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Analysis of total propionic acid in feed using headspace solid-phase microextraction and gas chromatography☆

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Abstract

A method is described to analyze total propionic acid content (free propionic acid + sodium, ammonium, calcium salts, etc.) in feed, using headspace solid-phase microextraction (HS-SPME) of a feed suspension in salted and acidified water. Optimization of the extraction variables was done by simplex method after choosing a polyacrilate fiber to enhance the response of this acid. Separation was made by capillary gas chromatography (GC), using a special free fatty acid phase (FFAP) column for acids and a flame ionization detector (FID). Some of the chromatograms were also done, injecting the SPME fiber in a GC–mass spectrometry (MS) system, working with some specific ions for propionic acid, to be selective enough to avoid confusing the propionic acid peak with interferences of those complex matrixes. The method was tested for linearity and repeatability. Detection and quantification limits were also calculated. The method was applied to commercial feed samples, very variable in composition, quantifying by standard addition method. No major interferences were observed.
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Keywords: Headspace analysis; Propionic acid

1. Introduction

Propionic acid and its salts have been used as mold inhibitors in feed at doses over 200 mg/kg. The analysis of total propionic acid content in this matrix has been traditionally difficult because feed is very variable in composition, with different proportions of total fat, protein, starch, etc. Usual ingredients could be as diverse as: corn, soybean, blood plasma and fishmeal. These raw materials are prone to generate interferences when applying chromatographic methods and could introduce important limitations to extraction procedures. In the literature, methods can be found based on the Wiegner distillation method or the gas chromatographic analysis of ethereal or aqueous solutions [1]. Also a Japanese Government Food Additive Regulation method can be found based on the gas chromatographic [2] or the HPLC analysis [3] of the distillate of a feed sample under acidic conditions. Other methods, can also be found, based on the HPLC analysis on an ODS column of *p*-nitrobenzyl derivative of propionic acid, using strong cation-exchange columns to separate interferences from the sample [4], or capillary electrophoresis of aqueous solutions

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deproteinized in basic medium by ultrafiltration [5]. Recently, a headspace solid-phase extraction (HS-SPME) method has been applied to the analysis of free propionic acid content of distillers grains, using a carboxen-polydimethylsiloxane (PDMS) fiber [6]. Propionates were not considered.

As seen, developed methods to determine the total quantity of propionic acid in feed, were tedious, sophisticated or solvent consuming. Then, a simple analytical method, sensitive enough to detect ppm quantities and selective enough to avoid confusing the propionic acid with interferences of these complex matrices is needed.

A new method is described in this paper that allows us to analyze the total propionic acid content (free + salts) in feed, by capillary gas chromatography with flame ionization (FID) or mass spectrometry (MS) detection, using HS-SPME of a suspension of feed in hot, salted and acidified water.

Separation was done with a special column for acids free fatty acid phase (FFAP) to improve the shape of the propionic acid peak, increasing its detectability and improving its quantification.

2. Experimental

2.1. Reagents

Propionic acid (analytical-reagent grade; Merck, Darmstadt, Germany), sodium propionate (Quimidroga, Barcelona, Spain), sodium chloride and sulfuric acid (both analytical-reagent grade; Panreac, Montcada i Reixach, Spain), acetonitrile (HPLC grade; Carlo Erba, Rodano, Italy), deionized water (Laboratorio de agua destilada, Badalona, Spain).

2.2. Samples

The method was developed using a propionic acid free commercially available standard feed, of known composition: corn 32, barley 24, pea 18, soya 12, meal byproducts 8, vitamin corrector 3, added vegetable fat 3 (total fat 5%), lysine 0.08, antioxidant 0.01%.

Commercial samples of different European origins were also used to test the applicability of the method. Both meal and grain mix were used.

2.3. Instruments

The SPME holder and polyacrylate 85 μm fiber were from Supelco, Bellefonte, PA, USA.

The chromatograph HP-6890, flame ionization detection system and the mass selective detector HP-5973 were from Hewlett-Packard, Palo Alto, CA, USA.

2.4. Material

The chromatographic column (HP-FFAP, 50 m × 0.32 mm, 0.52 μm) was from Hewlett-Packard. Further, stirrer/heater, water bath, centrifuge, balance, stir bars, pH paper, 120 ml glass vials and butyl/aluminum caps, beakers 100, 500 ml and centrifuge tubes were used.

2.5. Standard preparation

Exactly near 0.2 g propionic acid of >98% purity was weighted in a 100 ml beaker and was made up with acetonitrile. A 2000 mg/l solution was obtained, which was used to quantify, using the standard addition method.

2.6. Chromatographic conditions

2.6.1. Oven

Initial temperature: 60 °C, initial time: 0 min, rate: 4 °C/min, final temperature: 230 °C, final time: 20 min. Under these conditions, propionic acid was eluted near 21 min.

2.6.2. Injector

Injector pressure 80 kPa (near 1.5 ml/min helium flow), injection temperature: 250 °C, injection type splitless, purge on 2 min.

2.7. Detection

2.7.1. MS

MS transfer line temperature: 250 °C, MS source temperature: 230 °C, MS source quadrupole temperature: 150 °C. Ion employed to quantify: 74.1, ions employed to qualify: 45.1, 73.1.

2.7.2. FID

Detector temperature: 250 °C, hydrogen flow 40 ml/min, air flow 450 ml/min, make-up nitrogen 50 ml/min.

3. Results

The following results were obtained with FID except when indicated.

Table 1

Simplex no.	SPME exposition time (min)	Vial temperature (°C)	Salt conc. (%, m/v)	Propionic acid GC area
1	30	60	20	274967
2	45	80	30	405705
3	60	70	25	356174
4	40	70	40	483076
5	55	80	40	435003
6	30	80	40	499192
7	10	80	40	512495
8	5	80	40	611255[a]
9	1	80	40	347861

[a] Optimum.

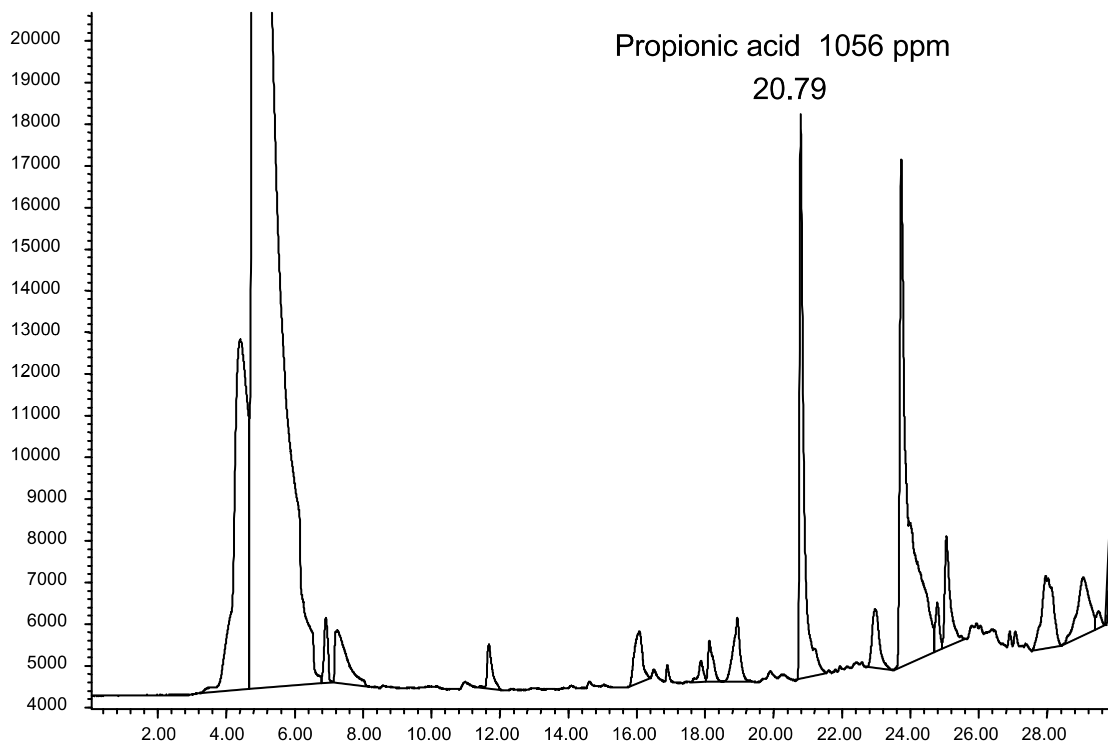

Fig. 1. HS-SPME–GC–FID chromatogram of a meal feed. Time scale in min.

Some preliminary tests were made with propionic acid standards in water to be sure the method was acceptable using all conditions and reagents, except the matrix and, in this way, evaluating better matrix interaction.

A first test, to choose the optimum SPME fiber, was done analyzing a standard propionic acid solution in water with three fibers: PDMS 100 μm, PDMS–carboxen–divinylbenzene (DVB) and polyacrylate in the same conditions. Twenty milliliter of a 20 mg/l standard propionic acid solution in water, were taken in a 120 ml glass vial sealed with a butyl/aluminum cap. Agitation was done by a magnetic stir bar. SPME fibers were exposed into the vial for 30 min at 20 °C. Chromatographic areas were in the order: polyacrylate > PDMS–carboxen–DVB > PDMS 100 μm. The polyacrylate fiber was chosen for the rest of the process.

Optimization, via simplex [7], of three different operational conditions: Vial temperature, SPME extraction time and NaCl concentration added, using a 20 mg/l standard propionic acid solution with the rest of conditions as mentioned above, is shown in Table 1. Criteria for simplex optimization were the combination of maximum propionic acid area and minimum SPME extraction time. The best results were obtained with higher temperature and salt concentrations.

Finally, a maximum vial temperature of 80 °C, to avoid problems with boiling water and vial overpressure, and saturation of salt (near 40% m/v), were chosen to standardize conditions. A maximum of chromatographic area was obtained with 5 min of exposition time under these conditions.

The optimum working method was applied to increasing concentrations of propionic acid standards in water (2, 20, 200 and 2000 mg/l) to calculate the linearity. A $r^2 = 0.99998$ was obtained.

The same working method was applied to three vials with 20 mg/l propionic acid standard in water each one, to calculate standard repeatability. A R.S.D. = 2.5% of GC area and a R.S.D. = 0.03% of retention time, were obtained.

The rest of tests were made with the standard feed, using the following *final feed sample preparation*

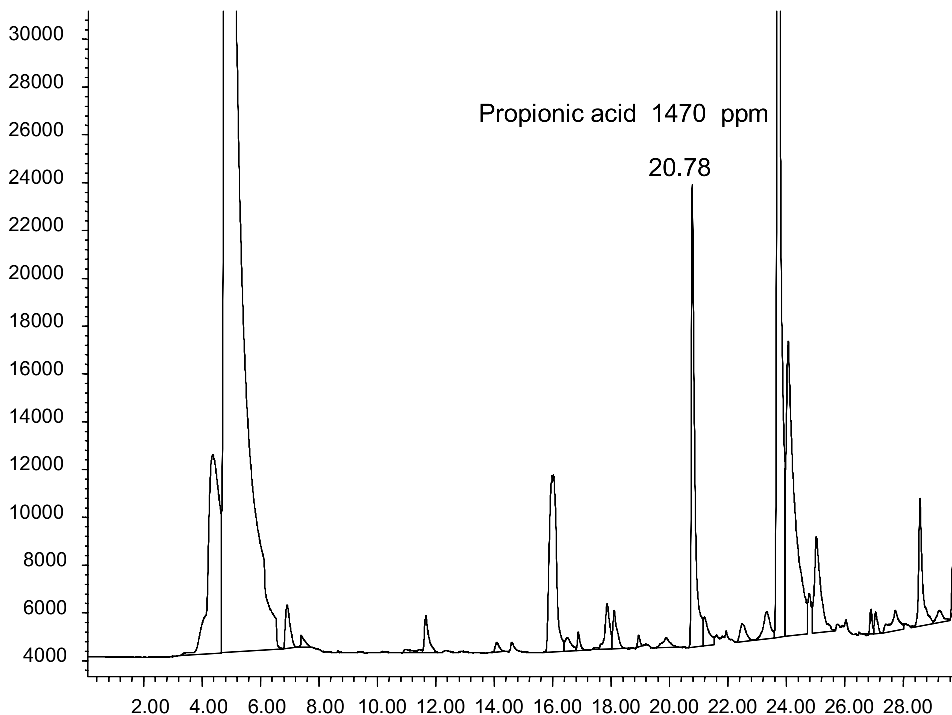

Fig. 2. HS-SPME–GC–FID chromatogram of a grain mix. Time scale in min.

method: for every experiment exactly near 10 g of each feed were weighted in a 500 ml beaker. One hundred gram of water and a magnetic stir bar were added. Agitation was left for 10 min. Two 10 ml centrifuge tubes were filled with the supernatant and were centrifuged for 2 min. A 120 ml glass vial was filled with 20 ml of the supernatant of the two centrifuge tubes and a magnetic stir bar was added. Some drops of 10% aqueous H_2SO_4 were added to be sure that the pH of the final solution was acid and to let all propionate salts to be transformed to free propionic acid. Finally, 8 g (saturation) of NaCl were added and the vial was capped with an aluminum cap and a butyl septum. The closed vial was introduced in a water bath at 80 °C, during 10 min with fast agitation to equilibrate the vial temperature and the headspace of the sample. A SPME syringe with a polyacrylate 85 μm fiber was inserted in the vial and was exposed for 5 min. The fiber was taken out from the vial, injected into the port of the chromatograph and exposed into the injector. Analysis was started and the syringe was left 30 min into the injector to clean the fiber for the next extraction avoiding memory effects.

To calculate linearity, the above final feed sample preparation was applied to four samples of the same propionic acid free standard feed, fortified with a total content of 45, 189, 454 and 1890 mg/l of propionic acid, added as sodium propionate (to avoid losses by volatility). A $r^2 = 0.9998$ was obtained.

To calculate sample repeatability, the same final feed sample preparation was applied to three commercial samples (in meal form) containing a mold inhibitor at total propionic acid doses of 1500 mg/l.

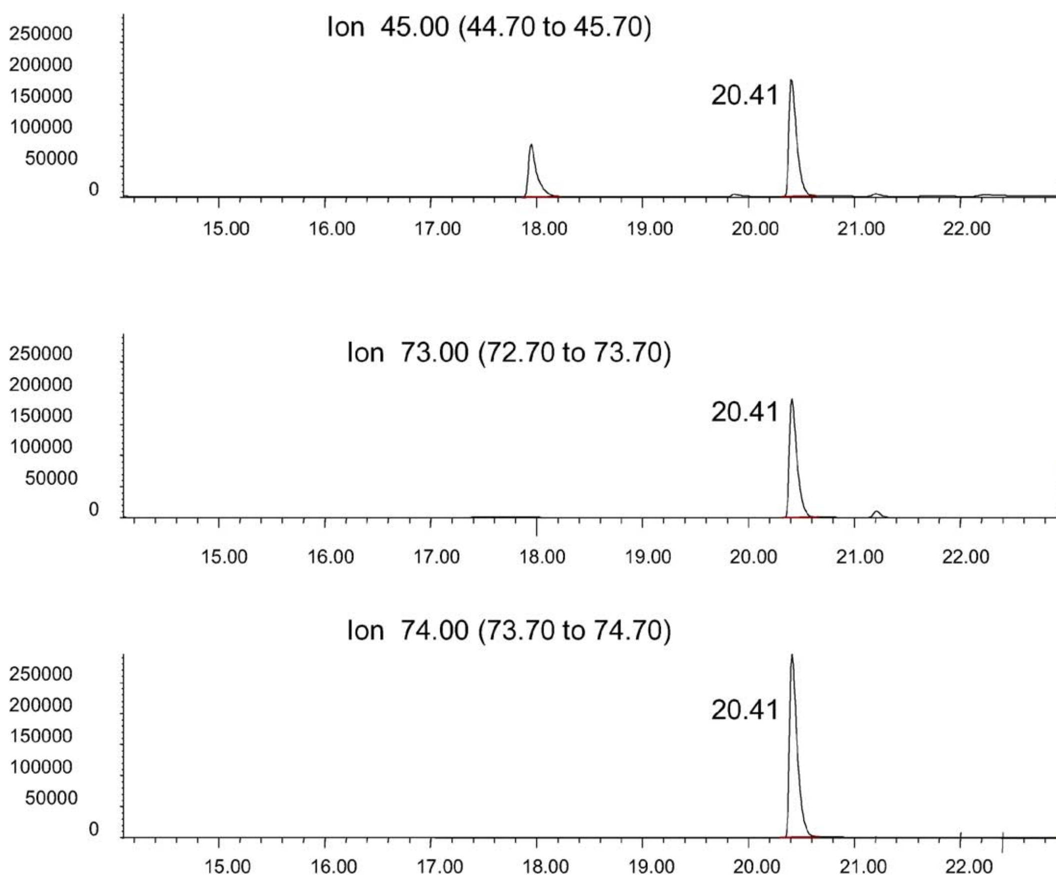

Fig. 3. HS-SPME–GC–MS (selected ion monitoring) chromatogram of a commercial sample feed (meal form) containing 324 mg/l of propionic acid. Time scale in min.

A R.S.D. = 7.6% of GC area and a R.S.D. = 0.07% of retention time were obtained.

To calculate detection and quantification limit, final feed sample preparation was applied to a sample of the same propionic acid free standard feed, fortified with a total content of 3 mg/l of propionic acid, added as sodium propionate. The height of the propionic acid peak, and the baseline noise were measured. A detection limit of 1.5 mg/l (two times baseline noise) and a quantification limit of 5 mg/l (three times detection limit) were obtained. Blank tests were made following the final feed sample preparation procedure without the addition of feed, using HPLC water, deionized water and tap water, giving all of them analogous results with an interfering peak corresponding to few counts of area, that was considered.

Recovery was calculated using standard addition method. The importance of the effect of the matrix on results was studied using the final feed extraction procedure but using the mass spectrometer as a detector. Comparing the GC area obtained with a propionic acid free standard feed fortified with a total content of 200 mg/l of total propionic acid, with the GC area of an aqueous solution of 20 mg/l of the same acid (the method dilutes sample 10 times) only a 16% of the chromatographic area of the aqueous solution was obtained when feed sample was analyzed. A strong influence of the matrix was shown with the decreasing of the propionic acid area in the headspace of the feed extract. Then an external standard cannot be used as quantitation procedure. Standard addition method is strongly recommended.

The method presented in this paper was applied to 20 commercial European feed samples of different origins to quantify the propionic acid added. No major interferences were observed in all chromatographic profiles from components extracted from samples, neither in meal feed, Fig. 1, nor in grain mix, Fig. 2. In these two figures, propionic acid contents near 1000 and 1500 mg/l were found.

Thinking in future more complex samples, the same analysis was done injecting the SPME fiber in GC–MS, working with some specific ions characteristic of propionic acid, as shown in Fig. 3.

Chromatographic profile was cleaner than the profile obtained with FID, and lower detection limits were expected. More work has to be done in the future with this configuration.

4. Conclusion

Propionic acid and its salts are used as mold inhibitor in feed to prevent the growing of molds. Usual methods to determine its quantity in feed are tedious and/or solvent consuming. A method able to analyze total propionic acid content (free + salts) in feed, using HS-SPME of a suspension of feed in salted and acidified water was described in this paper. A polyacrylate fiber was used to extract and enhance the response of the acid. Separation was done by capillary gas chromatography with a special column for acids (FFAP). MS was used, in addition to FID, to be selective enough to avoid confusing the propionic acid with other interferences of these complex matrices.

Using this new method, we were able to analyze ppm quantities of propionic acid with good linearity, repeatability and good detection and quantification limits. The method was applied to commercial samples, very variable in composition, doing the quantitation by standard addition method. No major interferences were found.

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