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Pungency evaluation of onion cultivars from the Venezuelan West-Center region by flow injection analysis–UV–visible spectroscopy pyruvate determination

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Abstract

A flow injection analysis (FIA) method was developed for the determination of pyruvate in onion cultivars (*Allium cepa* L.) from the West-Center region of Venezuela. The reference Schwimmer and Weston (1961) (J. Agric. Food Chem. 9 (1961) 301) Batch method was modified and adapted to FIA conditions. The formation kinetic of the 2,4-dinitrophenylhydrazine (DNPH)–pyruvate complex was evaluated at room temperature and at 37 °C. It was demonstrated the suitability of the chromopher formation at room temperature. The optimal values for the FIA parameters were: sample injection volume 3 mL, flow rate 6 mL min^{-1}, reactor length 1.5 m, sodium hydroxide concentration 1.0 mol L^{-1} and hydrochloric acid concentration 0.5 mol L^{-1}. The working calibration range was extended from 80 mg L^{-1} (Batch method) to 700 mg L^{-1} with the FIA set up. The sample dilution step is thus avoided, simplifying the whole analysis process. The pungency in representative samples of the cultivars Yellow granex 438, Ultra Hybrid and Red onion “Sangre de Toro” was evaluated by the flow injection analysis (FIA)–pyruvate method and the results were compared to the reference Batch pyruvate method and to the taste panel test. Non-significant differences were found at the 95% of confidence level between the FIA method and the Batch reference method. Correlation coefficient when comparing the FIA results to the taste panel test was $r^2 = 0.8353$. Significant differences ($P < 0.05$) were found in the pungency of the cultivars, the Ultra Hybrid having the highest pungency. The pungency order from minor to major was: Red onion, Texas Grano 438 and Ultra Hybrid.

Keywords: Pungency; Onion; Pyruvate; Taste panel; Flow injection

1. Introduction

The pungency of onion is one of the most important parameters to take into account in the quality evaluation of the cultivars [1]. This property is associated to enzymatic reactions, promoted by the enzyme allinasa [2]. In the process, the flavor precursors produce volatile compounds and pyruvates. Then the pyruvate content can be used for the indirect quantification of the pungency property [3,4] of this important at world level cultivar. The pungency is influenced by the soil physical–chemical characteristics, nutrients [5,6], storage [7–9] and kind of cultivar [10] among other variables. The pungency evaluation can be carry out by the determination of the volatile compounds generated after the action of the enzyme allinasa using chromatographic techniques [8], the lactate dehydrogenase mediated reduction of pyruvate [11] or most frequently by the spectrophotometric determination of pyruvates using the Schwimmer and Weston reaction of pyruvates with DNPH [3,4]. The studies on the analysis of onion pyruvates were focused in the simplification of sample preparation [3,4,12–15] but not on the spectrometric determination itself. On the other hand, being the onion one of the most important cultivars in the world, it becomes appropriate the automation of the analytical procedure by means of a flow injection analysis (FIA)

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manifold in order to minimize the use of glassware, the possibility of contamination, while reducing the whole analysis time. The use of modified carbon paste electrodes is reported for the FIA pyruvate determination in mammalian cell cultivation media with high selectivity and sensitivity (in the range of 5 M and 5 mM) [16]. Biosensors can also be used for the determination of pyruvates by means of luminescence detection using a FIA system with high sensitivity [17,18]. Although their selectivity and sensitivity, biosensors are composed of biological materia and could be inactivated by strong acids or bases [19]. Additionally, biosensors usually have a limited testing life due to the biological character of their design. The automatic analysis of onion samples for pyruvate determination using UV–vis detection and the Schwimmer and Weston reaction was approached by Yoo and Pike [13]. An automated method was developed using a high performance liquid chromatography (HPLC) system and pumps for reagents and sample delivery. The onion juice must be filtered through a 0.45 mm nylon filter. The mixture of sample—2,4-DNPH was heated in a 3 m long HPLC tubing coiled in a column heater set to 70 °C, following the addition of NaOH at the end of the coil. Since a HPLC system was used, the chromatographic process was matched using a column packed with glass beads. The obtained data resembles a true chromatogram. The results showed a bias respect to the HPLC and the spectrophotometric methods though the automation and the high sample throughput. In this work a FIA procedure is proposed using the reaction of pyruvate with 2,4-DNPH and UV–vis detection, simplifying the instrumental set up reported by Yoo and Pike and obtaining a good concordance between results compared to those obtained by the established method.

2. Experimental

2.1. Reagents

The calibration curves were performed using standards of sodium pyruvate (Merk, Germany), by serial dilution of the stock solution prepared dissolving the salt to a concentration of 1000 mg L^{-1}. The Swimmer and Weston reaction for formation of the colored compound was carry out using 2,4-dinitrophenylhydrazine (2,4-DNPH) (Scharlau Chemie S.A., Spain), Sodium hydroxide (Merk, Germany), Chlorhydric acid (Riedel de Haën, Germany) and de-ionized water.

2.2. Sampling and sample preparation

Three kind of onion cultivars from the region of “Valle de Quibor”, Lara state, Venezuela were selected for analysis namely, 438 (at two times after harvest), Ultra and Red onion “Sangre de Toro”. One hundred onions from each of these cultivars were carefully selected. For comparison to the sensorial test by panel, samples of the cultivars 430, Red onion and Texas Yellow granex were also analyzed.

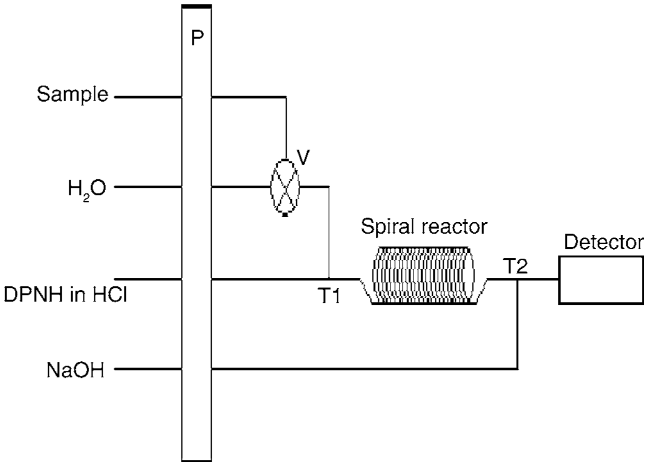

Fig. 1. Manifold for pyruvate determination.

Samples were prepared by weighing fresh onion in a proportion 1:2 with water, followed by homogenization in domestic blender and filtration of the supernatant, following the procedures reported in the literature [12–15]. For the FIA purposes non-additional dilution of the supernatant was performed.

The pyruvate background was estimated by taking the spectra of the inactivated samples. The inactivation procedure was performed in a microwave oven prior to homogenization as recommended by Ketter and Randle, 1998 [15]. Non-significant differences were found between the reagents blank and the inactivated samples. Therefore, determination of the pyruvate background levels was skipped in order to simplify the whole analytical procedure.

2.3. Instrumental

An ISMATEC peristaltic pump IPC model with four channels was used for sample and carrier introduction controlled by a Temporizer GrabLab model 900 and a control valve Cole-Palmer, model 625E Bunker CT. The detection was performed with the automatic wavelength scanning Cintra 10e spectrometer. The FIA manifold is shown in Fig. 1. It has four channels with Tygon tubing R-3606 of 0.0599 in. diameter. The de-ionized water is mixed with DNPH and goes through the spiral reactor. Then is mixed with the NaOH solution stream, and the base line is detected in the flow cell. In a second step the sample is injected into the de-ionized water stream, mixed in the reactor with the DNPH and finally after confluence with the NaOH stream the colored compound is formed and detected at 441 nm.

2.4. Determination of pungency by sensorial test

For the sensorial test a panel of eight persons was trained, following the procedure reported by Wall and Corgan, 1992 [4]. The rating scale used was 1: extremely mild, 2: mild, 3: slightly pungent, 4: pungent and 5: extremely pungent. Samples from each cultivar were randomly selected and analyzed for pyruvate content. Then the panel tasted the samples. To clear the palates water, crackers and apple slices were used. The experiment was repeated on different days.

3. Results

3.1. Study of reaction kinetic

The reaction kinetic was followed at two temperatures: 25 °C (room) and 37 °C using the Batch procedure for three experimental conditions of HCl and NaOH concentrations (HCl 2 mol L^{-1}–NaOH 0.6 mol L^{-1}, as reported in the literature, HCl 0.1 mol L^{-1}–NaOH 0.5 mol L^{-1} and HCl 0.5 mol L^{-1}–NaOH 1.0 mol L^{-1}). This assay was performed in order to determine the reliability of the FIA procedure without thermal bath and for the initial estimation of the reactor length. It was observed that after 2 min the reaction between pyruvates and DNPH in HCl was near 84% performed for both temperatures. This room temperature was considered optimal, since the thermal bath is avoided in the FIA set up, and a good signal was allowed for the analyzed samples. It is observed that the reaction was mostly completed after 5 min for the three tested reagents conditions. The reaction by FIA was then performed at room temperature, and the reactor length for mixing of sample and water with DNPH in HCl was adjusted on the basis of the kinetic study. The reaction with NaOH was almost instantaneously performed.

3.2. Optimization of reagent concentrations

The 2,4-DNPH concentration was fixed as reported in the literature (0.0125%) [4,20] after the evaluation of lower (0.0065%) and higher levels (0.0250%). The concentration of 0.0065% allows to low signal values while the concentration of 0.00250% of 2,4-DNPH allows to high background values. The reagent concentrations were optimized regarding to the HCl and NaOH concentration. Chlorhydric acid concentration less than 0.5 mol L^{-1} was not suitable due to the low signal values and to the fact that the 2,4-DNPH is not easy dissolved at acid concentration lower than 0.5 mol L^{-1}. Best results in sensitivity were obtained for the concentration of HCL 0.5 mol L^{-1} and NaOH 1.0 mol L^{-1} while having the lower reagents concentration to obtain the higher signal value (see Fig. 2).The optimal reagent concentrations were independent of the FIA parameters as sample volume, reactor length and flow rate. It was observed that signal depended on the initial HCl concentration and the ratio to the NaOH concentration.

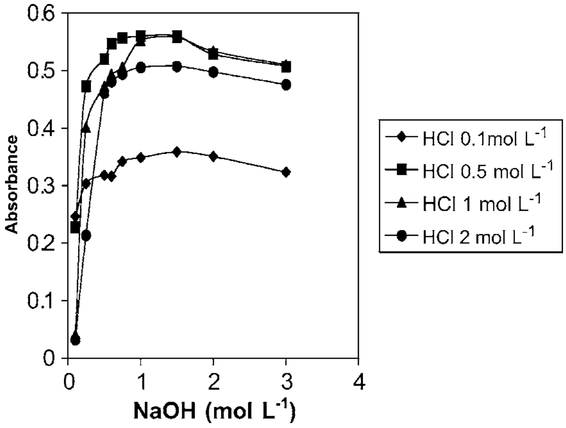

Fig. 2. Effect of HCl and NaOH concentration on the absorbance signal of 2,4-DNPH–pyruvate compound for an onion sample.

3.3. Optimization of FIA

The FIA system was optimized in terms of the sample volume, flow rate and reactor length.

3.3.1. Sample volume

This parameter was optimized fixing the reactor length at 2 m, using a flow rate of 6 mL min^{-1}. These values were selected taking into account the reaction kinetics at room temperature. The optimal value for sample volume was 6.0 mL. The sample volume must be higher than 3 mL in order to improve the absorbance signal and hence the sensitivity due to the dispersion in the system. This fact could be a disadvantage of the proposed FIA method, but it is necessary to take into account that sample amount does not become a restriction when determining pyruvate in onion samples.

3.3.2. Flow rate

This parameter was adjusted for HCl 0.5 mol L^{-1} and NaOH 1.0 mol L^{-1}, an injection volume of 6 mL and a reactor length of 2 m. The flow rate was changed from 2.5 mL min^{-1} to 10 mL min^{-1}. The value corresponding to the maximal absorbance was 4.5 mL min^{-1}, but the value of 6 mL min^{-1} was selected since the absorbance does not observe a significant decrement and a higher sample throughput is obtained. Higher flow rate values result in an undesirable increment and unstability of the base line signal.

3.3.3. Reactor length

The optimal reactor length was determined for HCl 0.5 mol L^{-1} and NaOH 1.0 mol L^{-1}, a sample volume of 6 mL and a flow rate of 6 mL min^{-1}. It is observed that this parameter does not affect significantly the absorbance signal if changed from 0.5 m to 3 m. Since the reaction is more than 70% completed after 2 min, small reactor lengths affect the absorbance signal due to the reaction kinetic restriction. On the other hand, for higher reactor lengths the absorbance signal decreases due to the dispersion on the system. It is important to point out that reactor length directly influences the sample throughput. A good compromise was found for a reactor length of 1.5 m.

3.4. Comparison between Batch and FIA

The determination of pyruvate levels was performed by the Batch procedures using the reagent concentrations

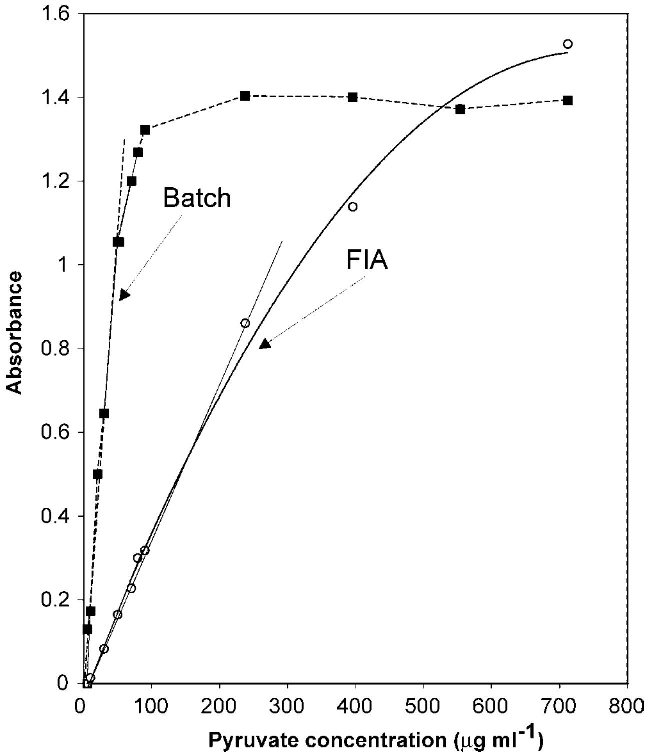

Fig. 3. Calibration curves for pyruvate determination by FIA linear and square fitting (solid line), Batch linear fitting and signal vs. concentration hystogram (dotted line).

recommended in the literature (HCl $0.6\,\mathrm{mol\,L^{-1}}$ and NaOH $2.0\,\mathrm{mol\,L^{-1}}$) and the optimized concentrations (HCl $0.5\,\mathrm{mol\,L^{-1}}$ and NaOH $1.0\,\mathrm{mol\,L^{-1}}$). A first important difference between the Batch and FIA methods was observed in the working calibration range. A narrow range was observed by Batch, up to $80\,\mathrm{mg\,mL^{-1}}$ being $r = 0.998$, as shown in Fig. 3. A wider working calibration range was found in the FIA method, up to $200\,\mathrm{mg\,mL^{-1}}$, being $r = 0.999$ if a straight curve is fitted and up to $700\,\mathrm{mg\,mL^{-1}}$, if a square fitting is used ($r = 0.999$) (see Fig. 3). Non-significant differences were found among the results obtained by using the different calibration curves by FIA (straight line or square fitting). This is not possible for the Batch methods. A plateau is observed in the curve above the concentration level of $80\,\mathrm{mg\,mL^{-1}}$ (see corresponding hystogram in Fig. 3), and samples with high concentration should be diluted prior to analysis. In the FIA system a wider range of samples with different concentration levels can be analyzed using the same sample to water dilution of the first step of sample preparation only. This fact becomes important when a high number of samples is being analyzed, as pointed by preceding works [13,14].

Each cultivar sample was analyzed for pyruvate determination by the Batch and FIA methods ($n = 5$, for each cultivar and method). The results obtained by the three methods (Batch reference, Batch optimized and FIA) were compared by analysis of variance and no significant differences were found ($F = 0.3799$ and critical value 3.8852, when applying the Scheffe test). The mean precision as relative standard deviation (% R.S.D.) were 5.04% in Batch reference, 4.75% in Batch optimized and 0.7% in the FIA method.

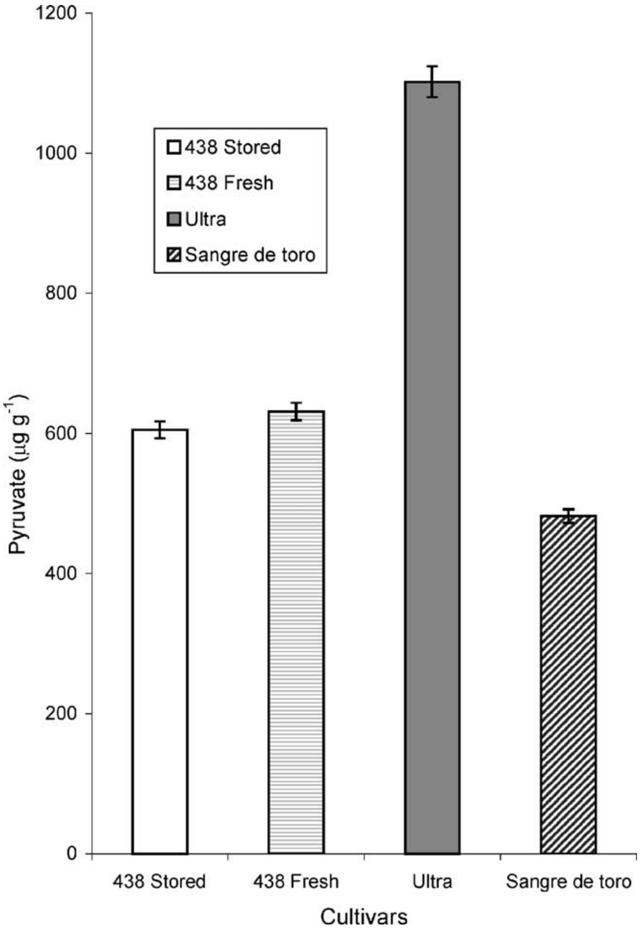

Fig. 4. Pyruvate concentration in onion cultivars from the West-Center region of Venezuela.

3.5. *Determination of pyruvate concentrations*

Pyruvate concentration in cultivars significantly differed at the 95% of confidence level depending on the cultivar. The higher concentration ($\mu\mathrm{g\,g^{-1}}$) was found for the Ultra cultivar (1102 ± 3). When compared to the 438 cultivar by the Student's t-test; $t = -77.27$ respect to stored 438 and -79.69 respect to fresh 438, being t critical $= 2.57$. The 438 cultivar has the middle value of concentration ($\mu\mathrm{g\,g^{-1}}$) (630 ± 13 for fresh and 605 ± 1 for stored). The lowest concentration ($\mu\mathrm{g\,g^{-1}}$) corresponded to the Red onion "Sangre de Toro" (482 ± 1). The pyruvate concentration of this cultivar was significantly lower compared to the 438 cultivar ($t = 25.38$ for fresh 438 and 28.47 for stored 438 being t critical $= 2.57$). No significant differences were found for the 438 cultivar depending on the time of storage ($t = 1.167$ and t critical $= 2.306$). Concentration levels are shown in Fig. 4. These results were compared to those obtained by

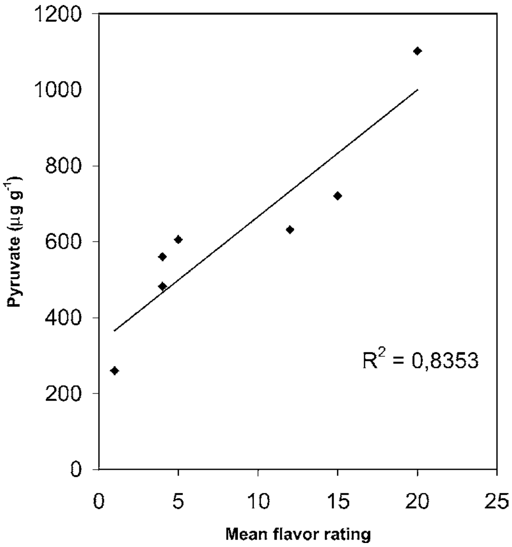

Fig. 5. Pungency by tasting panel (mean flavor rating) vs. pyruvate concentration.

the sensorial test (see Fig. 5). The correlation factor r^2 was 0.8353, which is similar to the reported in the literature by Wall and Corgan (1992) [4].

4. Conclusions

The flow injection analysis of onion samples for the indirect determination of pungency allows to non-significant differences respect to the analysis by the Batch reference and optimized methods. The optimal reagent concentrations are HCl 0.5 $\mathrm{mol\,L^{-1}}$ y NaOH 1.0 $\mathrm{mol\,L^{-1}}$. The reaction can be performed at room temperature simplifying the whole analytical procedure. The quantification procedures by Batch methods have the lack of small calibration range, and dilution of some onion sample extracts is mandatory. In the FIAS method the calibration range can be extended to 700 $\mathrm{mg\,mL^{-1}}$ by means of a square polynomial fitting. A significant improvement in the precision of the analysis is observed with the FIA method when compared to Batch methods. The pyruvate concentration is an adequate parameter for the onion pungency estimation. A high correlation with the sensorial test is found. Non-significant differences were found in the pungency of variety 438 fresh and stored. The less pungent onion was the Red variety and the most pungent was the variety Ultra.

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