Husbandry and Nutrition

Review

Gas Chromatography and High-Performance Liquid Chromatography in Agriculture

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ABSTRACT

Background: Chromatography permits the separation of chemical components, their identification, and quantification in complex mixtures. Chromatographic methods have various applications, including determining an agrochemical solvent percentage and its utilization in the food-processing industry. High-performance liquid chromatography (HPLC) and gas chromatography (GC) are essential for agriculture. Both types are used in numerous research studies today thanks to their versatility, sensitivity, reproducibility, and quickness. **Aim**: To discuss the main results of studies that include GC and HPLC in agriculture. **Materials and methods:** The study relied on a compilation of recent papers, where these techniques play a critical role in this area of science. **Results:** The utilization of GC and HPLC in agriculture contributed to relevant results, from the analysis of fatty acids profile in the formulation of a diet for animal nutrition, to the determination and quantification of agrochemical residues in plants, the detection of contaminants in the diets, and drug residues that compromise the lives of animals. **Conclusions:** The use of chromatography is an essential methodology for biochemical, toxicological, structural, and agro-industrial studies.

Keywords: chromatographic analysis, agriculture, quantification, identification (Source: AGROVOC)

INTRODUCTION

Chromatography is a process that comprises several separation techniques based on the physical properties of certain materials, which interact with substances or substance mixes, which are associated with their chemical properties, and permit breaking down a mix and analyzing its components. Technology has helped diversify chromatographic techniques, largely improving their capacity to deal with different sorts of mixes. No other separation method is so powerful and general.

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High-performance liquid chromatography (HPCL) and gas chromatography (GC) play a major role in agricultural results. GC is a chromatographic method applied to directly volatilized compounds, or transform them into a volatile derivative. Meanwhile, HPLC permits the separation of compounds under low vapor pressure, as well as heat-labile compounds or those needing previous chemical treatment (derivation) (Sgariglia *et al*., 2010).

Today, the application of chromatographic methods is broad. They include determining the solvent percentage in an agrochemical product, purity quality control of solvents used as raw materials in a food-processing plant, determining a medication's active principle contents, and its utilization in food-processing plants for quality control of products and raw materials, such as proteins, aromatics, and in determining colors (Sgariglia *et al*., 2010).

In agriculture, its use is associated with the advantages and versatility of these methods. Accordingly, the aim of this paper is to discuss the main results of studies that include GC and HPLC in agriculture.

DEVELOPMENT

HPLC and GC as analytical methods in agriculture

Regularly, scientists discover new techniques and procedures that make laboratory work more efficient, simple, and accurate. Nowadays, many traditional analyses have been replaced by novel techniques that can provide more satisfactory results in a shorter period of time. Both GC and HPLC constitute a pillar for multi-purpose studies, particularly in agriculture. Recently, these techniques have played a major role in research, due to their utilization in the study of alternatives to animal feeding, the evaluation of health indicators, product quality control, etc.

Usefulness in feeding economically important animals

The appearance and evaluation of antibiotic resistance against pathogenic bacteria encourage the banning of antibiotic use in animal feeding while creating incentives to search for new alternatives. Among the different choices are the utilization of probiotics, prebiotics, stimulators of immunity, and the use of plant extracts. The essential extracts and their compounds are a way of enhancing feeding efficiency and reducing nutrient loss. They are also important in optimizing the pig productive parameters of pre-fattening pigs. Guerra *et al.* (2008) conducted a study to evaluate the effect of oregano extract (*Oreganum vulgare*) on some productive parameters of weaned pigs. HPLC coupled with a mass detector was used to evaluate the chemical composition of essential oil. A total of thirty-three cross-bred piglets were included in the experiment to determine the effect of essential oil as a growth supplement. The results showed that the oil from oregano has greater effects in terms of weight gain and final weight than the control. However, this effect was lower when compared to the antibiotic treatment.

Parra *et al.* (2016) did a study to evaluate the nutritional value of non-conventional raw materials to manufacture low-cost animal feeds that do not compete with human nutrition. They analyzed the nutritional contents of ground cavies' abdominal viscera (*Cavia porcellus*) through proximal analysis and determination of fatty acid profile using gas chromatography. The results indicated that the ground viscera from cavies' can compete with different meals for its nutritional qualities. The chromatographic analysis permitted the identification of the fatty acid composition in the fresh cavies' viscera. Omega 3, 6, and 9 polyunsaturated fatty acids were identified, with the relevance of linoleic acid contents, which are costly and non-abundant in the animal diet. Therefore, this chromatographic technique, along with proximal analysis led to the conclusion that the nutritional components of the viscera from covies are a promising raw material in animal nutrition (birds, pigs, and double-purpose cattle), which encourages the inclusion of these residues.

Valverde (2020) did a study on the effect of the inclusion of citrus pulp sub-products in the diets of pigs to evaluate yields and the animal's intestinal health. The effect of this treatment on the intestinal microbiota and the fatty acid profile was evaluated through gas chromatography. The results did not show significant differences in any of the groups of microorganisms studied, though a rise in the critical pulp levels in the diet causes a reduction of fecal enterobacteria, total anaerobes, and lactobacillus count in the feces, and increases the number of bifid bacteria. The chromatographic technique permitted the analysis of fatty acids with the inclusion of citrus pulp in the diet, whose total concentration of total fatty acids, saturated fatty acids, and polyunsaturated fatty acids was lower, while the monounsaturated fatty acid concentration (24% citrus pulp inclusion) was higher. Hence, this study enabled the modification of fattening pig feed composition with the inclusion of 24% of citrus pulp, with no negative effects on intestinal health, meat, and carcass quality, and the fatty acid profile in the subcutaneous fat.

González-Torres *et al.* (2021) also used gas chromatography coupled with a flame ionization detector to determine the fatty acid profile in the diet of fattening pigs, containing sweet potato (30%) and evaluated the nutritional quality of meat. Seperiza *et al.* (2021) determined the composition of fatty acids in the eggs of Cuban hens, using gas chromatography to evaluate the effect of the inclusion of materials with bioactive compounds to feed these birds.

Usefulness in plant analysis

The inclusion of alternative nutritional sources in production animals is one of the most widely used technologies, as it provides large amounts of digestible nutrients that contribute to a country's import reduction. Unfortunately, pests affect this type of feed, and, therefore, it is important to use pesticides to control them and increase the production of plant-based feeds. Consequently, it is necessary to exert greater control over the presence of crop residues to ensure food safety and reduce the risks of their possible ingestion by consumers. HPLC and GC are key techniques for these kinds of research. Arrieta-Víquez (2016) conducted a gas chromatographic study using a mass spectrometry detector to determine aldrin, dieldrin, deltamethrin, dicloran, endozoan, chlorpyrifos, cypermethrin, lambda-cyhalothrin, pendimethalin, and lindane in fruits and other vegetables with high water contents. The results of the study showed that of the total of samples analyzed nationally, 5% went over its maximum residue limits for pesticides (MRLP) in feeds, with cypermethrin as the one with the most fails, whereas, on the import side, 1% fails was associated with endosulfan in apples.

Valentín *et al*. (2021) used HPLC to analyze the pesticide residues in tomato plants, identify such residues, and determine their toxicity level. Thanks to this technique, some level of *Chlorpyrifos* residues was identified, including some quantities of cadmium (Cd), Chrome (Cr), Copper (Cu), Manganese (Mn), and Zinc (Zn). However, the residue average of pesticides and heavy metals found in tomatoes did not surpass the permissible limits, according to the *Codex Alimentarius* and other international standards. Nevertheless, pesticides are known to be compounds that can alter the normal functions of the organism, and therefore, must ensure the product's harmlessness.

Dussac-Moreno (2021) used liquid and gas chromatography in the same study to analyze and detect pesticide residues in fresh fruits and garden vegetables. Both techniques helped established the residue levels of these products, as well as the risk quotients by ingestion. The results evidenced that the likelihood that consumers might be exposed to pesticide residues leading to unfavorable consequences to health, was low, though this topic should be further studied since the health of consumers might be compromised.

Gas chromatography could also be used to analyze and identify plant secondary metabolites known in the literature by their traditional use, but not demonstrated scientifically. Pyrowood acid has several applications in the forestry industry as a wood preserver, bio-repellent, bio-fungicide, and foliar fertilizer. It can also be used as an alternative to obtain organic products. Catacora *et al.* (2019) used gas chromatography to characterize the chemical components and active principles of pyrowood acid from woody bamboo (*Guadua sarcocarpa*), coral tree (*Erythrina ulei*), and Imbaúba Gigante (*Cecropia sciadophylla*), for agriculture. The utilization of this chromatographic technique helped find 12 chemical-organic compounds in the pyrowood acid from woody bamboo. In coral tree, a total of 19 compounds were found, and in Imbaúba Gigante, 23 compounds were observed. The identification and characterization of these compounds may explain the properties of this pyrowood acid for the lumber industry.

Usefulness in animal health

The utilization of medication in production animals is a controversial issue, since these medications often accumulate, not only affecting animal health, but also that of the consumers. Among the most commonly referred medications in the scientific literature, are the antithyroid agents, which lead to animal weight increases due to water retention, and are also capable of causing hypothyroidism. Injectable or patch hormone cocktails are usually employed and may cause cancer. Antibiotics in animal production are commonly used to cure, prevent, and promote animal growth. Despite the benefits for animal health, their utilization in animal production leads to a loss of efficiency over time, as well as bacterial resistance in humans.

The residues from these drugs in the meat and biological samples (feces and urine), can be tested through several different techniques. There are methods for quick detection of drug residues in farm animals, including the ELISA types. However, HPLC also constitutes a very effective tool for the detection of these substances, with high specificity and quickness, with a large number of samples analyzed at the same time. Today, it is one of the most commonly used techniques for this type of research (Moudgil *et al.*, 2019).

Many countries continue to develop alternative treatments without drugs, based on plant sources that not only contain nutritional factors for the animals, but also, other secondary metabolites that help reduce the colonization of pathogenic bacteria, and prevent meat contamination. Such is the case of guava leaf extract (*Psidium guajava*), whose broad-spectrum antibacterial activity thanks to quercetin, has been documented. Silva-Vega *et al*. (2021) did a study to characterize and determine the inhibiting effect of *Escherichia coli* O157:H7 motility on guava leaf extract since ruminants are known to be the main host of *E. coli* O157:H7, which causes the hemolytic uremic syndrome in these animals. The chemical composition of these extracts was analyzed by gas chromatography, which showed that quercetin was the compound in the alcoholic extract of guava leaves with the largest proportion, being effective in the inhibition of *E. coli* O157 H7 motility.

Contaminants also affect the health of production animals and that of consumers. These contaminants may be present in the feed supplied to the animal and in their products, such as milk.

One of the most widely used techniques to detect these harmful substances is HPLC. Bernate-Bobadilla *et al.* (2021) did a study in which they used this advanced technique to determine the presence of aflatoxin M1 on some dairy farms in the south of Bogota, being milk a highly demanded item that needs special care. This product can get contaminated with aflatoxins, which generate adverse effects on humans and animals. Particularly, a cancer effect is observed in the liver, and exposure to it may lead to parenchyma degeneration, followed by carcinoma and cirrhosis.

Usefulness in environmental studies

Animal feces accumulate in the soil and form part of the greenhouse effect gases (GHG). Gas chromatography coupled to several detectors is a tool to detect and quantify greenhouse gases (CH_4, CO_2, and N_2O) at different stages of livestock production. Saynes-Santillan (2018) conducted a study based on this type of chromatography to measure CH_4 (methane) concentrations in ruminants. Globally, these animals provide the largest source of anthropocentric emissions of CH_4 (Ripple *et al.*, 2014). Other studies refer that gas chromatography plays a major role in measuring CH_4 and N_2O (nitrous) oxide in animal stools. Most researchers focus on the emissions generated by enteric fermentation. However, the feces and urine piled up on the farms resulting from livestock raising is inevitably a significant source of GHG. Urine, mud, and feces contain inorganic nitrogen, carbon, and water that can be used by microorganisms to produce CH_4 and N_2O. Jungbluth *et al*. (2001), noted that 0.05-0.7 % nitrogen excreted in the feces of cattle is produced as N_2O, while pigs on thick hay bedding can reach 50-60 %.

Although the study of soils is mostly focused on the analysis of their chemical composition (minerals), another interesting topic for scientists has to do with the study of soil contamination by hydrocarbons, a common occurrence causing ecological and social catastrophes. A crude oil spillage on the ground permeates the sol with oil-based hydrocarbons, which affect the soil's physical, chemical, and biological characteristics. Contamination caused by hydrocarbons damages local systems, since the accumulation of contaminants in animal and plant tissues may lead to mutations or death. Several review articles have dealt with the different factors that affect the biodegradation speed of oil (Hassanshahian *et al*., 2020). Nevertheless, few bibliographic sources have compiled the diversity of analytical methods for the quantification of hydrocarbon biodegradation. In that case, GC and HPLC constitute analytical methods that help quantify total hydrocarbon biodegradation from oil, which once more shows the usefulness of these chromatographic techniques.

The usefulness of chromatography in agriculture at the Animal Science Institute

In Cuba, there are several research and educational facilities that tackle agriculture, which aim to generate and transfer updated knowledge, integrated technologies, and novel products from biotechnology, plant science, and sustainable systems, to increase agro foods. Among them are the National Health Center for Agriculture (CENSA), the National Agricultural Sciences Center (INCA), and the Animal Science Institute (ICA), in Mayabeque province. These facilities employ advanced research technology in agriculture.

The Animal Science Institute is engaged in animal nutrition and livestock improvements. The main goal of ICA's staff is to enhance animal feed production and achieve sustainable intensification of livestock raising.

ICA conducts research based on GC and HPLC analysis. Rodríguez *et al.* (1998) made long-chain fatty acid determinations in white mulberry plants. Pérez *et al.* (2005) were able to determine short-chain total fatty acids (AGCC) (acetic, propionic, butyric, and lactic) in cecum samples from broiler chickens using a gas-liquid chromatograph with a flame detector. In an *in vitro* evaluation of the sensitivity of Canavalia grains (*Canavalia ensiformis*) and black-eyed pea (*Vigna unguiculata*), alone, or mixed with sorghum grains (*Sorghum bicolor*), González *et al*. (2012) analyzed monomeric and dimeric sugars in water extracts, using HPLC coupled with a refraction index detector, lactic acid through HPLC in the UV detector, and quantified the short-chained fatty acids and ethanol separately, by gas chromatography. In the study of secondary metabolites of *Leucaena leucocephala* and its association with some elements of the weather, several different expressions of digestibility and primary metabolites, Herrera *et al*. (2017) quantified sugars by HPLC coupled with a refraction index detector. García *et al*. (2020) determined the concentration of lactic acid by HPLC, and the concentration of short-chain fatty acids individually (acetic, propionic, isobutyric, butyric, isovaleric, and valeric) through gas chromatography, in a study of chemical, physical, and microbiological characterization of feeds fermented for animal consumption.

CONCLUSIONS

High-performance liquid chromatography and gas chromatography are two essential pillars of scientific research nowadays. The high diversity of applications for the two techniques, their versatility, and quickness permit their utilization in various areas of science. Specifically, in agriculture, they are essential tools that produce relevant results, from the analysis of fatty acids profile in the formulation of a diet for animal nutrition, to the determination and quantification of agrochemical residues in plants, the detection of contaminants in the diets, and drug residues that compromise the lives of animals and consumers, including the analysis of secondary metabolites in extracts, as alternatives to replace antibiotics in farm animals.

The utilization of these techniques in ICA will enable further scientific research, and broaden the horizons for novel analytical techniques and the search for highly demanded compounds.

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AUTHOR CONTRIBUTION STATEMENT

Research conception and design: MHM, MAV; data analysis and interpretation: MHM, MAV; redaction of the manuscript: MHM, MAV.

CONFLICT OF INTEREST STATEMENT

The authors declare the existence of no conflicts of interests.