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Review on HPLC for the measurement of Food Flavonoids

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ABSTRACT: ***Flavonoids are botanical polyphenols that may be found in a variety of foods, including fruits, veggies, and grains. The anthocyanidins, pigments held to account for the blue and red colors in fruits, fruit drinks, sparkling wine, and floral; the catechins, focused in tea; the flavonols and flavonol glycosides, discovered in citrus and honey; as well as the flavones, flavone, and flavonol glycosides, found in tea, fruits, vegetables, and honey; and the flavones, flavonol glycosides, found in tea, fruits, vegetables Flavonoids are beneficial to human health because of their hydrogen-donating antioxidant action and ability to form divalent transition metal cations. HPLC (high-performance liquid chromatography) with computer control has become the analytical technique of choice. Many methods for detecting and quantifying flavonoids in one, two, or three classes have been developed. This overview tabulates the different HPLC and sample preparation techniques that have been used to quantify specific flavonoids inside a subclass or even across several subclasses.***

KEYWORDS: ***Chromatography, Food Flavonoids, Flowers, Polyphenols, Sample preparation.***

1. INTRODUCTION

Carotenes, tetrapyrrole compounds, and flavonoids are three of the most significant natural pigments. Flavonoids are pigments found in plants that are produced biosynthetically from phenylalanine. Chalcone synthetase catalyzes the condensation of three moles of malonyl-coenzyme A (CoA) from glucose metabolism to produce ring A (Figure 1). Rings B and C are similarly derived from gluconeogenesis, but through the shikimate route, which involves the conversion of phenylalanine to cinnamic acid, which is subsequently transformed to coumaric acid. In a single enzymatic step, coumaric acid CoA and three malonyl CoAs are combined to produce naringenin chalcone. 3-hydroxyflavonoids (e.g., catechins), 3,4-diol flavonoids (e.g., quercetin), and procyanidins are formed when the C-ring closes and gets hydrated [1].

There are about 4000 flavonoids classified into 12 subclasses. Water-soluble anthocyanins, which are reduced from yellow flavonoids owing to oxygen loss, are responsible for the orange, red, and blue hues seen in vegetables, fruits, flowers, and plant storage tissue. Anthocyanins aid seed dispersion and pollination by attracting animals. Two aromatic rings surround a heterocyclic six-membered ring containing oxygen in flavonoids (Figure 1). As a result, they may be classified as diphenylpropanes' derivatives. Isoflavones, found in soy and soy foods, have such a similar structure to isoflavones but a different connection to the propane bridge. (Figure 1) [2].

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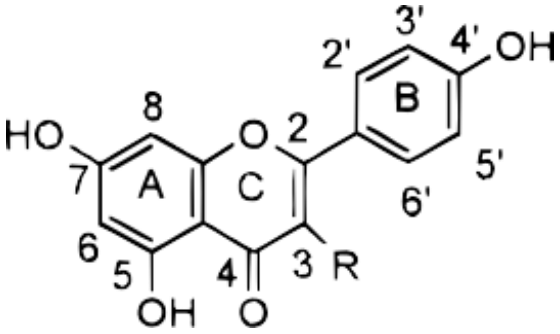

Figure 1: General structure of flavonoids (left: R) OH in flavonols, R) H in flavones) and isoflavones (right).

Anthocyanins, flavanols (catechin s), flavones, flavanones, and flavonols are the anthoxanthins, which are glycosylated flavonoid. Flavonoids may be found in almost all plants. Flavanones and flavones are often found together in the same plant (typically citrus), while flavones and flavonols, as well as flavanones and anthocyanins, are not. Because flavonoids are found in high quantities in legumes, particularly in diets containing soybeans, they are generally handled independently from the other five sub-classes. Minor categories of flavonoids, flavans, proanthocyanidins, neoflavonoids, bi- and triflavonoids, and the biological implications of flavonoids are all covered in a comprehensive book edited by Harborne. Harborne's book also contains detailed 1 H NMR spectrum information as well as 72 1 H NMR spectra.[3]

1. Health Benefits:

Flavonoids are radical scavengers that provide hydrogen (antioxidants). Flavonoids inhibit the superoxide-driven Fenton process by complexing iron ions. Certain flavonoids, particularly those with the carboxylate structure in the B-ring, have a significant copper complexation activity. Flavonoids renew R-tocopherol by decreasing the R-tocopheroxyl radical. Singlet oxygen is also quenched by flavonoids.

Formica and Regelson examined the biology of flavonoids, whereas Rice-Evans et al evaluated the structure-antioxidant activity correlations. Allergies, inflammation, infections, hypotension, arthritis, mutations, carcinogens, cancer, and AIDS have all been linked to flavonoids. Polyphenols inhibit phosphodiesterase of cGMP and cAMP, xanthine oxidase, and elastase. Catechins' antioxidative properties are believed to be related to radical scavenging. Catechins oxidize by transferring hydrogens from the phenyl rings' hydroxyl groups, blocking linoleic acid from oxidizid. In human lymphoid leukemia cells, catechin-rich persimmon extract causes cell death (apoptosis). Ascorbic acid (vitamin C) is regenerated by flavonoids, which in turn renews vitamin E. Despite the large number of in vitro experiments that have been published, there is limited evidence from real human feeding trials that may assist address the issue of whether antioxidant mechanisms are at work in people [3],[4].

Flavonoids and isoflavones are physically identical. They are mostly found in soy and may offer health benefits comparable to flavonoid. The structures of phytoestrogens and their metabolites are similar to those of mammalian estradiol. They are thought to impede estrogen receipt via

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com- petitive blocking at the hormone receptors and inhibit estrogen production as phytoestrogens. They may lower the risk of malignancies that are hormone-dependent, such as prostatic and breast cancer. In his study of the nutritional effects of legumes and soybeans, Messina includes the estrogenic effects of isoflavones. This overview of techniques for measuring flavonoids in foods is necessary because health consequences need comprehensive understanding of the total flavonoids of the food supply.[5]

2. Conditions of Chromatography:

Reversed-phase (RP) columns are almost entirely used, with depend largely from 100 to 300 mm and an internal diameter of 4.6 mm. Stereochemistry is seldom mentioned in contemporary research. However, in both reversed phase and normal phase mode, Cyclobond I, a â-cyclodextrin-bonded stationary phase, was utilized to separate the 2R and 2S diastereomers of flavanone glycosides and benzoylated flavanone glycosides, respectively.There has been documented research on the enantiomeric isolation of flavanones and the diastereomeric isolation of flavanone glycosides without the use of food extractions reviewed cyclodextrins and cyclodextrin synthesis, respectively.

An aquatic acidified liquid solution such as watery acetic acid, perchloric acid, phosphoric acid, or formic acid (solvent A) or a less polar organic solvent such as methanol or acetonitrile, potentially acidified, are used in most elution systems (solvent B). The National Institute of Standards and Technology (NIST) has discovered that using trifluoroacetic acid in both solvents improves catechin resolution and removes peak tailing. Isocratic, tertiary, and even quaternary systems have been recorded less often . Runs last around an hour on average, with equilibration in between. A notable exception is the 340-minute HPLC run utilized for pattern matching analysis of isoflavones in soy saucesTypical flow rates are 1.0 or 1.5 mL/min.[6]

2. DISCUSSION

Anthocyanins:

Anthocyanins are anthocyanidin acylglycosides and glycosides. C3 monosides, biosides, and triosides are the most common, although 3,5- and 3,7-diglycosides also exist. Fruit contains six types of anthocyanidins. Cyanidin is the most prevalent. Except for pelargonidin, blueberries have it all. Strawberry is mostly red due to pelargonidin 3-glucoside, but it also includes cyanidin 3-glucoside. Pomegranate juices containing mostly delphinidin are purple, while those containing mostly pelargonidin are red. Despite Hertog et alanalysis .'s of cranberry and Hakkinen et alanalysis .'s of strawberry and blackcurrant, no anthocyanin analysis was performed after hydrolyzing procedures based on Hertog's extraction method.Other extraction techniques, such as SPE, were employed instead, and MS was occasionally used to confirm the identity of the glycosides.[7]

Flavanols are a kind of flavonoid (Catechins):

Catechins are mostly present in brewed tea and red wine. Because green tea is produced from fresh leaf and black tea leaves contain dark components such as theaflavins and capsorubin owing to oxidation process of polyphenols, green tea has greater catechin concentrations than

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black or taiwanese tea. Oolong tea, which has been partly oxidized, nevertheless retains a significant amount of catechin.[8]

Flavones and flavonols are two types of flavonoids:

Flavones and flavonols are found as O-glycosides in plants. Where the flavones contain a hydrogen, the glycosides have a hydroxyl at C3. In vegetables, glycosides of the flavonol quercetin predominate, although glycosides of the flavonol kaempferol, as well as the flavones apigenin and luteolin, are also present. Quercetin glycosides are typically the sole flavonols found in fruits, with myricetin and kaempferol glycosides present in trace quantities. common flavones and flavonols, respectively. After lyophilization, vegetables, herbs, and teas including flavones, flavonols, and flavonol glycosides were often extracted using LLE and even SPE. It was simpler to extract teas and wines. For green beans, semipreparative HPLC was employed [9].

Isoflavones:

People consume around 20 of the 13000 species of legumes. Isoflavone content in soy and soy products has been the subject of the most research. Food contains at least 15 isoflavones, most of which are glycosides, but aglycons may be detected in fermenting soy products. Other legumes have low amounts of isoflavone. Boiling and filtering are common methods for analyzing teas, but Lin et al. have utilized LLE and even SPE. Catechins in wine have been studied without sample preparation has also been employed. After filtering, tannins in red wine, beers, apple cider, and fruit liqueurs were tested.

Understanding the function of flavonoids in plant metabolism and human health requires knowledge of their flavonoid concentration in plant-based diets. Furthermore, such knowledge has been used as the foundation for chemotaxanomic systems, which were extended to the detection of beverage adulteration, among other application. Although other modern separation systems, such as capillary zone electrophoresisand micellar electrokinetic capillary Hilh Reversed-phase C 18 column material was utilized to pack the most commonly used columns. C 8 packings have only been used in a small number of cases, and only when the flavonoids being separated were more polar, such as aglycons and glycosides of isoflavones.

Detachment systems for flavonoids in food products have indeed been oriented toward the measurement of all (usually several subclasses) of the prominent flavonoids in a single food, such as wine, tea , apples, etc. Many of these analytical methods have been used to look at various aspects of plant physiology, such as sensitive to environmental changes, variations between species and/or cultivars, changes during ripening, and so on. A few methods for measuring flavonoid content in a variety of frequently eaten foods have been developed Only one HPLC method has been devised to isolate and quantify significant dietary flavonoids from all five subclasses isoflavones are usually analyzed with independent systems because they are found almost exclusively in soybeans and soybased foods). Even using this method, only a small number of flavonoids and other phenolics were isolated. In general, acetonitrile and/or methanol in conjunction with water containing an acid have been used as mobile phases with reversed-phase HPLC columns.

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Tetrahydrofuran and 2-propanol have also been employed as nonpolar solvents on occasion. The kind of acid employed as a modifier to reduce peak tailing was the most significant change in the mobile phases. Acetic acid or formic acid were most often used, although phosphate buffer at low pH, ammonium acetate, citric acid, and trifluoroacetic acid (TFA) were also used. Dalluge discovered that using deactivated C18 columns and using TFA as the mobile phase's acidic modifier significantly enhanced peak shape and repeatability of catechin retention durations in tea.

Preliminary research from the authors' lab backs up their findings (Merken and Beecher, unpublished results). For the analysis of flavonoids, sample preparation methods vary from “filter and inject” in the case of various drinks to digestion, sample preparation (SPE column), filtering, and analysis for solid meals. It is not necessary to digest the glycosylated forms of flavonoids when they are of interest. When data on a large number of flavonoids is needed, the aglycon forms of the flavonoids are often tested.

For methanol/water combinations (50:50, v/v), hydrolysis of flavonoid glycosides needs relatively large concentrations (1-2 M) of mineral acids under reflux condition. Anthocyanidins and catechins are likewise degraded under these circumstances. Merken and Beecher, unpublished findings), and myricetin is partly destroyed (70 percent recovery) (Merken and Beecher, unpublished results). These findings emphasize the need of developing sample preparation methods that allow flavonoids to generate aglycons without destroying the flavonoids themselves [10].

3. CONCLUSION

Flavonoids have proven to be beneficial to human health in vitro. More in vivo research is required to determine the beneficial effects of flavonoids and to determine if there are any risks associated with potential overdosing. Hundreds of articles on flavonoids HPLC have been published in the last 20 years or more, although HPLC techniques may identify flavonoids from one, two, or even three subclasses in a single run. Foods may have many subclasses, and mixed diets can include all of them. A technique is required to measure all major flavonoids in food and drink at the same time.

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