use of more reagents and a spectrophotometer for quantification. The ACETimage method employs only sodium hydroxide, and the analytical signal is acquired using a Color Grab app (a free laboratory instrument). Given its portability and the fact that its LOD and LOQ were below the maximum permitted acetaldehyde content, it presents an affordable option for quality control during Pisco production at the pilot plant level.

The acetaldehyde content in Pisco head samples, measured by the ACETimage method and Gas Chromatography (GC-FID) (Table 1), exhibited a non-normal distribution according to the Kolmogorov–Smirnov test ($p < 0.05$). Thus, Spearman's nonparametric correlation analysis was used to validate the ACETimage method, giving a correlation coefficient (r) of 0.94 ($p < 0.0001$) (Figure 8A). Bland–Altman analysis showed that the differences in acetaldehyde content measured by the ACETimage and GC-FID methods across all samples fell within the 95% limits of agreement (Figure 8B). The Passing–Bablok regression analysis

of the acetaldehyde concentration results obtained with ACETimage (y) compared to those obtained by GC-FID (x) is presented in Figure 8C. Because the 95% CI for the intercept includes the value zero, it can be concluded that there is no significant difference between the obtained intercept value and zero, and there is no constant difference between the two methods. Conversely, because the 95% CI for the slope does not include the value of one, there is a proportional difference between the ACETimage and GC-FID methods [31,47,48]. Furthermore, no significant deviation from linearity was observed (p = 0.58).

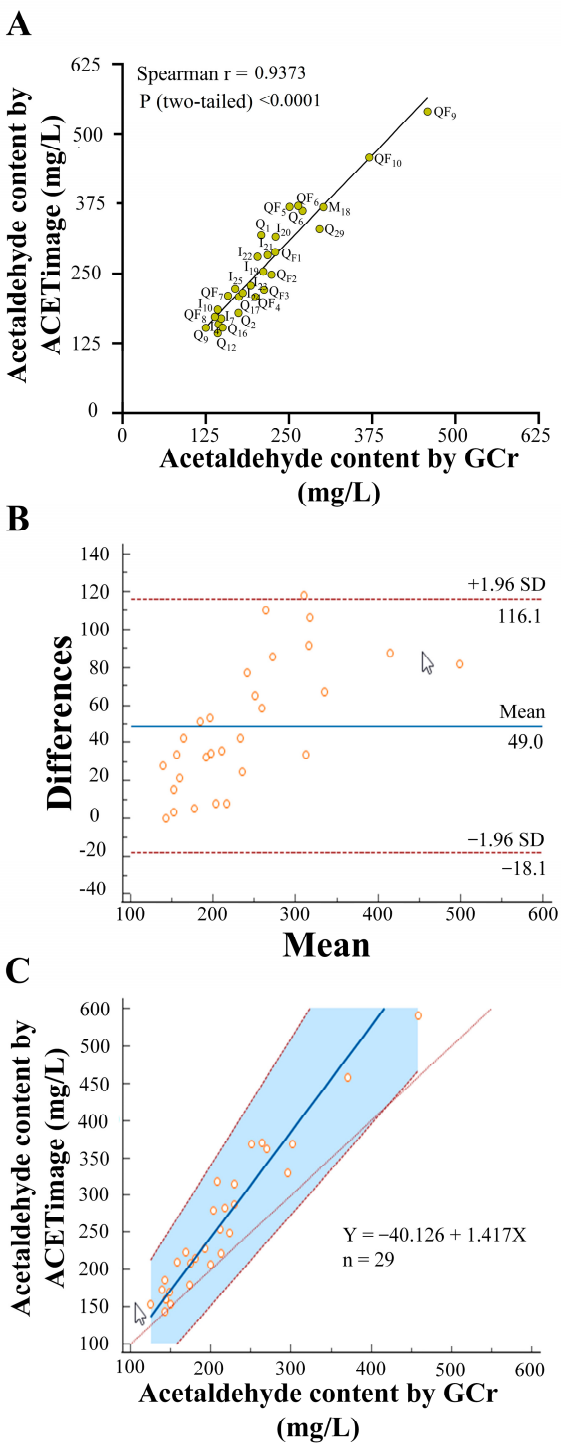


Figure 8. Correlation analysis (**A**), Bland–Altman analysis (**B**), and Passing–Bablok Regression analysis (**C**) were conducted on the acetaldehyde content in Pisco head samples, as measured by the ACETimage and gas chromatography reference (GC-FID) methods.

Table 1. Acetaldehyde content in Pisco head samples measured by the ACETimage method and the gas chromatography reference (GC-FID) method. The values are the mean of three replicates.

Samples	Acetaldehyde Content (mg/L)			
	ACETimage		GC-FID	
	Mean	RSD	Mean	RSD
Q_1	318.4	1.1	208.4	3.1
Q_2	179.4	1.0	174.1	0.8
I_4	159.5	4.6	144.2	0.7
Q_6	361.9	3.7	270.3	0.6
I_7	169.8	3.9	148.1	2.8
Q_9	153.0	0.6	125.0	1.7
I_{10}	185.1	1.6	142.9	0.8
Q_{12}	143.3	0.5	142.9	0.9
Q_{16}	153.5	2.3	150.3	1.2
Q_{17}	207.7	1.7	174.9	2.5
M_{18}	368.4	2.5	301.8	0.3
I_{19}	253.5	0.9	211.4	1.5
I_{20}	315.6	11.2	229.9	1.9
I_{21}	282.8	1.1	218.1	0.8
I_{22}	279.8	16.3	202.8	0.5
I_{23}	228.2	1.8	192.7	0.5
I_{24}	214.8	0.8	180.6	0.9
I_{25}	222.6	21.8	169.1	0.5
Q_{29}	329.6	4.0	295.9	1.2
QF_1	287.8	12.6	229.4	0.4
QF_2	248.2	0.4	223.7	0.4
QF_3	220.6	0.5	212.6	1.1
QF_4	207.6	0.5	199.9	0.7
QF_5	368.8	5.8	251.1	0.7
QF_6	370.6	1.2	264.0	0.9
QF_7	209.7	1.3	158.3	1.0
QF_8	172.6	0.9	138.8	1.3
QF_9	540.2	0.2	458.4	1.2
QF_{10}	458.4	0.5	370.8	2.0

RSD = Relative Standard Deviation. Q = Quebranta, I = Italia, M= Mixture and QF = Quebranta fraction.

In general, the ACETimage method tended to overpredict the acetaldehyde content in Pisco head samples compared to the GC-FID method (Table 1) and this was confirmed by the Passing–Bablok regression analysis (Figure 8C). All Pisco head fractions from the aromatic Italia grapes (I_4, I_7, I_{10}, and $I_{19–25}$) were overpredicted by the ACETimage method, while some Pisco head samples produced from the non-aromatic Quebranta grapes gave similar acetaldehyde content values (Q_2, Q_{12}, Q_{16}, Q_{F3}, and Q_{F4}); to the GC-FID method. The other head fractions from Quebranta Pisco (Q_1, Q_6, Q_9, Q_{17}, QF_1, QF_2, and $Q_{F5–10}$), were overestimated by the ACETimage method (Figure 9A). One possible explanation for the ACETimage method overestimating the acetaldehyde content in Pisco head samples is the presence of terpenoids, which could also participate in the aldol condensation/crotonization/resinification reaction (Figure 9B). This result is consistent with the predominant terpenoid content in Pisco samples made from Italia and Quebranta grapes [48,49].

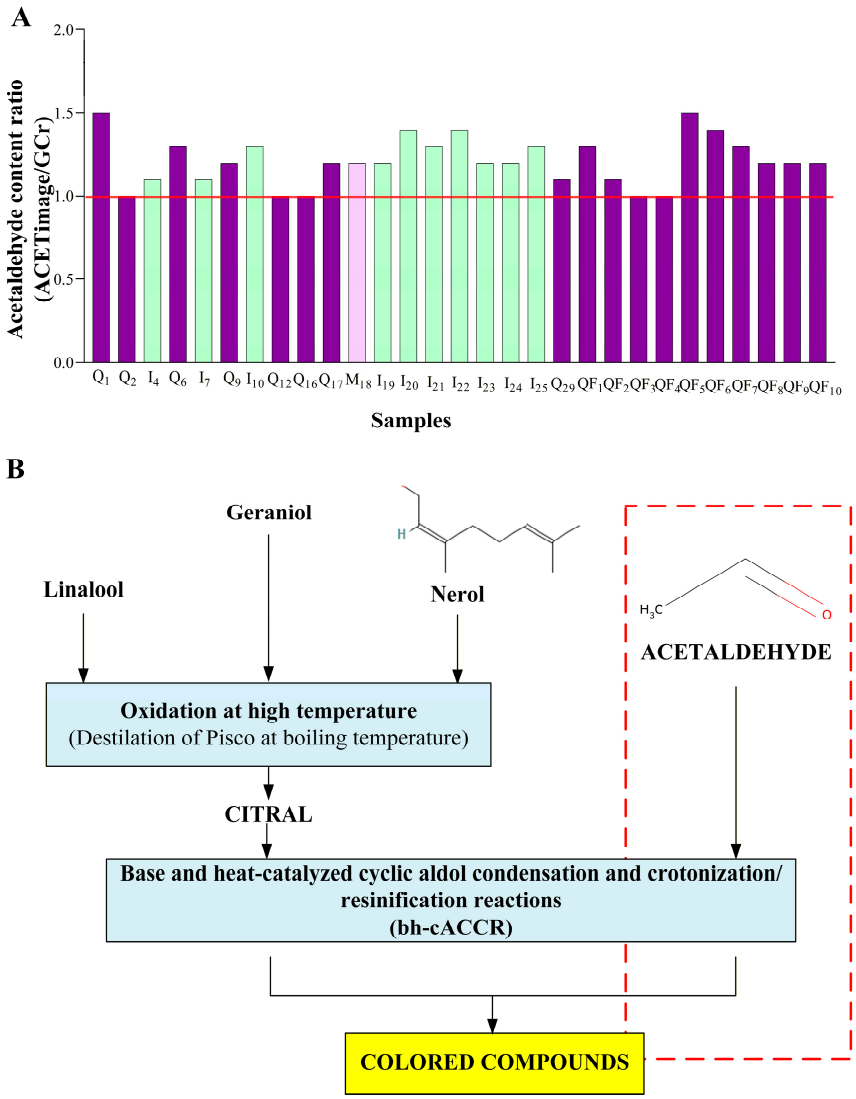


Figure 9. The aldehyde content ratio of the Pisco head samples was assessed using both the ACETimage method and the gas chromatography reference (GC-FID) method (**A**). A proposed theoretical mechanism is presented that could explain the interference and reaction of terpenoids in the quantification of acetaldehyde using the ACETimage method, which is based on bh-cACCR and image analysis (**B**).

4. Conclusions

A colorimetric method, ACETimage, was developed and validated for quantifying acetaldehyde in Pisco head fractions produced during distillation. The optimal conditions for the colorimetric reaction involved placing an aliquot of Pisco head fraction in headspace vials, adding an equal volume of a NaOH (20% *w*/*w*) solution, and heating the Pisco solution in boiling water for 2 min. The RGB color information was acquired by the Color Grab App for Smartphone using an optimal brightness set of 0.5 within 10 min after the completion of the reaction period, during which the color remained constant. The relationship between the Euclidean distance based on RGB values and acetaldehyde concentration showed a quadratic relationship in the 0–300 mg/L. The detection limit (LOD) of the ACETimage method was 29.96 mg/L, and the quantification limit (LOQ) was 95.32 mg/L. The ACETimage method showed a significant correlation with GC-FID, but it tended to overestimate the acetaldehyde content, possibly because of the presence of terpenoids that might interfere with the aldol condensation reaction.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/chemosensors14040084/s1, Figure S1: Heating systems for colorimetric reactions; Figure S2: The image acquisition and analysis system; Figure S3: Color parameters calculated from the RGB and CIELab color space at different acetaldehyde concentrations.

Author Contributions: Conceptualization, L.E.R.-S. and L.C.-H.; Methodology, B.H.-S., M.M.G., L.E.R.-S. and L.C.-H., Software, B.H.-S. and L.E.R.-S.; Validation, B.H.-S., L.E.R.-S. and L.C.-H.; Formal Analysis, B.H.-S., M.M.G., L.E.R.-S. and L.C.-H.; Investigation, B.H.-S., L.E.R.-S. and L.C.-H.; Resources, L.C.-H.; Data Curation, B.H.-S. and M.M.G.; Writing—Original Draft Preparation, B.H.-S.; Writing—Review and Editing, L.E.R.-S. and L.C.-H.; Visualization, B.H.-S.; Supervision, L.C.-H.; Project Administration, L.C.-H.; Funding Acquisition, L.C.-H. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Acknowledgments: We thank the San Nicolás winery in the Pisco-Ica Valley, which provided the samples for this research; Yalan Wu, a doctoral student at Ohio State University, who assisted with the chromatographic analyzes of the samples; and the Vibrational Spectroscopy Laboratory at The Ohio State University, where part of the research analyzes were conducted.

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

References

1. Hatta-Sakoda, B.; Guevara-Pérez, A.; Morales-Soriano, E. Evolution of Acetaldehyde, Methanol, and Furfural in Pisco Distillation. *ACS Food Sci. Technol.* **2024**, *4*, 889–894. [CrossRef]
2. Palma, J.C.; Fabian-Campos, J.; Dioses-Morales, J.J.; Arias-Durand, A.D.; Espinoza-Cordova, G.; Gonzales-Uscamayta, M.; Rengifo-Maravi, J.C.; Chire-Murillo, E.T.; Caro Sanchez-Benites, V.A.; Jorge-Montalvo, P.; et al. Pisco, an Appellation of Origin from Peru: A review. *Heliyon* **2025**, *11*, e42251. [CrossRef]
3. Ernesto, R. Historia actualizada del pisco. *Rev. Investig. Univ. Cordon Bleu* **2025**, *12*, 42–54. [CrossRef]
4. Wardencki, W.; Orlita, J.; Namieśnik, J. Comparison of extraction techniques for gas Chromatographic determination of volatile carbonyl compounds in alcohols. *Fresenius' J. Anal. Chem.* **2001**, *369*, 661–665. [CrossRef]
5. Wardencki, W. Alcoholic Beverages. In *Encyclopedia of Analytical Science*, 3rd ed.; Elsevier: Amsterdam, The Netherlands, 2018; pp. 1–10.
6. Paiano, V.; Bianchi, G.; Davoli, E.; Negri, E.; Fanelli, R.; Fattore, E. Risk assessment for the Italian population of acetaldehyde in alcoholic and non-alcoholic beverages. *Food Chem.* **2014**, *154*, 26–31. [CrossRef]
7. Guittin, C.; Maçna, F.; Barreau, A.; Poitou, X.; Sablayrolles, J.M.; Mouret, J.R.; Farines, V. The aromatic profile of wine distillates from Ugni blanc grape musts is influenced by the nitrogen nutrition (organic vs. inorganic) of Saccharomyces cerevisiae. *Food Microbiol.* **2023**, *111*, 104193. [CrossRef]
8. Afkhami, A.; Parham, H.; Rezaei, M. Kinetic spectrophotometric determination of acetaldehyde. *Anal. Lett.* **2000**, *33*, 527–538. [CrossRef]
9. Avigad, G. A simple spectrophotometric determination of formaldehyde and other aldehydes: {Application} to periodate-oxidized glycol systems. *Anal. Biochem.* **1983**, *134*, 499–504. [CrossRef] [PubMed]
10. Mohamed, A.A.; Mubarak, A.T.; Marestani, Z.M.H.; Fawy, K.F. Highly sensitive and selective catalytic determination of formaldehyde and acetaldehyde. *Talanta* **2008**, *74*, 578–585. [CrossRef]
11. Liu, Y.; Liu, C.; Xu, X.; Niu, C.; Wang, J.; Zheng, F.; Li, Q. Development of a Rapid and Sensitive Fluorescence Sensing Method for the Detection of Acetaldehyde in Alcoholic Beverages. *Foods* **2022**, *11*, 3450. [CrossRef]
12. Yang, C.; Li, Y.; Wang, J.Z.; He, J.; Hou, H.; Li, K. Fast and highly selective detection of acetaldehyde in liquor and spirits by forming aggregation-induced emission luminogen. *Sens. Actuators B Chem.* **2019**, *285*, 617–624. [CrossRef]
13. Li, Z.; Fang, M.; LaGasse, M.K.; Askim, J.R.; Suslick, K.S. Colorimetric Recognition of Aldehydes and Ketones. *Angew. Chem. Int. Ed.* **2017**, *56*, 9860–9863. [CrossRef]
14. Rodríguez, D.M.; Wrobel, K.; Wrobel, K. Determination of aldehydes in tequila by high-performance liquid chromatography with 2,4-dinitrophenylhydrazine derivatization. *Eur. Food Res. Technol.* **2005**, *221*, 798–802. [CrossRef]
15. Charapitsa, S.V.; Kavalenka, A.N.; Kulevich, N.V.; Makoed, N.M.; Mazanik, A.L.; Sytova, S.N.; Zayats, N.I.; Kotov, Y.N. Direct determination of volatile compounds in spirit drinks by gas chromatography. *J. Agric. Food Chem.* **2013**, *61*, 2950–2956. [CrossRef]

16. Charapitsa, S.V.; Sytova, S.N.; Korban, A.L.; Sobolenko, L.N. Single-laboratory validation of a gas chromatographic method of direct determination of volatile compounds in spirit drinks: Need for an improved interlaboratory study. *J. AOAC Int.* **2019**, *102*, 669–672. [CrossRef] [PubMed]
17. Coldea, T.E.R.; Socaciu, C.; Pârv, M.; Vodnar, D. Gas-Chromatographic Analysis of Major Volatile Compounds Found in Traditional Fruit Brandies from Transylvania, Romania. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2011**, *39*, 109–116. [CrossRef]
18. Shin, K.S.; Lee, J.H. Acetaldehyde contents and quality characteristics of commercial alcoholic beverages. *Food Sci. Biotechnol.* **2019**, *28*, 1027–1036. [CrossRef]
19. Thang, N.Q.; Tan, L.V. Determination of Methanol, Acetaldehyde, and Ethyl Acetate in Thousand Folds of Ethanol Sample by Headspace Gas Chromatography with Mass Spectrometry. *Int. J. Anal. Chem.* **2023**, *2023*, 8851265. [CrossRef] [PubMed]
20. Anjos, O.; Santos, R.; Estevinho, L.M.; Caldeira, I. FT-RAMAN methodology for the monitoring of honeys' spirit distillation process. *Food Chem.* **2020**, *305*, 125511. [CrossRef]
21. Anderson, J.B.; Peters, M.S. Acetaldehyde Aldol Condensation Kinetics. *J. Chem. Eng. Data* **1960**, *5*, 359–364. [CrossRef]
22. Bell, R.P.; McTigue, P.T. 603. Kinetics of the aldol condensation of acetaldehyde. *J. Chem. Soc.* **1960**, 2983–2994. [CrossRef]
23. Oliver, J. Explicación teórica de la reacción coloreada, fundamento de la reacción colorimétrica del enranciamiento de grasas. *ARS Pharm* **1965**, *6*, 271–273.
24. Oliver, J.; Suñé, J.M. Metodo colorimetrico para determinar el enranciamiento de aceites y grasas sólidas. *ARS Pharma* **1965**, *6*, 243–257.
25. Menevseoglu, A.; Aykas, D.P.; Hatta-Sakoda, B.; Toledo-Herrera, V.H.; Rodriguez-Saona, L.E. Non-Invasive Monitoring of Ethanol and Methanol Levels in Grape-Derived Pisco Distillate by Vibrational Spectroscopy. *Sensors* **2021**, *21*, 6278. [CrossRef] [PubMed]
26. Marinho, O.R.; Lima, M.J.A.; Rocha, F.R.P.; Reis, B.F.; Kamogawa, M.Y. A greener, fast, and cost-effective smartphone-based digital image procedure for quantification of ethanol in distilled beverages. *Microchem. J.* **2019**, *147*, 437–443. [CrossRef]
27. Minh-Huy, D.; Anh-Dao, L.T.; Thanh-Nho, N.; Nhon-Duc, L.; Cong-Hau, N. Smartphone-based digital images as a low-cost and simple colorimetric approach for the assessment of total phenolic contents in several specific Vietnamese dried tea products and their liquors. *Food Chem.* **2023**, *401*, 134147. [CrossRef]
28. Anh-Dao, L.T.; Thanh-Nho, N.; Huu-Trung, B.; Tien-Giang, N.; Dong, T.U.T.; Quoc-Duy, N.; Quang-Hieu, N.; Le-Vy, N.; Thanh-Dieu, N.T.; To, D.V.T.; et al. A portable colorimetric tool using a smartphone camera applied for determining total phenolic contents in coffee products. *Chin. J. Anal. Chem.* **2023**, *51*, 100228. [CrossRef]
29. Soares, S.; Fernandes, G.M.; Rocha, F.R.P. Smartphone-based digital images in analytical chemistry: Why, when, and how to use. *TrAC Trends Anal. Chem.* **2023**, *168*, 117284. [CrossRef]
30. Vucāne, S.; Cinkmanis, I.; Šabovics, M. Colorimetric Measurements of Vegetable Oils by Smartphone-Based Image Analysis. *Proc. Latv. Acad. Sci. Sect. B Nat. Exact Appl. Sci.* **2022**, *76*, 110–115. [CrossRef]
31. Abderrahim, M.; Arribas, S.M.; Condezo-Hoyos, L. A novel high-throughput image based rapid Folin-Ciocalteau assay for assessment of reducing capacity in foods. *Talanta* **2016**, *152*, 82–89. [CrossRef]
32. Abderrahim, M.; Arribas, S.M.; Condezo-Hoyos, L. A novel pyrogallol red-based assay to assess catalase activity: Optimization by response surface methodology. *Talanta* **2017**, *166*, 349–356. [CrossRef] [PubMed]
33. Ordomsky, V.V.; Sushkevich, V.L.; Ivanova, I.I. Study of acetaldehyde condensation chemistry over magnesia and zirconia supported on silica. *J. Mol. Catal. A Chem.* **2010**, *333*, 85–93. [CrossRef]
34. Geng, Z.; Sheng, L.; Zhang, K.; Shi, F.; Gong, H. Study on the process of producing crotonaldehyde from acetaldehyde catalyzed by Zr-β zeolite. *Sep. Purif. Technol.* **2024**, *335*, 126123. [CrossRef]
35. Franco, M.O.K.; Suarez, W.T.; Rodrigues Penha Pereira, G.; Castelo Bandane, V.; Robaina Vieira, M.C.; Bezerra Dos Santos, V.; Barbosa de Almeida, J.P. Using colorimetric spot test and digital imaging-based technique for volatile acidity determination in cachaça with the aid of a smartphone. *Microchem. J.* **2023**, *187*, 108416. [CrossRef]
36. Franco, M.O.K.; Suarez, W.T.; Bezerra dos Santos, V. Digital Image Method Smartphone-Based for Furfural Determination in Sugarcane Spirits. *Food Anal. Methods* **2017**, *10*, 508–515. [CrossRef]
37. Pires Dos Santos Benedetti, L.; Bezerra Dos Santos, V.; Almeida Silva, T.; Benedetti-Filho, E.; Lacerda Martins, V.; Fatibello-Filho, O. A digital image analysis method for quantification of sulfite in beverages. *Anal. Methods* **2012**, *7*, 7568–7573. [CrossRef]
38. Nozière, B.; Dziedzic, P.; Córdova, A. Formation of secondary light-absorbing "fulvic-like" oligomers: A common process in aqueous and ionic atmospheric particles? *Geophys. Res. Lett.* **2007**, L21812. [CrossRef]
39. Noziere, B.a.; Cordova, A. A Kinetic and Mechanistic Study of the Amino Acid Catalyzed Aldol Condensation of Acetaldehyde in Aqueous and Salt Solutions. *J. Phys. Chem. A* **2008**, *112*, 2827–2837. [CrossRef]
40. Franco, M.O.K.; Suarez, W.T.; Valentin Maia, M.; Bezerra Dos Santos, V. Smartphone Application for Methanol Determination in Sugar Cane Spirits Employing Digital Image-Based Method. *Food Anal. Methods* **2017**, *10*, 2102–2109. [CrossRef]
41. Fay, C.D.; Wu, L. Critical importance of RGB color space specificity for colorimetric bio/chemical sensing: A comprehensive study. *Talanta* **2024**, *266*, 124957. [CrossRef]

42. Nozière, B.; Chabert, P. Abiotic C–C bond formation under environmental conditions: Kinetics of the aldol condensation of acetaldehyde in water catalyzed by carbonate ions (CO_3^{2-}). *Int. J. Chem. Kinet.* **2010**, *42*, 676–686. [CrossRef]
43. Martin, J.D.; Gracia, A.R.; Asuero, A.G. Fitting Nonlinear Calibration Curves: No Models Perfect. *J. Anal. Sci. Methods Instrum.* **2017**, *07*, 1–17. [CrossRef]
44. INDECOPI. Reglamento de la Denominación de Origen Pisco. 2024. Available online: https://www.gob.pe/es/i/5805882 (accessed on 10 December 2025).
45. Khanmohammadi, M.; Vahid, H.; Moazzen, N.; Garmarudi, A.B.; Alijanianzadeh, R. Kinetic spectrophotometric determination of acetaldehyde in medical ethanol. *J. Anal. Chem.* **2008**, *63*, 1164–1167. [CrossRef]
46. Papaefstathiou, I.; Bilitewski, U.; de Castro, M.D.L. Determination of acetaldehyde in liquid, solid and semi-solid food after pervaporation-derivatization. *Fresenius' J. Anal. Chem.* **1997**, *357*, 1168–1173. [CrossRef]
47. Cacho, J.; Culleré, L.; Moncayo, L.; Palma, J.C.; Ferreira, V. Characterization of the aromatic profile of the Quebranta variety of Peruvian pisco by gas chromatography–olfactometry and chemical analysis. *Flavour Fragr. J.* **2012**, *27*, 322–333. [CrossRef]
48. Cacho, J.; Moncayo, L.; Palma, J.C.; Ferreira, V.; Culleré, L. Characterization of the aromatic profile of the Italia variety of Peruvian pisco by gas chromatography-olfactometry and gas chromatography coupled with flame ionization and mass spectrometry detection systems. *Food Res. Int.* **2012**, *49*, 117–125. [CrossRef]
49. Locko, G.A.; Trenton, N.J.; Frank, W.C.; Pa, H. Oppenauer Oxidation of Geranol/Nerol. U.S. Patent 4,663,488, 5 May 1987.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.