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NON-NUTRIENT BIOACTIVE SUBSTANCES IN FOOD OF PLANT ORIGIN CAUSING BITTERNESS AND ASTRINGENCY

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The manuscript is an overview of natural bioactive non-nutrient substances causing sensation of astringency and bitter taste in food products. These compounds were discussed following their general division into three classes: nitrogen compounds (alkaloids, glycoalkaloids, glucosinolates), terpenoids (saponins), and phenolic compounds (phenolic acids, flavonoids, tannin polymers). Groups of compounds from the classes described were characterised individually with reference to the occurrence and biological activity. Moreover, threshold detection/recognition values (established in water or measured in other media than water) of these selected substances were presented and their chemical structures displayed.

INTRODUCTION

In a variety of forms living on the earth, plants constitute as little as *ca.* 320,000 species, whereas insects alone have to date been identified in a multitude of 8,750,000 species [Gibbs, 2002]. Under these circumstances, it is striking that so far plants have not been completely destroyed. This is due to the fact that the plants generated secondary metabolites which serve to protect the plants against survival-threatening factors. Structural diversity of the secondary metabolites hinders their systematization. Generally, they can be divided into three major classes: nitrogen compounds (alkaloids, amines, non-protein amino acids, cyanogenic glycosides, glucosinolates), terpenoids (monoterpenes, sesquiterpene lactones diterpenoides, saponins, limonoides), and phenolic compounds (mono-phenols, flavonoids, quinones).

Secondary metabolites present in food of plant origin are often referred to as natural non-nutrient substances, phytochemicals or bioactive compounds. Recent studies have provided information that a number of these substances exert a favourable effect on human health and are recommended as components of health-promoting food. Apart from playing multi-oriented positive biological functions, phytochemicals are found to display some negative activity, namely evoking bitterness and astringency in food. It should be emphasised, though, that in some beverages and food products a small intensity of these attributes may bring benefit to their overall sensory quality, like for instance in tea, red wine, vermouths, cocoa, beer, and nuts.

So far, no physicochemical methods have been elaborated that would enable determining the intensity of bitterness and astringency of food products evoked by the presence of phytochemicals. A sensory effect can be predicted based on the knowledge of concentrations at which these compounds are detected or recognised. The detection and the recognition thresholds are absolute thresholds. The first being the minimum concentration which can be detected without any requirements to identify or recognize the stimulus, while the latter is the minimum concentration at which a stimulus can be identified or recognized.

Bitter taste of food and the sensation of astringency are evoked by non-nutrient bioactive substances, with highly differentiated chemical structure, belonging to alkaloids, glucosinolates, saponins, and phenolic compounds. The most typical of these have been discussed below.

ALKALOIDS

Alkaloids are numbered as compounds from a nitrogen base group characterised by abundance of forms. Almost all compounds belonging to this group demonstrate a bitter taste. Alkaloids held a special position among natural substances due to a wide range of their medical applications, spanning a couple of last centuries. A human diet is poor in alkaloids, whose most common representatives therein are purine alkaloids, such as: caffeine (1,3,7-trimethylxanthine) (Figure 1 A), theobromine (3,7-dimethylxanthine) and theophylline (1,3-dimethylxanthine), occurring in coffee, tea and cocoa. Coffeine content in coffee is a significant factor affecting consumer's acceptance of this drink. The Arabika coffee, containing 1.5% of coffeine and described as "mild", is more preferred and rated high in terms of the sensory quality than the Robusta coffee with a higher coffeine content ranging from 2.4% to 2.8% [Illy 2002; www.coffeescience.org.].

There is extensive literature referring to the values of the absolute threshold for coffeine, whereas there are no

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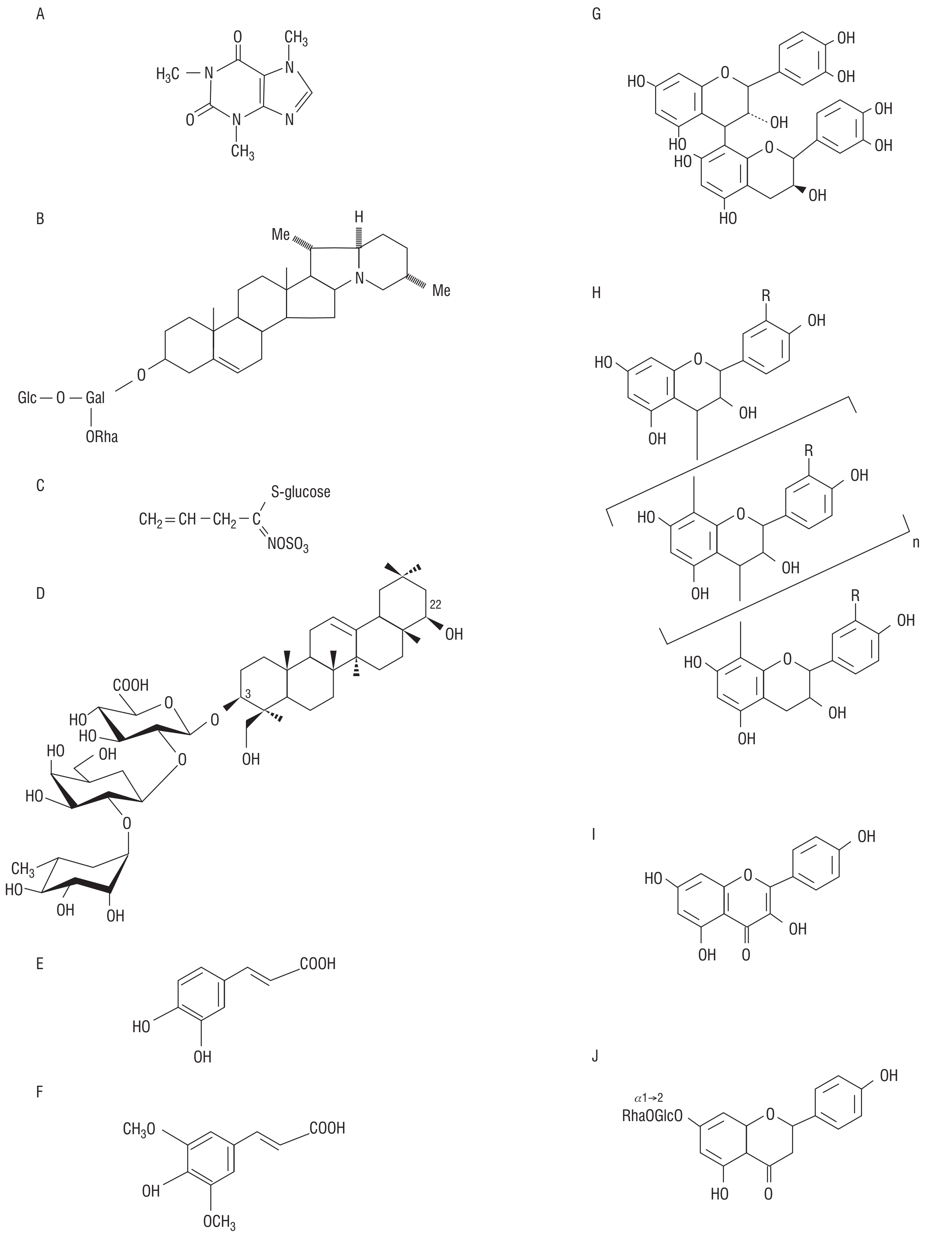

FIGURE 1. Chemical structures of selected non-nutrient bioactive compounds generating bitterness and astringency in food: A – caffeine; B – solanine; C – sinigrin; D – soya saponin; E – caffeic acid; F – sinapic acid; G – proanthocyanidins; H – condensed tannins; I – quercetin; and J – naringin.

such data for theobromine and theophylline. The recognition threshold values of coffee established in water have been reported in an extensive range of values, from 1.940 mg/kg [Sanders *et al.*, 2002] to 777 mg/kg [Langan & Yearick, 1976]. A great differentiation of results is caused by a number of factors, mainly by different research methods and the age of people taking part in experiments. Scientific investigations have indicated that in elderly people the threshold values of different tastes are likely to increase, with the highest increase reported for astringency [Weiffenbach *et al.*, 1986; Schiffman, 1993].

Investigations carried out recently proved coffeine to be a very desirable bioactive dietary component. Together with phenolic compounds, it is responsible for strong antioxidant properties of roasted coffee [Richelle *et al.*, 2001]. It has been implied that – as a strong antioxidant – coffee may counteract colon cancer [Nissen *et al.*, 2000; Daglia *et al.*, 2000; Nicoli *et al.*, 1997].

GLYCOALKALOIDS

Another group of alkaloids with a bitter taste are glycoalkaloids. In food, this group of compounds is represented by tomatine that occurs in tomatoes. A tomatine molecule consists of tomatidine aglycone linked to tetrasaccharide (glucose, xylose, galactose) [Raddick, 1974]. The recognition threshold of tomatine established in water accounts for 100 mg/kg [Skorikova *et al.*, 1985]. According to Kajderowicz-Jarosińska [1965], tomatine concentration in green tomatoes ranges from 0.086% to 0.089%. With tomato fruits ripening, a rapid decline in tomatine concentration proceeds and mature tomatoes contain only trace amounts of that compound. The bitter taste of tomatoes may also be generated by solanine (a glycoalkaloid occurring also in potatoes) (Figure 1 B). A solanine molecule is made up of solanidine aglycone linked to tetrasaccharide (glucose galactose-rhamnose). The content of this compound in green tomatoes fluctuates between 0.01 and 0.03%, whereas in appropriately-stored potatoes – between 0.002 and 0.004% [Nikonorow & Urbanek-Karłowska, 1987; Siemekova & Horcin, 1980]. The recognition threshold of solanine astringency established in 0.02% lactic acid has been reported to reach 62.6 mg/kg [Zitnak & Filadelfi, 1985].

Both tomatine and solanine are acknowledged as harmful to people. Thus, these both glycoalkaloids may affect not only the sensory quality of food products but also even determine their toxicity.

GLUCOSINOLATES

Glucosinolates, similarly as alkaloids, belong to the group of natural substances built of nitrogen-containing molecules. They are thioglycosides with β-(D)glucose as a saccharide component. Generally, glucosinolates can be divided into three groups: aliphatic, arylic, and indole. Unlike alkaloids, these compounds are common ingredients of a human die. Especially high contents of glucosinolates have been reported in cruciferous vegetables, including cabbage (white, red, savoy, Chinese), Brussels sprout, cauliflower, red small radish, turnip, broccoli, white and red radish, kale, and cress [Ciska *et al.*, 2000]. According to the updated state-of-the-art, implementation of the cruciferous vegetables in a diet contributes to a reduced incidence of some types of cancer, especially of the colon. The anti-carcinogenic properties of the breakdown products of glucosinolates (produced by chewing, cutting, crushing, and normal processing of the vegetables, which releases the enzyme myrosinase) have been reviewed recently [Verhoeven *et al.*, 1997]. It has been shown that indoles and isothiocyanates are effective modulators of the biotransformation of enzymatic systems. Stimulation of the activity of phase I and phase II biotransformation enzymes, which are responsible for metabolizing xenobiotics, is one of the powerful strategies of the action of anticarcinogens [MacDanell *et al.*, 1988; Verhoeven *et al.*, 1997]. With respect to the above-mentioned beneficial effects of glucosinolates on the human health, it seems reasonable to increase their contents in a diet.

A typical (pungent, burning) flavour of cruciferous plants is generated by the breakdown products of glucosinolates, mainly isothiocyanates. Whereas the bitter taste appearing in *Brassica* vegetables may be evoked by glucosinolates and products of their degradation as well. It has been demonstrated that sinigrin, progoitrin, gluconapin, (aliphatic glucosinolates), and 5-vinyloxazolidine-2-thione (breakdown product) are involved in bitter taste [Fenwick *et al.*, 1983; Griffiths & Fenwick, 1984]. The detection threshold of sinigrin (Figure 1 C) established in water accounts for 106 mg/kg. According to Doorn *et al.* [1998], sinigrin and progoitrin correlated negatively with taste preference of Brussels sprout when their combined content was higher than 2.2 g kg^{-1}. The available literature provides no data on the sensory activities of other glucosinolates and products of their degradation.

SAPONINS

Saponins (sapogenin glycosides) represent another group of bioactive compounds evoking the astringency sensation in food products. Each saponin consists of a sapogenin, which constitutes the aglucon moiety of the molecule, and a sugar [Price *et al.*, 1987]. The sapogenin may be steroid or a triterpene and the sugar moiety may be glucose, galactose, pentose, or methylpentose. A great structural diversity of these compounds contributes to a high variability of their biological activities [Oleszek *et al.*, 1994]. Although practically non-toxic to man upon oral ingestion, they act as powerful hemolytics when injected into the blood stream, dissolving the red corpuscles even at extreme dilution [Price *et al.*, 1987]. On the other hand, dietary saponins lower the blood cholesterol level and prevent atherosclerosis [Oakenfull *et al.*, 1984; Nikonorow & Urbanek-Karłowska, 1987]. In some papers, they have additionally been reported to demonstrate the anticarcinogenic activity [Messina & Barnes, 1991].

In food products, the best recognised saponis in terms of composition, content and biological activity are these of soybean, characterised by a terpenoid-like structure and referred to as sapogenoles (Figure 1 D) [Irland *et al.*, 1986]. A reach source of these compound are also other grain legume seeds [Price *et al.*, 1986], sugar beet, spinach, potatoes and asparagus. All saponins foam strongly when shaken with water (a common trait of this group of compounds is their ability to lower surface tension). They form oil-in water emulsions and act as protective colloids, thus they are applied in the production of some food agents. The available literature lacks data on their threshold values.

PHENOLIC COMPOUNDS

Of the natural bioactive substances discussed, phenolic compounds play the most significant role from the viewpoint of the sensory quality of food. They are ascribed to demonstrate a number of health-promoting properties resulting mainly from their strong antioxidant activity. These substance occur commonly in food and their especially rich sources include vegetables, fruit, seeds of some plants, some cereals, and also wines, tea, coffee, fruit juices and a number of spices [Herman, 1976; Hertog *et al.*, 1992; Frankel *et al.*, 1995; Yen & Chen, 1995]. Phenolic compounds may be generally divided into phenolic acids, flavonoids and highly-polymerised compounds. Each of the group mentioned contains substances responsible for introducing bitterness and astringency into food products.

Phenolic acids

In food, phenolic acids occur as derivatives of benzoic and cinnamic acids. They predominantly exist as esters of organic acids or glycosides, with only a small part of them being present as free forms. The most common derivatives of the benzoic acid include the following phenolic acids: 3-hydroxybenzoic, salicylic [2-hydroxybenzoic acid], gentisic [2,5-dihydroxybenzoic acid], protocatechuic [3,4-dihydroxybenzoic acid], and vanillic [4-hydroxy-3-methoxybenzoic acid]. Whereas the most important phenolic acids of cinnamic acid derivatives are represented by: caffeic [3,4-dihydroxycinnamic acid], *trans-o*-coumaric [*trans*-2-hydroxycinnamic acid], *p*-coumaric [4-hydroxycinnamic], ferulic [3-(4-hydroxy-3-methoxyphenyl)-2-propenic acid], and sinapic [4-hydroxy-3-5-dimethoxycinnamic acid]. Sensory attributes of food products are determined to a greatest extent by the derivatives of cinnamic acids than by those of the benzoic acid. Caffeic acid (Figure 1 E), which forms chlorogenic acids with quinone acid, has been found especially active. The concentration of chlorogenic acids affects considerably the sensory quality of food, since they contribute to enzymatic browning of food products, thus evoking their astringency [Oszmiański & Lee, 1990; Nicolas, 1994]. The phenolic acids: sinapic (Figure 1 F), ferulic and coumaric, can form esters with choline, which in turn generate bitterness, astringency, pungent taste and irritating flavour in some products made of vegetables and seeds of the *Cruciferae* family [Maga & Lorenz, 1973; Durkee & Thivierge, 1975; Ismail *et al.*, 1981]. This can be exemplified by sinapine (3,5-di-methoxy-4-hydroxy cinnamic acid) – being an ester of sinapic acid and choline [Fenwick *et al.*, 1982; Durkee & Thivierge, 1975].

According to Dadic and Belleau [1973], threshold values established in 5% aqueous ethanol for the following phenolic acids: caffeic, *trans-o*-coumaric, *p*-coumaric, ferulic and sinapic, account for 50, 50, 20, 10, and 20 mg/kg, respectively.

Derivatives of the cinnamic acid are known for their high antioxidant activity. Caffeic acid has been shown to be a potent antioxidant *in vitro* in different oxidation system [Cuvelier *et al.*, 1992; Chen *et al.*, 1997; Moon & Terao, 1998]. Also the antioxidant activities of ferulic and coumaric acids have been widely documented [Maillard & Berset, 1995; Maillard *et al.*, 1996; Fantozzi *et al.*, 1998; Ohata *et al.*, 1997].

Flavonoids

Of flavonoids, bitterness and astringency are demonstrated by catechins, and mostly by their oligomeric (Figure 1 G) and polymeric forms (Figure 1 H).

Catechins are precursors of proanthocyanidins (oligomeric forms) and condensed tannins (polymeric forms). In food products, catechins are predominated by (+) catechin and (-) epicatechin. The other monomers, including (+) gallocatechin and (-) epigallocatechin, are less common. In food, the oligomeric and polymeric forms of catechins may appear simultaneously. The composition and content of particular forms, especially the polymer size, determine the intensity of bitterness and astringency in food. Larger molecules tend to be less bitter and more astringent [Arnold *et al.*, 1980; Lea & Arnold, 1978]. In beverages, bitter taste and astringency are also affected by other factors, including: pH and the level of ethanol, sweetness or viscosity [Smith *et al.*, 1996; Fischer & Noble, 1994].

Bitterness is one of four basic tastes, and is perceived by taste receptors on the tongue [Kinnamon, 1996; Noble, 1990]. In contrast to bitterness, astringency is a tactile sensation perceived as dryness, puckering and roughness throughout the oral cavity. The physiology of astringency is still not well defined. Bate-Smith [1973] suggested the action of astringent compounds on the protein of the saliva. He claimed that the polyphenolic compounds, such as tannins, form complexes with salivary proteins and/or mucopolysaccharides, either precipitating them or causing sufficient conformation changes, so that they lose their lubricating power, thus making the mouth feel rough and dry. Later investigations have shown that polyphenols demonstrate a high affinity to binding with proline-rich protein in the saliva [Hagerman & Butler, 1981]. Direct binding of phenols to epithelial protein may also play an important role especially when the ratio of the concentrations of astringent substances to salivary protein is particularly high [Guinard *et al.*, 1986].

According to Sanderson *et al.* [1976], the threshold recognition values of bitter taste established in water for catechin, epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate reach 600; 600; 350, 200; and 300 mg/kg, respectively. Whereas the threshold recognition values of epicatechin gallate and epigallocatechin gallate astringency account for 500 and 600 mg/kg, respectively. On the contrary, there are no reports that would refer to the threshold values of detection and recognition of the highly--polymerised molecules. One of the obstacles in gathering such data is a lack of commercial standards of proanthocyanidins. A typical trait of these compounds is the ease of oxidation, which is the reason of their numerous enzymatic and non-enzymatic transformations [Oszmiański & Lee, 1990; Oszmiański *et al.*, 1996]. Due to a low stability of proanthocyanidins, only few laboratories deal with their purification and isolation. Significant limitations result also from the necessity of using large amounts of the experimental material in sensory assays and from the difficulty of carrying out assessments of bitter taste and astringent sensation in humans usually aversed to these types of stimuli.

Another group of flavonoid compounds generating bitterness in food are flavanols. They are much more stable than the above-discussed compounds as they occur in the form of glycosides. Flavanols manifesting bitter taste include quercetin (Figure 1 I) which is an aglucone of quercitrin, of rutin and of other glycosides. According to Dadic and Belleau [1973], the recognition threshold of quercetin in 5% aqueous ethanol and in beer is low and accounts for 10 mg/kg. Whereas the detection threshold value of quercitrin (quercetin 3-rhamnoside) in beer is considerably higher and reaches 250 mg/kg [Meilgaard, 1975]. Another intensively bitter compound belonging to flavonoles is naringin (Figure 1 J) which is responsible, among others, for the bitter taste of grapefruits as well as tomatoes and their juices [Guadagni *et al.*, 1973]. According to Pfeilsticker *et al.* [1978], the recognition threshold value of naringin bitterness accounts for 25 mg/kg.

All the above-mentioned flavonoids responsible for bitter taste and astringency sensation in food are characterised by a high antioxidative activity. Epidemiological studies have indicated that a flavonoid-rich diet reduces the risk of heart ischaemia, neaoplasm and osteoporosis incidence as well as delays aging processes [Block *et al.*, 1992; Hertog *et al.*, 1993; Halliwell, 1996; Diaz *et al.*, 1997]. The antioxidant activity of flavonoids is well documented both with respect to pure standards and different plant extracts. Flavonoids have been reported to inhibit lipid peroxidation, to effectively scavenge free radicals and to chelate ions of prooxidant metals, and their activity was shown to be determined by the chemical structure of a compound. The best known antioxidant properties have been these of tannins of grape seeds, wines and green tea [Yen & Chen, 1995; Teissedre *et al.,* 1996; Muramatsu *et al.*, 1986; Frankel *et al*., 1995; Tebib *et al.*, 1994a; 1994b; 1997]. Data indicate that their ability to scavenge free-radicals and block lipid peroxidation may be of significance to the prevention of cardiovascular diseases. The protective effect of tannins may be mediated through inhibition of the oxidative modification of LDL-C.

SUMMARY

Recently, much attention has been paid to the role of bioactive non-nutrient compounds in food. A number of investigations have been carried out to isolate and identify them, and to characterise their biological activity. Ample reports underline the significance of phytochemicals in prevention of numerous civilisation diseases. Nutritionists recommend higher implementation of raw materials and products rich in some polyphenols, glucosinolates and saponins to diets. Taking this into account, it may be assumed that the content of phytochemicals in a human diet will be increasing. It may be proved even by ongoing investigations aimed at obtaining high-phenolic raw materials with biotechnological methods, and by the developing industry of health-promoting food offering, among others, polyphenolic antioxidant preparations.

Increasing the content of antioxidants in raw materials and products and the production of antioxidant-supplemented health-promoting food requires deepening the knowledge on the sensory activity of these substances. Of special significance is the recognition of relationships between the sensations of bitterness and astringency and the antioxidant activity of phytochemicals. It is a new issue, so far not recognised scientifically, with a great potential practical application in designing of health-promoting food.
A lack of data on that subject has inspired us to undertake a research entitled "The relationship between the content of phenolic compounds and their antioxidant activity and the negative attributes of sensory quality (bitterness, astringency) in selected plant materials", carried out under the project "Methodological bases of complex evaluation of the quality and safety of new-generation food" co-ordinated by Prof. Dr. Henryk Kostyra (Project No. PBZ-KBN/020/P06/1999).

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FINAL REPORT

Title of the research ordered project:
THE METHODOLOGICAL BASES OF THE EVALUATION OF THE QUALITY AND SAFETY OF THE NEW GENERATION FOOD (PBZ-KBN-020/P06/1999).

Title of the individual project:
The relationship between the content of phenolic compounds and their antioxidant activity and the negative attributes of sensory quality (bitterness, astringency) in selected plant materials.

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Key words:
Phenolics, tannins, sensory quality, astringency, bitterness, antioxidant activity, antiradical properties, HPLC

SYNTHESIS OF RESULTS

A valuable biological activity of phenolic compounds (especially their antioxidant activity) is the reason of recent tendencies of increasing their content in a human diet. On the other hand, these compounds introduce to food some negative sensory attributes, *i.e.* bitterness and astringency, which may limit their applications. This project aimed at identifying relationships between the content and antioxidant activity of phenolic compounds and negative sensory attributes (bitterness, astringency). This is a new issue, that has not been recognised scientifically so far but of significant practical importance in designing of health-promoting food.

Experiments were carried out on extracts obtained from tannin-rich seeds of leguminous plants, including: faba bean (*Vicia faba* var. *minor*), broad bean (*Vicia faba* var. *major Harz*), pea (*Pisum sativum* L), Adzuki and red bean (*Phaseolus vulgaris* L), green and red lentil (*Lens culinaris*), and other tannin-rich plants, such as: walnut (*Juglans regia* L.), hazelnut (*Corylus avellana* L.), kernel of almonds (*Amygdalus communis* L.), seeds of vetch (*Vicia sativa* L.), buckwheat (*Fagopyrum esculentum* Möench), and buckwheat grits. The experiments were run in two stages: the first stage involved examinations of extracts obtained from seeds of grain legumes belonging to one family (*Leguminoseae*); whereas the second stage aimed at analysing extracts of the other raw materials originating from different families.

Extracts of phenolic compounds (in both stages) were subjected to sensory and chemical analyses.

The sensory experiment involved: (1) training of the sensory panel into discrimination of differences and repeatability of results referring to the assessments of bitterness and astringency (referential standards accepted included: tannic acid and aluminium potassium sulfate for bitterness, as well as coffeine and chinine for astringency); (2) elaboration of a standard procedure for the preparation and presentation of experimental extracts; (3) determination of the total astringency of the extracts with the scalling method, and determination of astringency indices (AI) and astringency coefficients (AC) for the extracts obtained; and(4) profile characteristics of extracts by means of the Quantitative Description Analysis (QDA).

All the above-described stages of sensory analyses were carried out in a laboratory complying with requirements of the international standard PN-ISO 8589 : 1998. A computerised system for sensory analysis was used to prepare assessments, collect individual results and analyse them.

With the sensory experiment, lyophilisate of panelists saliva was prepared additionally to determine the ability of saliva protein precipitation by polyphenolic extracts.

Within chemical experiments phenolics from the crude extracts were separated into fraction I of low molecular phenolic compounds and fraction II of tannins using a Sephadex LH-20 column chromatography with ethanol and water-acetone (1:1; v/v) as mobile phases. Crude extracts and their fractions were assayed for: the content of total phenolics, tannins determined using vanillin method, UV spectra, Total Antioxidant Activity (TAA), antioxidant activity using a β-carotene-linoleate model system, DPPH radical scavenging activity and reducing power. Additionally, the crude extracts were subjected to the analysis of tannins using the precipitation method of BSA and salivary proteins, anthocyanidin content after *n*-butanol/HCl hydrolysis, as well as contents of phenolic acids and flavonoids using the HPLC method.

The relationship between sensory astringency (AI and AC) and the content of phenolic compounds and their antioxidant properties was investigated with single regression analysis. Additionally, extracts of grain legume seeds were subjected to multiple regression analysis.

The results of sensory analyses indicated that the detection thresholds of tannic acid and coffeine (accepted as standards of astringency and bitterness) were low and accounted for 0.0024% and 0.0063%, respectively. Of the six modified soluble starches used to neutralise the astringency sensation in the mouths of panelists, only two potato starches (pudding and gelling ones) were able to reduce that sensation negligibly. It has also been shown that of the waters analysed: cooked water from water supply system, distilled water and mineral water, the last one (Tip, by Wosana S.A.) appeared to be the most neutral in terms of taste. Difficult problems in preparing the polyphenolic extracts for sensory analysis were posed by their incomplete solubility in water. Ethanol's addition improved the solubility of extracts, still it suppressed their astringency and intensified their bitterness. The detection threshold of ethanol was found to be low and reached 0.0025%. The results obtained were used to prepare the extracts for examinations of the intensity of their astringency. Based on the extracts' astringency, the AI and AC values were determined for individual extracts. The astringency intensity of grain legume seed extracts was found to follow the descending order: red bean>Adzuki bean>red lentil>green lentil>>pea>broad bean>faba bean. The AI values of these extracts ranged from 3.84 (faba bean) to 7.93 (red bean), whereas the AC values – from 5.56 (faba bean) to 10.79 (red bean). The intensity of astringency of the extracts obtained from plants originating from different families may be ordered as follows: walnut>hazelnut>kernel of almonds>>buckwheat>vetch>buckwheat grits. The AI values of these extracts fluctuated from 4.39 (vetch) to 9.62 (walnut), and these of AC – from 4.39 (buckwheat grits) to 7.84 (walnut). The QDA indicated that sensory profiles of the extracts were differentiated. Nevertheless, in all profiles a dominating attribute, that accompanied astringency, was bitterness. In extracts of legume seeds the highest intensity of that attribute was reported in Adzuki bean (*ca.* 6 conventional units in 10-point scale). In the other extracts, the intensity of bitterness fluctuated at a similar level of 3–4 conventional units. Apart from bitterness, the following attributes were identified in the sensory profiles obtained: bean-like (in legume extracts), sweet sour, mealy, and walnut skin.

Chemical analyses have demonstrated that the content of total phenolic compounds in crude extracts, expressed in catechin equivalents, was diversified and ranged from 22.6 mg/g of extract (pea) to 109.2 mg/g of extract (walnut). The content of tannins in the extracts determined using the vanillin method and expressed as absorbance at 500 nm/g ranged from 5.96 (broad bean) to 422 (buckwheat grits). The highest ability of BSA and salivary proteins precipitation was found for the extract of walnuts. After acid *n*-butanol hydrolysis the extracts of broad bean and Adzuki bean were characterised by the lowest (0.156) and the highest (3.367) values of absorbance at 550 nm/mg. All investigated extracts demonstrated the antioxidant and antiradical activity, as well the reduction power. The extracts of walnut and buckwheat were characterised by the highest TAA values: 5.25 and 2.48 μmol Trolox/mg, respectively. The extracts of walnut and buckwheat were the most effective scavengers of DPPH radical and their reduction power was the highest. The highest antioxidant activity in β-carotene--linoleate model system was noted for the extracts of buckwheat and buckwheat grits.

The results of TAA and DPPH scavenging activity of tannins were higher than those of the crude extracts and low molecular fractions. The content of total phenolics in fraction II was several times higher than that in fraction I. The relatively lower antioxidant activity of tannin fractions was observed in the case of the β-carotene-linoleate model system.

The HPLC analysis enabled identification of such phenolic acids as vanillic, caffeic, *p*-coumaric, ferulic, and sinapic. The highest content of ferulic acid was found in the extract of red bean (1575 μg/g). The highest content of quercetin was determined in the extract of Adzuki bean (2210 μg/g). The extract of the red lentil showed the highest content of caempferol (2146 μg/g).

For the legume seeds extracts, the statistically significant correlation was found for AI *vs*. tannins determined using both precipitation methods, and anthocyanidins content after *n*-butanol/HCl hydrolysis, and for AC *vs*. the abovementioned results and additionally *vs*. tannins determined using the vanillin method, Total Antioxidant Activity and results of the β-carotene-linoleate assay. In the case of the extracts of other plants, AI correlated with the results of tannin content determined using both precipitation methods, Total Antioxidant Activity, and reduction power. For the same material, the statistically significant correlation was found for AC *vs*. results of the β-carotene-linoleate assay.

The results of multiple regression analysis have shown that the AI values were affected the most by tannins determined using the precipitation method (54% of total variability) and Total Antioxidant Activity (37.8% of total variability). The effect of these variables was statistically significant. The other variables (tannins determined using the vanillin method and *n*-butanol/HCl hydrolysis, and total phenolic content) caused together 8.24% of total variability and were statistically insignificant. Multiple regression analysis of AC values have also demonstrated a statistically significant effect of Total Antioxidant Activity (*ca.* 65% of total variability), whereas tannins determined with the vanillin method caused *ca.* 27.5% of total variability.