Organoleptic and Nutritional Effects of Phenolic Compounds on Oilseed Protein Products: A Review

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ABSTRACT

In many new oilseed protein sources, phenolic compounds are as important as unsaturated lipids, carbonyl compounds, and nonenzymatic browning in the development of adverse flavors and colors in food products. The free phenolic acids are of particular concern because of enzymatic oxidation to *o*-quinones and subsequent binding to lysine and methionine in the proteins. Numerous free phenolic acids have been identified in all oilseed flours with syringic, ferulic and vanillic being the major components in cottonseed, peanut and soybean flours. Gossypol in cottonseed, chlorogenic acid in sunflower, and sinapine in rapeseed are microconstituent phenolics which cause unique problems in the utilization of these defatted flours and their protein isolates in food applications. The roles of bound phenolics and tannins in the binding of essential nutrients or altering chemical and functional properties require further investigation.

INTRODUCTION

Phenolic compounds are widely distributed in plant parts from the roots to the seeds. Some phenolic compounds are essential metabolites, others have complex structures of unknown function and may be unique to a particular plant family or genera. Much of the present discussion will deal with the occurrence of simpler compounds such as the phenolic acids and their esters which are common microconstituents in most oilseed, legume and cereal seeds. During industrial and food processing, the aqueous, temperature, and pH conditions may be conducive to extensive enzymic and autolytic reactions that lead to changes in nutrition, flavor, color and odor. Recent research on new oilseed protein sources has demonstrated that phenolic compounds may be as important as nonenzymatic browning or oxidation of unsaturated lipids in the development of adverse flavors, colors, odors, and antinutritive effects on proteins and vitamins.

The analysis, chemistry and physiology of plant phenolics as a group have been reviewed by Mabry et al. (1), Pierpoint (2), Ribereau-Gayon (3) and Van Sumere et al. (4). The objectives of the present review are to collate the more scattered literature on phenolic compounds in oilseed protein products and to provide information on the qualitative and quantitative differences in these important microconsitutents among oilseed protein products.

CLASSIFICATION AND STRUCTURE

A variety of classifications of phenolic compounds has been proposed: for the present purposes the common constituents in oilseed products appear to have six or seven basic structures as shown in Figure 1. These are the hydroxylated derivatives of benzoic and cinnamic acids, coumarins, flavonoids, and the polyphenolic tannins and lignin (3).

Phenolic acids include the benzoic acid (C_6+C_1) and cinnamic acid (C_6+C_3) based phenolics (Fig. 2). The benzoic acids are widely distributed in nature. The simpler types include *p*-hydroxybenzoic, protocatechuic, vanillic, gallic and syringic acids plus the *o*-hydroxy salicylic and gentisic acids.

The cinnamic acids *p*-coumaric, caffeic, ferulic, and sinapic are found in most oilseeds and occur frequently in the form of esters with quinic acid or sugars (Fig. 2). The well known chlorogenic acid is an ester of caffeic and quinic acids and is found in several isomeric and derivatized forms. The amino acids, tyrosine and dihydroxyphenylalanine, can be considered as modified forms of cinnamic acid and have phenolic functions. Apparently, the presence of an acrylic acid group conjugated with the aromatic ring facilitates the oxidation of cinnamic acids to the corresponding *o*-quinones. The oxidation potential of these monocyclic phenolics varies widely from the very easily oxidized 2,4,5-trihydroxy compounds to the more stable methylated phenolics and finally the least reactive monophenols (5).

The coumarins possess the same C_6-C_3 configuration of the cinnamic acids, but the C_3 chain is formed into an oxygen heterocycle (Fig. 1). The couramins are less widely distributed in seeds and occur primarily as glucosides. They exhibit limited chemical reactivity and have little effect on organoleptic or nutritive value of seed products.

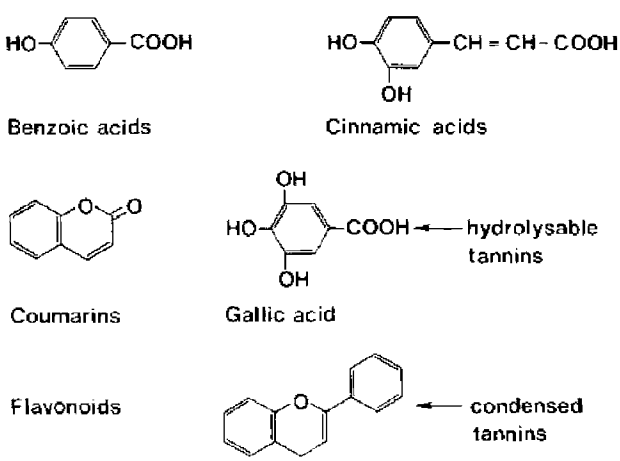


FIG. 1. Structures of the basic phenolic compounds found in oilseed protein products.

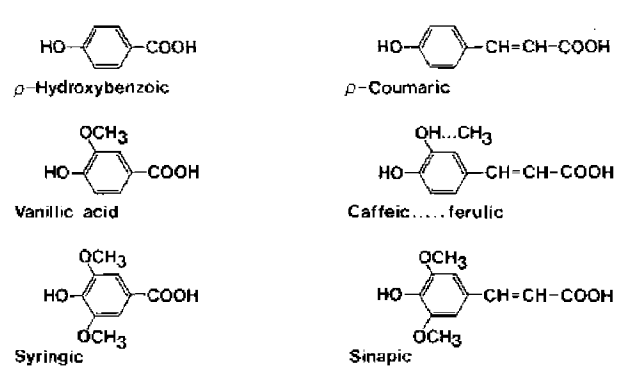


FIG. 2. Structures of common phenolic acids which occur in oilseed protein products.

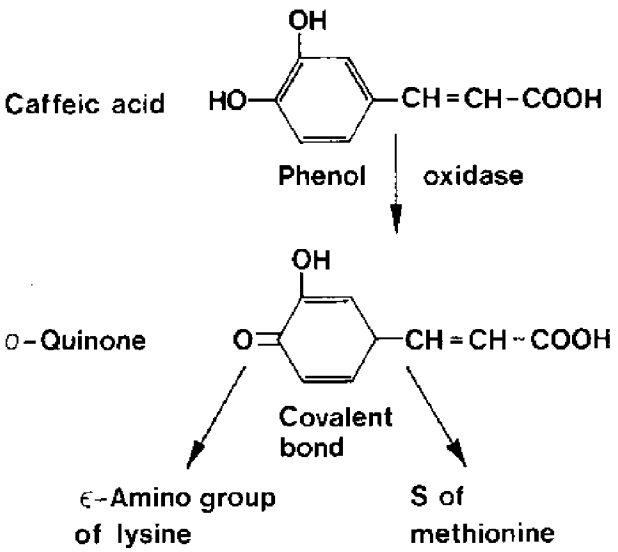


FIG. 3. Enzyme-catalyzed oxidation of caffeic acid to caffeoquinone followed by autolytic bonding to amino and thiol groups in proteins.

The flavonoids are a major group of plant phenolics having the C_6-C_3-C_6 structure in common (Fig. 1). The flavones, flavonols, flavanols, anthocyanidins, chalcones, aurones, etc., differ primarily in the nature of the C_3 group. Of these compounds, the flavonols are by far the most common: quercetin and kaempferol are widely distributed in seeds, especially as glycosides. The flavanols are unique in that they do not occur as glycosides but show greater reactivity especially through polymerization into "condensed tannins" (Fig. 1).

Polymeric phenols are distinguished into three groups on the basis of the products recovered by heating with acid or alkali. Hydrolyzable tannins yield phenolic acids (gallic or ellagic acids) plus glucose on acid hydrolysis while condensed tannins yield flavanols plus a brown residue. Alkaline hydrolysis of lignin releases a variety of benzoic and cinnamic acid derivatives as well as other unrelated compounds.

INTERACTION OF PHENOLIC COMPOUNDS WITH PROTEINS AND AMINO ACIDS

Atmospheric or enzyme-catalyzed oxidation of phenols in seeds results in quinoidal production and the formation of hydrogen peroxides. Both products are destructive of labile amino acids, denature proteins and inhibit enzymes such as indole acetic acid oxidase (6), trypsin and lipase (7) and arginase (8). In oilseeds, the cinnamic acids and their esters are of particular significance because they are the preferred substrate for phenol oxidase (phenolase, polyphenoloxidase) (2). The *o*-dihydroxyphenols, especially caffeic and chlorogenic acids, are oxidized to *o*-quinones by copper-containing enzymes which occur as ubiquitously in plant materials as the cinnamic acids (Fig. 3). Once formed, *o*-quinones (e.g., chlorogenoquinone) react nonenzymatically to polymerize, are reduced or bond covalently to amino, thiol and methylene groups. The ϵ-amino group of lysine and the thioether group of methionine are commonly attacked to render them nutritionally unavailable to the monogastric digestive system.

The implications of quinoidal formation are particularly serious in sunflower which is reported to contain 3 to 3.5 g of phenolic compounds per 100 g of flour with chlorogenic and caffeic acids representing ca. 70% of the total phenolics

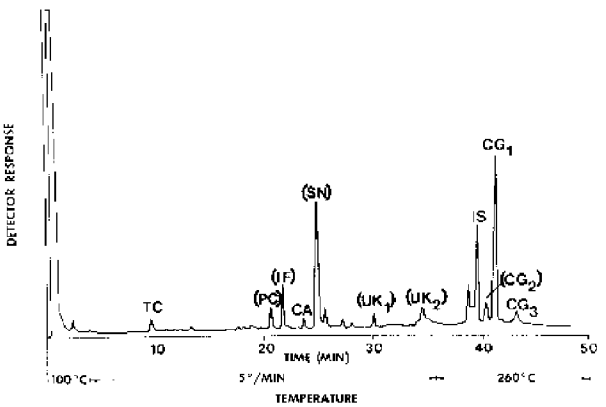


FIG. 4. GLC chromatogram of TMS derivatives of the free phenolic compounds in sunflower flour. *Trans*-cinnamic (TC), *p*-coumaric (PC), isoferulic (IF), caffeic (CA), sinapic (SN), unknowns (UK_1, UK_2), internal standard (IS) and chlorogenic acids (CG_1, CG_2, CG_3) (9).

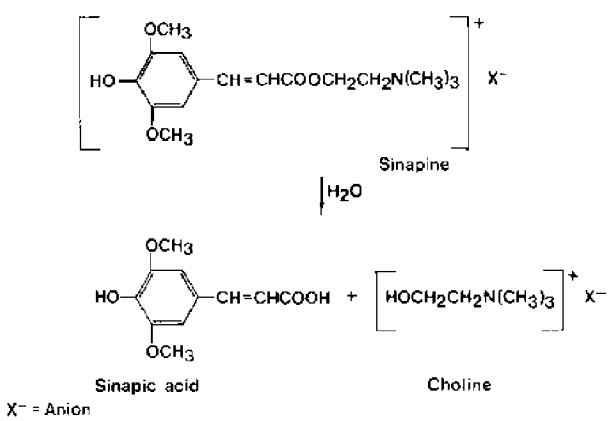
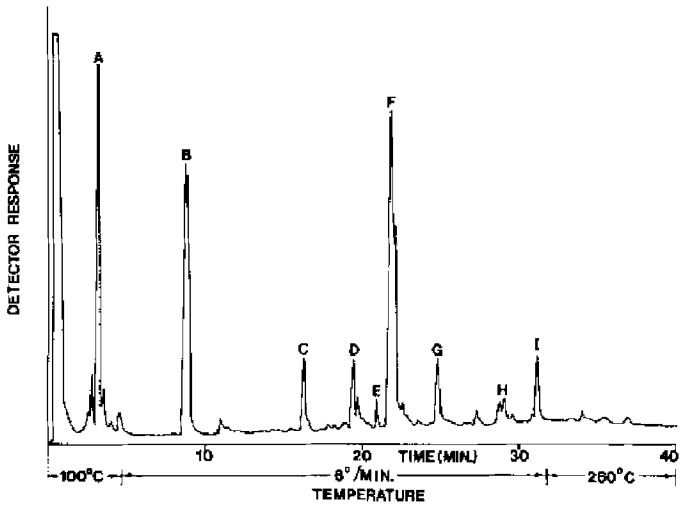


FIG. 5. Sinapine and its hydrolytic cleavage products (21).

(9). Under alkaline conditions, sunflower protein solutions develop dark green and brown colors due to oxidation of polyphenolic compounds and, when the proteins are precipitated at pH 4.7, the green colors cannot be washed from the protein isolate (10). However, the nutritive values of sunflower flour and concentrate were proportional to their lysine contents (11), and alkaline hydrolysis to release indigestible lysine and methionine may compensate in part for phenolic binding in protein isolates. Sabir et al. (12) demonstrated that, in sunflower protein solutions extracted with neutral salt solutions, all of the major protein fractions were free of phenolic associations. About one-third of the chlorogenic acid was bound to low molecular weight polypeptides and oligonucleotides (MW≤5000).

Generally, phenolic compounds are not toxic, but cottonseed resin glands contain a high concentration of the yellow pigment, gossypol, which is toxic to pigs and poultry but not ruminants. The gossypol will oxidize and esterify like other phenols but usually the aldehyde groups react with the ϵ-amino groups of lysine in the cottonseed globulins, especially during high temperature oil extraction or meal processing (13). Bound gossypol is undigestible and of limited toxicity, but the biological availability of lysine in the meal is reduced. Much of the commercial supply of cottonseed meal is utilized in monogastric nutrition by binding gossypol, iron supplementation, etc., to reduce free gossypol content and increase tolerance to the polyphenolic compound.



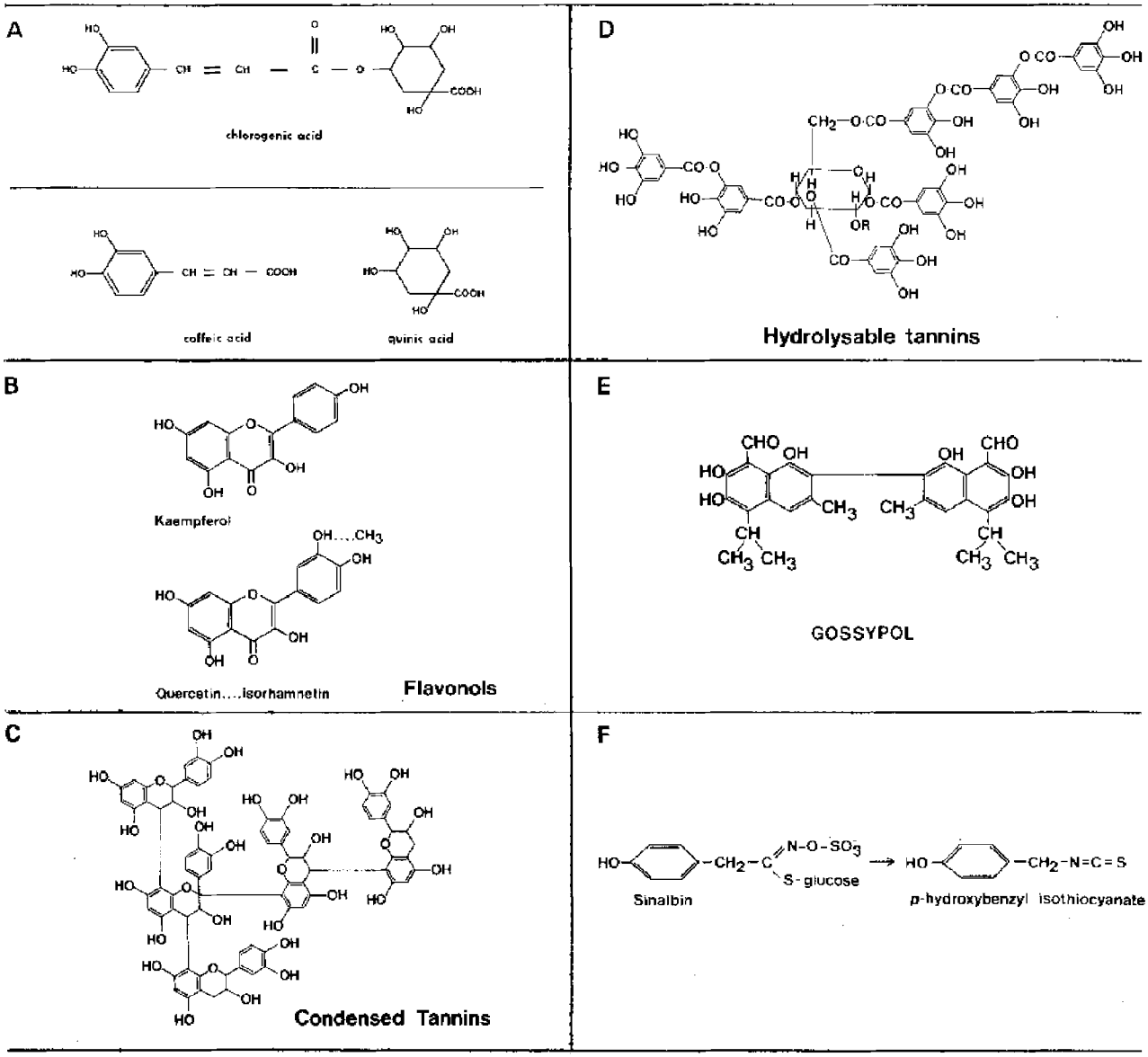


FIG. 6. GLC chromatogram of TMS derivatives of the free phenolic compounds in rapeseed flours. *p*-Hydroxybenzoic (A), *trans*-cinnamic (B), coumaric (C), ferulic (D), caffeic (E), sinapic (F), unknown (G,H) and chlorogenic (I) acids (24).

Tannins bind to enzymes and other proteins by hydrogen bonding to amide groups to form insoluble complexes. Generally, hydrolyzable tannins are more reactive than condensed tannins, and their oxidized derivatives form covalent bonds with proteins that are resistant to enzymic and microbial digestion (4). The combined reaction of quinones, polyphenols and tannins on the ϵ-amino groups of lysine and their subsequent polymerization into tannin-protein complexes will make large blocks of amino acids, including other essential amino acids, resistant to digestive enzymes of the monogastric and ruminant animals. Thus, a low level of oxidation can result in a substantial decrease in protein nutritive value (14).

PHENOLIC FLAVOR AND COLOR EFFECTS

The bitter and beany flavors of oilseed flours, including commercial soy flours, concentrates and isolates (15), have been a persistent problem in expanding their food uses. Commonly, the beany, bitter, chalky and astringent flavors of legumes have been attributed to enzymic and nonenzymic deterioration of unsaturated lipids to hydroperoxides and, ultimately, carbonyl compounds such as hexanal, pentyl furan and ethyl vinyl ketone (16).

Arai et al. (17) reported that a strongly phenolic-flavored ethanol extract of defatted soybean flour contained at least seven phenolic acids including syringic, vanillic, ferulic, gentisic, salicylic, *p*-coumaric and *p*-hydroxybenzoic acids. The main phenolic component was syringic acid. Maga and Lorenz (18) measured taste thresholds of phenolic acids, alone and in combinations, to show that the latter condition resulted in much lower taste thresholds of 40 to 90 ppm as compared to the individual acids. At these levels, phenolic acids would contribute significantly to objectionable astringent flavors in oilseed flours. By means of a 500 ft. capillary column, 27 free phenolic acids were separated by GLC of soy, cottonseed and peanut flours (19). The major free phenolic acids in all flours were syringic, ferulic and vanillic acids, which occurred in concentrations above the taste thresholds for phenolic acids.

Despite the high concentrations of chlorogenic acids, sunflower flours have much blander flavors and odors than soybean, cottonseed, or peanut flours. Sabir et al. (9) used GLC methods to identify *trans*-cinnamic, *p*-coumaric, isoferulic, caffeic, sinapic and chlorogenic acids in sunflower flour (Fig. 4). The color problem with chlorogenic acid under alkaline conditions represented a serious impediment to food utilization of sunflower protein products. Cater et al. (20) established that chlorogenic acid in sunflower seed is responsible for the green to brown color of alkaline extracts while caffeic produced only a slight pink, and quinic acid had no effect on color. Under slightly acid conditions, even freeze-dried or texturized samples develop a grey color. At the pH of bread (pH=5.5), sunflower protein supplementation with flours and concentrates caused breads to develop light to medium brown colors in the crumbs depending on the levels of phenolic compounds present.

The pungent, mustardy flavor and odor of rapeseed, mustard, and crambe flours are due to the hydrolysis products of glucosinolates which include several isothiocyanates and oxazolidinethione that are not phenolic compounds. However, the conversion of sinalbin in white mustard by the enzyme myrosinase to *p*-hydroxybenzyl isothiocyanate provides a particularly pungent flavor which is popular in prepared mustards.

Another bitter component in *Brassica* species and *Crambe* is sinapine, the choline ester of sinapic acid (Fig. 5). Austin and Wolff (21) demonstrated that rapeseed meals contained ca. 1% of sinapine, which was twice the level present in crambe meal. These levels of sinapine are quite high and would account for the bitter flavor of glucosinolate-free flours and products supplemented with these flours (22). Sinapine is not subject to hydrolytic cleavage except during germination when the appropriate esterase is activated. Therefore, techniques for detoxification or removal of the toxic glucosinolates should also be assessed for their ability to reduce the content of bitter sinapine.

Durkee and Thivierge (23) reported that the major phenolics in rapeseed meal, including hulls, were sinapine, kaempferol and isorhamnetin glycosides and esters. While no free phenolic acids were detected by their procedures, hydrolysis of the glycosides yielded sinapic, *p*-coumaric, caffeic, *p*-hydroxybenzoic and ferulic acids. The same constituent phenolic acids were found in dehulled rapeseed flours as free phenolic acids by Kozlowska et al. (24). In order of importance, the principal free phenolic acids in dehulled rapeseed flours have been identified as sinapic, *trans*-cinnamic, *p*-hydroxybenzoic, *p*-coumaric, ferulic, and caffeic acids. There were also two unidentified components and a small quantity of chlorogenic acid. In other rapeseed flours, low concentrations of vanillic and gentisic acids were also isolated as free phenolic acids.

Free phenolic constituents have been identified and quantitated in most oilseed flours. However, their specific effects on amino acids, odors, flavors, and colors are unknown in most cases. The bound phenolic compounds have been largely ignored by aqueous processing such as in breadmaking and extrusion cooking has shown the effects of released compounds that appear to have phenolic functions. Further studies are required on the levels of these bound and esterified phenolic compounds in new oilseed protein products and their potential effects on processed food quality.

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