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FOOD FLAVONOIDS

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Flavonoids are secondary metabolites widely distributed in the plant kingdom. They belong to a large group of compounds with highly diversified structure – referred to as polyphenols. This diversity determines a variety of life functions they play in plants. As indispensable components of plant food products, these widespread bioactive compounds are consumed by humans in amounts significant from the physiological point of view. Species- and varietal variability, vegetation season, light, climatic conditions, technological processing, and the way of preparing meals are the main factors determining their content in food and consequently their intake. Flavonoids get to the human's alimentary tract and then into the general blood circulation. Thus, while estimating their physiological functions, consideration should be given to transformations they undergo in metabolic processes. Although knowledge on the absorption and metabolism of flavonoids is still incomplete, it substantiates the statement that – after absorption – these compounds occur in the blood circulation as glucuronised, and/or sulfated or methylated conjugates. Therefore, most of the effects shown in *in vitro* experiments with aglycones cannot be directly extrapolated to *in vivo* systems. Despite the fact that conjugation is one of the stages of detoxification process and elimination of xenobiotics from the organism, the metabolites formed may still affect consumer organism. This concerns also the antioxidant properties of flavonoids which may be significantly reduced or even lost. However, it does not mean that flavonoids simultaneously lose their positive impact on consumer health. Even after being metabolised they may act locally or systemically indirectly influencing redox balance by inhibition of oxidative enzymes, inducing antioxidative and detoxifying enzymes, or compounds which may be involved in sustaining homeostasis. This, at least in part, may explain their beneficial physiological function resulting from epidemiological studies, especially in the prevention of atherosclerotic lesions development. Nevertheless, it is unclear whether metabolised flavonoids are capable of coming into contact with cellular membranes and penetrate the cell's interior. It is known, however, that a considerable part of absorbed flavonoids is rapidly excreted back to the alimentary tract with bile. Thus, the not-absorbed flavonoids and these excreted with bile may act as agents protecting the surface of the epithelial cells of the alimentary tract and prevent their degenerative changes.

INTRODUCTION

Constantly increasing pollution of the natural environment, improper nutrition, smoking, alcohol abuse, leading a stress-bearing and non-hygienic lifestyle pose a serious risk of health loss. Of the factors mentioned above, especially dietary habits have been claimed to exert the strongest impact on proper functioning of the human organism. Apart from physical activity, rational diet is one of the core elements in the prevention of chronic diseases.

Well-balanced diet should include fruit and vegetables whose consumption is recommended all year round, when possible in the fresh form. Unfortunately, under Polish climatic conditions most of them occur seasonally and only some, *i.e.* apples, *Allium*-, *Brassica*- and root vegetables may be stored and thus consumed up to few months after harvest.

Results of epidemiological studies point to a favourable effect of a diet rich in fruit and vegetables on human health in the aspect of disease development. The protective action of these products is ascribed to their bioactive substances, with polyphenols and especially one of their classes – flavonoids – playing a key role.

In 1936, while studying citrus fruits, Ruszynak and Szent-Györgi [1936] isolated two compounds currently classified to flavonoids: hesperidin and rutin. Investigations have indicated that these compounds demonstrate traits that affect mechanical properties (fragility and permeability) of humans blood capillaries. They have been suggested to be included into a group of vitamins (vitamin P), still a lack of results confirming all parameters that should be met by essential food ingredients hindered including the flavonoids into this group. Successive years of research have brought a number of interesting data referring to the biological activity of flavonoids. Their anti-inflammatory, antiallergenic, antiviral, thrombocyte aggregation-inhibiting, anti-hypertensive, anticarcinogenic, and antioxidant properties have been discovered [Formica & Regelson, 1995; Kinsella *et al.*, 1993; Pace-Asciak *et al.*, 1995; Terao & Piskula, 1998; Peterson & Dweyer, 1998; Guardia *et al.*, 2001; Manach *et al.*, 1996; Harborne & Williams, 2000].

These multiple physiological activities of flavonoids, especially their antioxidant activity, are determined by the type and structure of a compound. It is of significant importance, since in damage of structures and life functions of a cell a considerable role is ascribed to a disturbed balance

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of free radical reactions. Pathological levels of free radicals may result from the activities of endogenous and exogenous factors, including a diet.

CHEMISTRY OF FLAVONOIDS

As it was mentioned, flavonoids belong to a large group of compounds referred to as polyphenols, differentiated in respect of their structures and properties, and widely distributed in the plant kingdom [Iwashina, 2000; Herrmann, 1976, 1988]. A typical characteristic of the chemical structure of these compounds is the presence of an aromatic ring with hydroxyl groups. A number of polyphenolic compounds possess two or more hydroxyl groups substituted with a variety of functional groups. Taking into account the structure of the basic carbon skeleton, polyphenols may be divided into: phenylcarboxylic acids (C_6-C_1), phenylpropene acids (C_6-C_3), and flavonoids (C_6-C_3-C_6). The phenylcarboxylic acids are derivatives of benzene carboxylic acid, and the phenylpropene acids – derivatives of cynnamic acid. In food products, both the first and the latter occur mainly as esters or glycosides. Phenolic acids with o-diphenol structure are capable of chelating metal ions, which is highly significant in biological systems. In addition, they readily oxidize into respective quinones, which results in the formation of important oxidation-reduction systems and enables polymerization to high-molecular components.

Most frequently occurring phenylcarboxylic acids include: p-hydroxybenzoic acid, salicylic acid, protocatechuic acid, vanillic acid, and gallic acid. Whereas the most important phenylpropene acids include: caffeic acid, p-coumaric acid, ferulic acid, sinapic acid, and a group of chlorogenic acids (esters of caffeic and quinic acids).

The structure of flavonoids – a large sub-group of polyphenols – is based on a 2-phenyl-benzo-α-pyrone skeleton formed by two phenyl rings (A and B) linked with a heterocyclic pyrone ring (C) (Figure 1). Hence, the structure of diphenylpropene (C_6-C_3-C_6, flavan) may be distinguished in all flavonoids. It is formed upon condensation of 3 molecules of malonylo-CoA with 4-coumaroylo-CoA [Aoki *et al.*, 2000; Horbowicz 2000; Aherne & O'Brien, 2002]. The A ring is made from three malonylo residues formed during a metabolic pathway of glucose. The B ring is formed from 4-coumaroylo-CoA produced on the shikimic acid pathway from phenylalanine. With the share of specific synthase, chalkone is formed which under the influence of chalkone isomerase is transformed into flavanone. The compounds formed remain in equilibrium and constitute major substrates for a number of flavonoids. Further transformations: oxidative cyclization, bioreduction, transfer of an arylic group, dehydrogenation, hydroxylation, polymerization, mediated by specific enzymes (isomerases, transferases, oxidases, hydroxylases, synthases, reductases) result in the formation of representatives of the particular flavonoid classes (Figure 2).

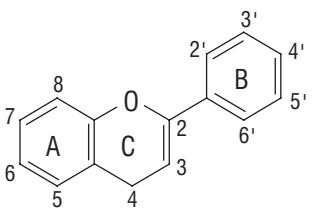

FIGURE 1. Structure of flavan.

To date, there have been classified around 6500 flavonoids [Harborne & Williams, 2000], which – depending on the oxidation degree of the C ring, and the number, and localization of functional groups – have been divided into several classes: aurones, chalcones, anthocyanins, catechins, flavones, flavanones, isoflavones, and flavonols [Rice-Evans, 2000]. Most often glucose is the sugar substituent, but xylose, arabinose, galactose rhamnose and glucuronic acid serve this purpose as well. Usually, glycosidation proceeds in the C-3 position, rarely in the C-4', C-3', C-5 or C-7 positions [Herrmann, 1988].

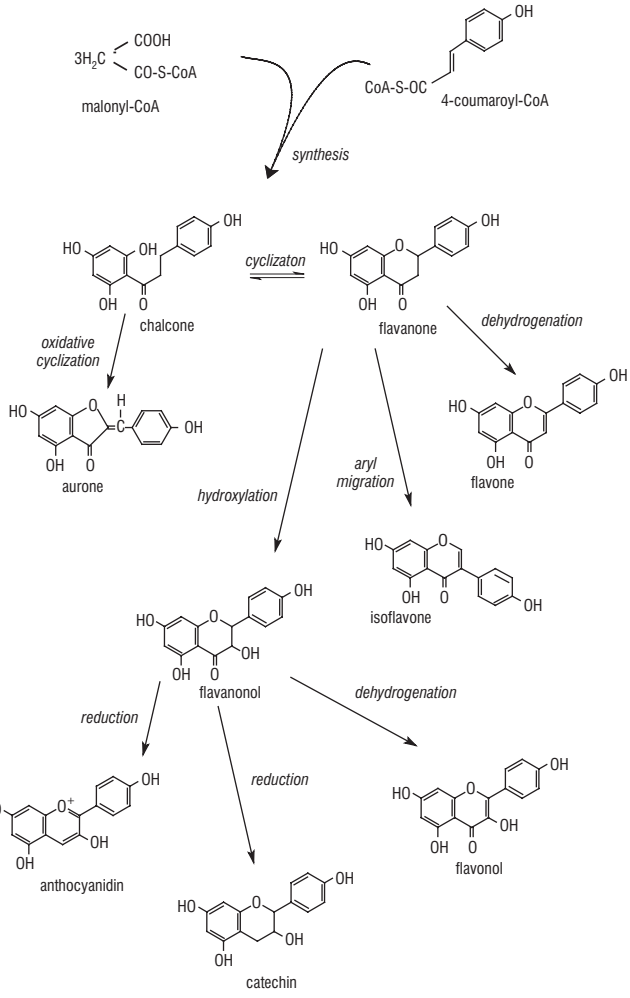

FIGURE 2. Scheme of biosynthesis of major flavonoid groups.

FLAVONOIDS IN PLANTS

Polyphenols are plant secondary metabolites derived from basic substances active in the basic metabolism and are formed in main pathways branches of the higher plants biosynthesis [Winkel-Shirley, 2001a, b]. High concentrations of flavonoids are typical of plants and plant products. Their presence was identified in all parts of a plant: in leaves, flowers, fruits, seeds and lignified tissues [Iwashina, 2000; Formica & Regelson, 1995; Cook & Samman, 1996; Herrmann, 1976, 1988].

In the plant kingdom, flavonoids appear most often as β-O-glycosidic derivatives [Herrmann, 1988; Horbowicz, 2000]. In leaves, flowers and fruits, flavonoids occur in the form of glycosides and esters with organic acids, whereas lignified tissues contain mainly free forms of flavonoids. Seeds may contain both the glycosidic forms and aglycones. With reference to polarity, plant flavonoids may be divided into lipophylic and hydrophilic ones [Rice-Evans, 2000]. The first occur in the outer living cells of plants. They have been identified in the waxy layer of leaves and leaf buds. Usually, flavonoids isolated from these parts of a plant appear to be O-methylated flavones and flavonols, and free hydroxyflavones. Hydrophilic flavonoids accumulate in vacuoles of plant cells. They occur most often as glycosidic derivatives where glycosylation increases the polarity of the flavonoid molecule, which is necessary for storage in plant cell vacuoles [Aherne & O'Brien, 2002].

Flavonoids serve various important physiological functions in plants. They actively participate in defence systems counteracting diseases and pests, affect biosynthesis of auxins, or function as substances protecting plants against competition of other plants. They are used as chemical signals enabling contacts of plants with desirable partners present in the phytosphere. They act also as antioxidant substances, thus serving as blocking and eliminating free radicals formed upon stresses that plants are subjected to during vegetation [Formica & Regelson, 1995; Aoki *et al.*, 2000; Harborne & Williams, 2000; Winkel-Shirly, 1996, 2001a, b; Simmonds, 2001; Wojtaszek, 1993].

The presence of flavonoids is one of the factors that determine plant pigmentation. Anthocyanins, chlorophylls and carotenoids are responsible for colouration of plants: their stems, leaves, flowers and fruits. The colour of flowers is mainly affected by pigments present in chromoplasts or flower tissue vacuoles. Flavonoid compounds occurring in flower lobules determine their colour: from light-cream, through orange, to blue and red. Pelargonidin (orange-red colour), cyanidin (purple colour) and delphinidin (violet-yellow colour) are the chemical basis of the colour of flowers. Additional factors, including: position of hydroxylation of the enumerated anthocyanidins, the presence of acylated aromatic substituent linked through a glucose residue to anthocyanidin, glycosilation or hydroxylation of the B ring; methylation; the presence of metals; the presence of flavones or flavonole co-pigments and other pigments, may modify the basic colour [Harborne, 1997]. Some groups of flavonoids (flavones and flavonols) are colourless. In flower lobules they very often form links with anthocyanidins as co-acting and stabilizing substances. To a smaller extent they are responsible for the yellow colour of flowers. Only yellow flavones (gossipetin, quercetagetin and their derivatives), owing their colour to the presence of an additional hydroxyl or methoxyl group in the C6 or C8 position of the A ring, influence the colour of flowers of plants from the *Compositae* family [Harborne, 1997].

Flavones (luteolin and apigenin) and flavonols (quercetin and kaempherol) occurring in most of white- or cream-coloured flower lobules probably act as attracting agents, making the flowers attractive to bees and other insects, which is of key importance in the biology of pollination [Harborne, 1997].

Radiation of the highest UV-B energy (280–315 nm) may penetrate through the ozone layer and potentially contribute to damage of plant life functions. Plants' resistance to this radiation is linked to their flavonoids which by absorbing radiation at these wavelengths may act as UV filters, thus protecting the photosynthesising tissues [Harborne & Williams, 2000]. This ability explains the highest content of flavonoids in the outer tissues of plants, *e.g.* leaves or flowers. Their concentration is observed to decrease along with approaching the stem of a plant [Kuhnau, 1976; Harborne & Williams, 2000]. Flavonoids are mainly accumulated in the aboveground parts of plants, except for *Allium* plants whose underground parts constitute a rich source of flavonols [Hertog *et al.*, 1992; Horbowicz & Kotlińska, 1998].

Very often flavonoids are accumulated in plant skin (apple, grapes, onion) [Wiczkowski *et al.*, 2003; McDonald *et al.*, 1998; Herrmann, 1976; Horbowicz & Bąkowski, 2000]. Two outer storage scales of onion contain *ca.* 70% of total quercetin in the bulb. Especially high concentrations of quercetin have been identified in dry skin of onion [Patil & Pike, 1995, Horbowicz & Bąkowski, 2000]. This part of onion was found to contain 5-fold higher level of quercetin compared to the fleshy scales. In the edible part of onion, the dominating form of quercetin are its glycosides which constitute 99.3% of total quercetin, and as little as 0.7% is made by free quercetin. The main glycosides of the flesh scales of onion include: 4'-O-β-quercetin-glucoside and 3,4'-O-bis-β-quercetin-glucoside [Bączek *et al.*, 2002; Tsushida & Suzuki, 1996; Hirota *et al.*, 1998; Price *et al.*, 1997]. On the other hand, a completely different configuration of quercetin derivatives was noted in dry skin where aglycone accounted for 53.2% of total quercetin [Wiczkowski *et al.*, 2003].

Observations of flavonoid concentrations in vegetables through the months of vegetative season have indicated their different levels. Results of a study by Hertog *et al.* [1992] point to 3–5-fold higher levels of kaempferol and quercetin in lettuce, leek and endive in the summer season compared to spring or autumn. The level of flavonoids in plants depends on weather conditions. Considerable differences have been observed in quercetin levels of onions between two summer seasons differing significantly in terms of weather. The average quercetin content of four onion varieties (Dawidowska, Błońska, Grabowska, Sochaczewska) in the vegetative season of 1997 (high insolation, high temperatures, low precipitation) accounted for 503 mg/kg fresh weight (f wt), and in the year 1998 (high precipitation, lower temperatures) – for 346 mg/kg f wt [Horbowicz & Bąkowski, 2000].

FOOD CONTENT AND INTAKE OF FLAVONOIDS

Species- and varietal diversity, vegetative season, light, climatic conditions, technological processes and manner of preparing meals are the major factors that determine the content and thus the intake of flavonoids.

Flavonoids are natural components of plant-derived foodstuffs: fruit, vegetables, nuts, seeds of cereal-, leguminous- and oil plants [Hertog *et al.*, 1992; Crozier *et al.*, 1997; Aherne & O'Brien, 2002]. Wide spread in these food products and consequently commonly-consumed flavonoid is quercetin (3,3',4',5,7–penthahydroxyflavone), compound reconed to flavonols – one of flavonoid classes [Herrmann,

1976]. Quercetin is present in tea, wine, onion, lettuce, savoy cabbage, broccoli, bean, apples, potatoes, peaches, buckwheat, and string bean. In plants, quercetin appears mainly in the glycosidic form [Herrmann, 1976, 1988]. Most often occurring quercetin glycosides include: 3-O-β-glucoside (apples, pears, plums, quince, cherries, grapes, rhubarb, apricots, lettuce, strawberries, parsnip, tomatoes, leek, onion), 3-O-β-rutinoside – rutin (apples, cherries, sweet cherries, apricots, rhubarb, parsnip, tomatoes, buckwheat, bean, broad bean), 3-O-β-rhamnoside (apples, plums, rhubarb, sweet cherries), and 3-O-β-galactoside (apples, quince, cherries, strawberries) [Herrmann, 1976, 1988; Macheix *et al.*, 1990].

High intakes have also been reported for another flavonol possessing one hydroxyl group less in its B ring than quercetin, namely kaempferol (3,4',5,7-tetrahydroxyflavone) and its glycosides. In vegetables and fruits, high contents of this compound have been found in strawberries, cherries, black-currant, bean, leek, raspberries, grapes, broccoli, chives and onion [Herrmann, 1976, 1988; Hertog *et al.*, 1992; Macheix *et al.*, 1990]. Another widely-discussed but appearing in low amounts flavonol is miricetin (one hydroxyl group more in the B ring than in quercetin – 3,3',4',5,5',7–hexahydroxyflavone) present in dark grapes, broad bean, red wine, and tea infusions [Hertog *et al.*, 1992; Wang & Halliwell, 2001; Tsanova-Savova & Ribarova, 2002; Trichopoulou *et al.*, 2000; Hertog *et al.*, 1993c]. An important group of flavonoids is represented by flavones, including apigenin occurring in celery, parsley, carrot and chicory, and luteolin appearing in celery, carrot, chicory and lettuce [Lugasi & Hovari, 2000; Hertog *et al.*, 1992; Trichopoulou *et al.*, 2000; Crozier *et al.*, 1997]. Other often occurring flavonoids include: hesperidin and naringin isolated from citrus fruits [Macheix *et al.*, 1990; Gil-Izquierdo *et al.*, 2001], as well as genistein and daidzein – two isoflavones present in soybean and its products [Coward *et al.*, 1998; Barnes *et al.*, 1994].

A number of studies have indicated that in vegetables the highest levels of flavonols can be observed in onion (284–843 mg/kg f wt), broccoli (36–137 mg/kg f wt), kale (20–300 mg/kg f wt), lettuce (4–273 mg/kg f wt), string bean (34–59 mg/kg f wt), "cherry" tomatoes (17–203 mg/kg f wt), and broad bean (46 mg/kg f wt). In fruits, high contents of flavonols have been reported in apples (21–72 mg/kg f wt) [Hertog *et al.*, 1992; Herrmann, 1976; Crozier *et al.*, 1997]. Lower levels of these compounds have been demonstrated in cherries (26–40 mg/kg f wt), apricots (27 mg/kg f wt), redcurrant (15 mg/kg f wt), grapes (17–19 mg/kg f wt), and strawberries (17–25 mg/kg f wt). A good source of flavonols are also beverages, especially red wine (5–26 mg/L), tomato juice (13 mg/L) and tea infusion (black tea: 18–47 mg/L, green tea: 28–50 mg/L) [Hertog *et al.*, 1992; Tsanova-Savova & Ribarova, 2002; Crozier *et al.*, 1997]. It should be emphasised that tea is rich most of all in catechins, which when combined with a high consumption of this beverage in Poland places it in a group of food products constituting the main dietary source of polyphenols of a Polish diet. Also in diets of other European populations and the USA, one of the basic/significant sources of flavonoids are *Allium* plants: brown onion, red and white onion, shallot, chives, leek, and garlic. In these vegetables, the highest levels of quercetin have been reported in fleshy storage scales of brown onion (185–1187 mg/kg f wt), in red onion (195–1917 mg/kg f wt), and lower levels – in shallot (256–393 mg/kg f wt) [Wiczkowski *et al.*, 2003; Hertog *et al.*, 1992; Horbowicz & Bąkowski, 2000; Tsushida & Suzuki, 1996; Price *et al.*, 1997; Price & Rhodes, 1997; Horbowicz & Kotlińska, 1998].

It seems that tea, apples and onion play a major role in providing flavonoids to a consumer of a standard Polish diet. Determination of an average daily flavonoids intake with food is very difficult because of great diversity of flavonoids (recent reports inform about 6500 identified compounds belonging to this group), high variability of flavonoid level in particular products of food origin, difficulties with precise determination of dietary habits of populations surveyed, a small group of compounds explored in details, and a lack of food composition tables that would list those compounds in respect of varietal differences of raw materials and effects of their processing technologies so that the data would refer to the content of these compounds immediately before consumption.

Taking into account the above-mentioned great diversity of flavonoids, their daily intake has been estimated to account for *ca.* 1 g and originate from the following groups of food products: cocoa, cola, coffee, tea, beer, wine – 420 mg; fruits and fruit juices – 290 mg; vegetables and herbs – 162 mg; potatoes – 79 mg; nuts – 45 mg; and cereal products – 44 mg [Formica & Regelson, 1995]. Hence, the daily intake of flavonoids determined on the basis of 5 dominating compounds only was found to reach 23 mg, including: 16 mg of quercetin, 4 mg of kaempferol, 1.4 mg of miricetin, 0.9 mg of luteolin, and 0.7 mg of apigenin [Hertog *et al.*, 1993b].

FOOD PROCESSING AND FLAVONOIDS

It is still not fully explained how flavonoids behave during storage of plant materials. Most of research indicate that the content of flavonoid compounds decreases with storage time. Onion stored for 24 weeks at a temperature of 4°C lost 30% of the initial quercetin content [Price *et al.*, 1997], while black-currant stored for 9 months at -20°C *ca.* 40% of initial quercetin [Häkkinen *et al.*, 2000].

Technological treatment of the plant material may diminish the content of flavonoids even to 50%. The main processes lowering this level included: peeling, dehulling, trimming and shredding the leaves [Aherne & O'Brien, 2002; Peterson & Dwyer, 1998]. After homelike peeling red onions contained 79% of the original total content of quercetin-4'-glucoside and only 27% of the anthocyanins [Gennaro *et al.*, 2002]. Quercetin-3,4'-diglucoside (Q3,4'G) and quercetin-4'-glucoside (Q4'G) were unaffected by chopping of onions, whereas rutin content in asparagus decreased by 18.5% in 60 min. This decline was not accompanied by an increase in free quercetin, therefore the authors speculated that the hydrolysis of rutin was possibly followed by an immediate oxidative cleavage of quercetin [Makris & Rossiter, 2001]. Quercetin-3,4'-glucoside was rapidly degraded in macerated onion tissues with a 50% loss after 5 h resulting in the production of quercetin monoglycoside and aglycone [Price & Rhodes, 1997], which could be explained by the activity of onion glucosidases [Tsushida & Suzuki, 1996]. On the other hand the application of irradiation as the method for onion preservation resulted in a significant increase in both free and total quercetin concentra-

tions [Patil, 2004]. Shredding lettuce leaves with the subsequent exposure to light for 48 h resulted in dramatic flavonoid decline ranging from 6 to 94% depending on the variety [DuPont *et al.*, 2000].

The flavonoid content of tea depends on the type of tea and process it was subjected to. Teas can be divided into two groups: fermented tea (black and oolung) and non-fermented tea (green tea). Total flavonoid content of teas fluctuates at a similar level and significant differences refer to their qualitative composition. Green teas contain mainly catechingallates, gallocatechingallates, gallocatechins and catechins. Black teas include teaflavins and tearubigins produced as a result of oxidation and polymerisation of catechins, both proceeding during fermentation of green tea [Hara *et al.*, 1991]. Generally, fermentation processes applied in the food industry increase the level of flavonoid aglycones, since in their course flavonoid glycosides undergo enzymatic hydrolysis.

Of all processes involved in meal preparation, cooking is the one to be blamed the most for decreasing the content of flavonoids. Losses are caused by boiling water which very strongly washes out flavonoids. This unfavourable phenomenon may be minimized by consumption of decoction (soups) [Nemeth *et al.*, 2003]. The cooking process is often accompanied by partial thermal degradation, in total a decrease in flavonoid content of a product may reach 75%, and *ca.* 30–40% of these lost compounds pass to water. A similar phenomenon was observed during microwave cooking of onion in water when losses, most likely linked to washing out of compounds, accounted for 60% [Crozier *et al.*, 1997]. Moreover, the process of cooking was shown to be a diminishing factor of vegetables antioxidative activity [Agostini *et al.*, 2004]. On the contrary, microwave heating for 1 min without water added, resulted in a 1.5 fold increase in total quercetin content probably due to their better extractability [Ioku *et al.*, 2001].

The lowest losses have been reported to occur during frying, and in the case of onion they reach *ca.* 20%. The total flavonoid content of onion remained unaltered during frying with oil and butter for 40 min [Ioku *et al.*, 2001]; however, the composition pattern of quercetin derivatives after frying was not reported. Still, extensive elongation of the frying period resulted in diminished contents of quercetin glycosides. Technological processing increased the amount of quercetin in sweet cherries, whereas freezing and canning halved the flavonoid levels in processed foods compared to those in fresh products [Hertog *et al.*, 1992]. Cereal grains and oil plant seeds also contain compounds from the flavonoid group, however technological processes they are subjected to almost completely reduce the contents of these compounds.

NUTRITIONAL STUDIES ON FLAVONOIDS BIOAVAILABILITY

In the last decade, the number of investigations into bioavailability of flavonoids has increased considerably. Absorption of flavonoids has been explored in both the *in vivo* and *in vitro* systems [de Vries *et al.*, 2001; Murota *et al.*, 2002; Gee *et al.*, 1998; Manach *et al.*, 1998; Walgren *et al.*, 2000; O'Leary *et al.*, 2003]. Studies have been carried out on rats [Manach *et al.*, 1997; Piskula, 2000; Wiczkowski *et al.*, 2003], pigs [Cermak *et al.*, 2003; Ander *et al.*, 2000], and humans [Hollman *et al.*, 1997; Olthof *et al.*, 2000; Graefe *et al.*, 2001]. Flavonoids have originated from plants, their extracts, plant products with a high content of the compounds analysed, and pure substances in the crystalline form [Hollman *et al.*, 1996, 1999; Aziz *et al.*, 1998; Sesink *et al.*, 2001; Nielsen *et al.*, 1997]. Most often explored flavonoid was quercetin and its derivatives, the source of which were: fresh, cooked or fried onion, apples, beans, broccoli, berries, black-currant, and red wine. Investigations have also been carried out into the absorption of the catechin-group flavonoids [Manach *et al.*, 1999; Kuhnle *et al.*, 2000], originated from tea infusions and red wine. Bioavailability of isoflavones (daidzein, genistein and their glycosides) has also been determined [Andlauer *et al.*, 2000a, b; Piskula, 2000].

Gugler *et al.* [1975] observed low absorption (<1%) of quercetin aglycone after its oral administration to humans. Opposite results with the same compound in experiment on rats were obtained by Ueno *et al.* [1982] who observed 20% absorption of this flavonoid. Results confirming the absorption of quercetin and its glycosides were also reported by Hollman *et al.* [1995, 1996, 1997, 1999], Manach *et al.* [1997, 1998], McAnlis *et al.* [1999], Crespy *et al.* [1999], Olthof *et al.* [2000], Ander *et al.* [2000], and Cermak *et al.* [2003]. In addition, papers by Piskuła and Terao [1998], Manach *et al.* [1999], and Donovan *et al.* [2001] report on the availability of catechins, and these of Piskula [2000], Andlauer *et al.* [2000 a, b], and Setchell *et al.* [2001] – on the effective absorption of isoflavones.

Results of the first investigations into the bioavailability of flavonoids carried out on animals indicated that most of flavonoids present in food are not absorbed in the small intestine as they occur in a glycosidic form [Kuhnau, 1976]. The authors postulated, partly being right, that only flavonoids occurring in the free form may cross the barrier of biological membranes of the small intestine.

It has been long since the early research on flavonoid absorption. Ever since, a great progress in analytical methods has been observed and knowledge on the properties of bioactive compounds and physiology of living organisms has been greatly extended. New research methods have appeared and the existing ones have been improved. Nevertheless, the mechanisms of flavonoid absorption have not been fully elucidated.

In a study on volunteers, Hollman *et al.* [1995] compared the absorption of different forms of quercetin. Analyses indicated quercetin absorption at a level of 52% when it originated from fried onion where quercetin occurs as glucosides, at a level of 24% when pure quercetin was administered with a meal, and at a level of 17% after a meal supplemented with standard quercetin 3-O-β-rutinoside. The authors concluded that quercetin is readily absorbed in the small intestine, and its absorption improves when glucose is bound to a quercetin molecule. Contrary results were obtained by Wiczkowski *et al.* [2003] in comparing the bioavailability of quercetin from flesh scales and dry skins of onion. In the experiment on rats, all quercetin originated from dietary sources. The source of quercetin glycosides were fleshy scales of onion (0.7% of quercetin in a free form and 99.3% in the form of glycosides), and the source of quercetin aglycone were dry skins of onion (53.2% of quercetin in a free form and 46.8% in the form of glyco-

sides). The results obtained indicated that quercetin bioavailability from dry skins was significantly higher than from the flesh scales of onion, which – as suggested by the authors – may point to a better absorption of quercetin in a free form than its glucosidic derivatives. In other experiment, Hollman *et al.* [1997] reported that when 9 volunteers were administered with: an onion portion containing 225 μmol of quercetin (mainly in the form of quercetin 4'-O-β-glucoside and quercetin 3,4'-O-bis-β-glucoside), a portion of apples containing 325 μmol of quercetin (in the form of quercetin 3-O-β-galactoside, quercetin 3-O-β-rutinoside, quercetin 3-O-β-rhamnoside, quercetin 3-O-β-arabinoside, quercetin 3-O-β-xyloside, and quercetin 3-O-β-glucoside), and 331 μmol of quercetin 3-O-β-rutinoside in a crystalline form, the bioavailability of quercetin from apples and rutin constituted 30% of quercetin bioavailability from onion. The maximum quercetin concentration in blood plasma was noted 0.7 h after consumption of onion (c_{max}=0.74 μmol/L), 2.5 h after consumption of apples (c_{max}=0.30 μmol/L), and 9 h after consumption of rutin (c_{max}=0.30 μmol/L). On the basis of pharmacokinetic data obtained, a conclusion was drawn that quercetin glucosides are rapidly absorbed as early as in the small intestine, whereas quercetin-3-O-β-rutinoside and other non-glucosidic derivatives of quercetin present in apples are absorbed in the large intestine where, probably as a result of the activity of bacterial enzymes of local microflora, non-glucosidic residues are removed and quercetin is slowly absorbed. Investigations aimed at a comparison of the absorption of quercetin and rutin in rats were also carried out by Manach *et al.* [1997]. Their results indicated a substantially slower absorption of quercetin-3-O-β--rutinoside compared to quercetin aglycone. Similarly, the authors suggested that first rutin must be hydrolysed by the colonic bacteria, and then the released quercetin may be absorbed, whereas free quercetin administered to rats is absorbed in the first section of their small intestines. In other study Hollman *et al.* [1999] compared the bioavailability of quercetin-4'-O-β-glucoside and quercetin-3-O-β--rutinoside. The aim of this experiment was to determine the effect of the type of glucose bound to a quercetin molecule on its absorption rate. The experiment was conducted on 9 volunteers who were administered with 311 μmol of pure quercetin-4'-O-β-glucoside and 5 days later with 311 μmol of pure 3-O-β-rutinoside. Volunteers' blood plasma analysis indicated that the bioavailability of quercetin-4'-O-β-glucoside was fivefold higher than that of 3-O-β-rutinoside. The maximum quercetin concentration in blood plasma after the intake of glucoside (c_{max}=3.5 μmol/L) was observed after 0.5 h and was 20 times higher than after the intake of rutinoside (c_{max}=0.18 μmol/L). In the case of rutin, the maximum concentration was reported as late as 6 h after the intake. Similarly as in their previous papers [Hollman *et al.*, 1995, 1997] as well as that by Manach *et al.* [1997], the authors concluded that glucoside is absorbed in the small intestine, whereas rutinoside is not absorbed in the small intestine and passes to the large bowel where it is hydrolysed by enzymes produced by bacterial flora present therein.

The reports enumerated indicate that quercetin administered in the form of glucosides originated from onion was absorbed better than that administered in the glucosides originated from apples or that in the form of a crystalline substance added to meals. The fact of rapid absorption of quercetin from glucosides points to the effect of sugar molecules bounded on the absorption rate. Olthof *et al.* [2000] carried out a research to examine the effect of glucose residue position in a quercetin molecule on the rate of its absorption. In the experiment, two quercetin glucosides were used: quercetin-4'-O-β-glucoside and quercetin-3-O--β-glucoside. The result obtained did not indicate any differences in the bioavailability of the compounds examined.

The above-discussed results of studies on flavonoid bioavailability denote that onion is a very good source of quercetin. deVries *et al.* [2001] compared quercetin bioavailability from three different raw materials: fried onion, wine, and black tea. Twelve volunteers were given 750 mL of red wine, 50 g of fried onion, and 375 mL of black tea infusion, which corresponded to 14–16 mg of quercetin. The results obtained revealed that quercetin present in red wine, black tea and onion is absorbed. The authors claim, however, that red wine and tea, after consumption of which quercetin concentration in blood plasma of volunteers reached c=26 nmol/L, are a poor sources of quercetin compared to onion (c=53 nmol/L).

ABSORPTION AND METABOLISM OF DIETARY FLAVONOIDS

Epidemiological studies reveal a positive impact of a flavonoid-rich diet on the prevention of numerous diseases [Keli *et al.*, 1996; Knekt *et al.*, 1996; Hertog *et al.*, 1995, 1993a, b]. Consumption of food rich in flavonoids does not imply flavonoids good bioavailability and in turn their consumer protective function, although their direct beneficial activity in the alimentary tract should not be forgotten. Factors determining flavonoid absorption that could be described as "structural" include the presence or a lack of glycosylation of the basic flavonoid structure, site of glycosylation, and the number and type of sugar molecules bound. On the other hand, physiological factors that should be considered while exploring absorption processes include: pH of the alimentary tract, character of a carrier, stomach contents, intestinal peristalsis, blood and lymph flow, and pathological changes.

Bioavailability of a compound is defined as a part of a dose that may absorbed after administration. In the case of food, an analysis of bioavailability enables quantitative determination of organism exposition to the substance consumed with a diet. Releasing, absorption, distribution, metabolism and excretion are physiological processes which determine the bioavailability of a given substance. Those processes ensue in a large time interval and usually proceed simultaneously [Rice-Evans, 2000].

The gastrointestinal system includes oral cavity, oesophagus, stomach, small and large intestines, digestive glands (salivary glands, pancreas and liver). In particular sections of the alimentary tract, flavonoids consumed with food undergo the same processes as the whole ingested food. In the first section, the oral cavity, food is mechanically disintegrated. An appropriate treatment of a bite is of significant importance to enzyme availability for nutrients in consecutive sections of the alimentary tract. As it was mentioned earlier, flavonoids occur usually in the outer, the oldest and very often partially-lignified tissues. Proper disintegration of food in the oral cavity may affect further release of

flavonoids to digestive fluids. A research by Piskula and Terao [1998] indicated solubility to be one of the parameters limiting quercetin absorption. When food is being disintegrated, ingesta mix with saliva and saccharides are partly digested by ptyalin present in the saliva. So far, there are no explicit reports that would indicate the oral cavity as the site of flavonoid transformation.

Flavonoids present in food demonstrate different chemical structures [Herrmann, 1976, 1988; Iwashina, 2000]. The presence or a lack of glucose, methyl or acyl substituents in a flavonoid molecule is an essential parameter determining their absorption and consequently indicating directions of their metabolism [Scalbert & Williamson, 2000].

The next section of the gastrointestinal tract is stomach. The most typical trait of gastric juice is a high concentration of H^+ ions, pH of gastric juice accounts for *ca.* 1.0. Flavonoids reaching the stomach are resistant to the activity of gastric juice and enzymes present therein. Investigations of Gee *et al.* [1998] demonstrated the resistance of structures of quercetin-3-O-β-rutinoside and quercetin-3-O-β-glucoside on pH value approaching that of the stomach. Absorption surface area of stomach is small compared to the intestinal section of the alimentary tract. The total surface area of mucous membrane of a human stomach makes *ca.* 0.8 m^2, whereas the absorption surface area of the small intestine is several hundred times larger and ranges from 200 to 300 m^2. According to Piskuła *et al.* [1999], daidzein and genistein aglycones, but not their glucosides, are absorbed in a rat's stomach. The same conclusions were drawn by Crespy *et al.* [2002] who compared gastric absorption of quercetin and its two glycosides: 3-O-β-rutinoside and 3-O-β-glucoside, in rats.

Further digestion of dietary ingredients and absorption of the end-products of digestion are the major functions of the small intestine. Digestion may proceed in the intestinal lumen and on the surface of mucous membrane where the end-products are being absorbed. Processes of intestinal transport can be divided into two main types: passive and active. In intestinal absorption, a key role is played by simple diffusion. It applies to low-molecular-weight substances which penetrate through pores with water and to high-molecular-weight substances soluble in a lipid layer of the membrane. The other products of digestion are absorbed by means of active transport, which requires energy input and the presence of specific carriers.

Flavonoid aglycones are characterised by hydrophobic properties and can penetrate to enterocytes through biological membranes as a result of passive transport. The presence of a glycoside substitution in a flavonoid molecule increases flavonoid's hydrophilicity, thus limiting the possibility of its absorption as a result of diffusion. Two mechanisms are postulated that enable passing the intestinal barrier by flavonoid glycosides present in the intestine. According to the first mechanism, glycosides may be hydrolysed in the small intestine lumen by β-glycosidases present therein, for instance phlorizin lactase (LPH, EC 3.2.1.23 and 62), which is capable of hydrolysing flavonoid glucosides, and aglycone produced may be absorbed by an enterocyte [Day *et al.*, 2000]. What is more, papers by Day *et al.* [2000, 2003] and Sesink *et al.* [2003] indicate that LPH-mediated hydrolysis is the main pathway enabling the absorption of quercetin-3-glucoside. The second mechanism postulated assumes the possibility of transferring intact flavonoid glycosides through intestinal cell wall by means of an SGLT1 carrier being a glucose transporter dependent on Na^+ concentration gradient [Gee *et al.*, 1998, 2000]. Reports published suggest that the transport of quercetin-4'-glucoside [Walgren *et al.*, 2000; Day *et al.*, 2003] and quercetin-3-glucoside [Gee *et al.*, 1998, 2000] follows the mechanism described. Next, the absorbed glycosides are hydrolysed by β-glycosidases present in cytosole of small intestine mucosa cells. Investigations of Day *et al.* [1998] indicate that these β-glycosidases hydrolyse quercetin-4'-glucoside, daidzein-7--glucoside, and genistein-7-glucoside.

Phenolic compounds which were not absorbed in the upper part of the gastrointestinal tract and these which underwent detoxification process in liver and were returned to the small intestine with bile reach the large intestine where they are modified by bacterial enzymes [Formica & Regelson, 1995; Justesen *et al.*, 2000; Rechner *et al.*, 2002, 2004] and alkaline medium. They may be hydrolysed by β-glucosidases, α-rhamnosidases and β-galactosidases produced by bacteria colonising the colon [Rechner *et al.*, 2004], and the produced free flavonoids may be absorbed by mucous membrane of the large intestine [Manach *et al.*, 1998; Hollman *et al.*, 1995] or be metabolised by bacterial microflora to low-molecular-weight derivatives [Rechner *et al.*, 2002, 2004]. As a result of this metabolism, the A ring detaches from a flavonoid molecule and a heterocyclic C ring opens, which leads to the formation of a number of low-molecular-weight phenolic compounds [Aura *et al.*, 2002]. This process is accompanied by the production of phenylacetic and phenylpropionic acids and their derivatives [Scalbert & Williamson, 2000].

Some researchers assume that deglycosilation is the first stage of flavonoid metabolism. The successive stages of metabolism of hydrolysed compounds include: glucuronisation, methylation, sulfation, and hydroxylation. It seems that liver, intestine mucous membranes and kidneys are organs that serve the key role in these transformations. In cells of intestinal epithelium proceeds quercetin conjugation with glucuronic acid mediated by UDP-glucuronic transferase (UGT, EC 2.4.1.17) [Manach *et al.*, 1998; Piskula *et al.*, 1998a]. This transformation results in the production of quercetin-3-glucuronide and quercetin-7-glucuronide [Gee *et al.*, 2000]. Moreover, catechol-O-methyltransferase (COMT, EC 2.1.1.6) present in cells of rat small intestine runs methylation of a hydroxyl group of the quercetin C ring catechol moiety [Manach *et al.*, 1998]. After being absorbed in intestines, flavonoids are bound with blood albumin and transported *via* portal vein to liver [Manach *et al.*, 1996]. In liver, flavonoids and their metabolites are subject to further modifications, which leads to the formation of quercetin-3-, 7-, 4'-, 3'-glucuronide [Day *et al.*, 2000, 2001; Mullen *et al.*, 2002; Manach *et al.*, 1996], and 3'- and 4'-O-methylated derivatives [Manach *et al.*, 1997]. In cytosole of liver cells there appear also enzymes from a group of sulfotransferases (PST, EC 2.8.2.1) which catalyse conjugation of quercetin and its conjugates with sulfuric acid [Manach *et al.*, 1996]. Enzymes participating in the metabolism of flavonoids may modify several hydroxyl groups simultaneously [Day *et al.*, 2000]. Metabolites produced in liver are excreted to bile and with bile they return to the small intestine and are reabsorbed, thus forming flavonoids enterohepatic circulation.

FLAVONOIDS AS ANTIOXIDANTS *IN VITRO*

Damages of biological systems caused by reactive oxygen species belong to processes directly linked with development of cardiovascular and malignant diseases. Human organism possesses systems controlling oxidation processes posing a threat to structures and functions of cells. Three defence mechanisms has been developed [Bartosz, 1995], including: prevention of reactions of reactive oxygen species with biologically-significant compounds, breaking free-radical chain reactions and undesirable non-radical oxidation reactions, scavenging the products of free radicals reactions with biological substances and repair of damages. An important function in these processes play antioxidant enzymes (superoxide dismutase E.C.1.15.1.1, catalase E.C.1.11.1.6, glutathione peroxidase E.C. 1.11.1.9) and low-molecular-weight antioxidants (gluthatione, uric acid, vitamins C and E, carotenoids, and flavonoids).

In vitro investigations have demonstrated that the antioxidant properties of flavonoids are linked with their capability for scavenging free radicals, chelating metals and inhibiting the activity of oxidases [Terao & Piskula, 1998; Ioku *et al.*, 1995; Terao *et al.*, 1994; Bors *et al.*, 1990; Chen *et al.*, 1990; Afanas'ev *et al.*, 1989]. Flavonoids' activity as antioxidants refers to their ability to transfer a hydrogen atom or an electron and to the possibility of their interactions with other antioxidants [Rice-Evans, 2000]. Quercetin may serve as a hydrogen donor for α-tocopherol radical, thus regenerating α-tocopherol – a key element of redox balance in biosystems. The quercetin radical formed may be reduced by vitamin C which is converted into ascorbyl radical [Frankel *et al.*, 1993; Bors *et al.*, 1990]. A significant property of quercetin is its capability for blocking the oxidative activity of systems with transition metal ions (Cu^{+}/Cu^{2+}, Fe^{2+}/Fe^{3+}) that play an essential role in the formation of reactive oxygen species in Fenton's reactions. The antioxidant properties of flavonoids result from their chemical structure: 3',4'-o-dihydroxyl (catechol) system in the B ring, reciprocal configuration of the double bond C2-C3 and the 4-carbonyl group of the C ring, and configuration of the 3-hydroxyl group and the double bond C2-C3 of the C ring with the 5-hydroxyl group of the A ring [Bors *et al.*, 1990; Cos *et al.*, 1998, Manach *et al.*, 1996; Terao & Piskula, 1998] (Figure 3). All the mentioned structural conditions may be found in a quercetin molecule which, in the *in vitro* systems efficiently scavenges hydroxyl radical ($OH^{\bullet}$), superoxide radical ($LOO^{\bullet}$), superoxide anion radical ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), and nitrogen oxide ($NO^{\bullet}$). Results of some assays indicate that by scavenging free radicals or blocking transition metal ions quercetin efficiently inhibits lipids oxidation, especially of low density lipoproteins (LDL) [Terao & Piskula, 1998]. In living cells there occur two media with different physical properties, in which free-radical processes proceed: (1) liquid phase consisting of cytoplasm and medium inside cellular organelles, and (2) hydrophobic phase which includes cellular lipid membranes. Hydrophilic compounds (glutathione, vitamin C) accumulate in the liquid phase, whereas hydrophobic compounds (vitamin E, carotenoids) – in the lipid phase. Compounds with intermediate polarity accumulate at the border of both phases. As it results from investigations of Ioku *et al.* [1995] and Terao *et al.* [1994], flavonoid aglycones appear to be such interphase antioxidants.

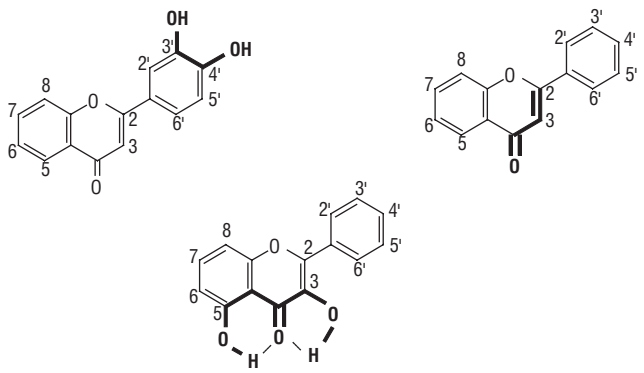

FIGURE 3. Structural elements of flavonoids determining their antioxidant properties.

In food of plant origin, flavonoids occur mainly in the glycosidic form (except for catechins appearing as aglycones). *In vitro* explorations have implied that quercetin aglycone demonstrates stronger antioxidant properties compared to its glycosides [Ioku *et al.* 1995; Afanas'ev *et al.*, 1989]. In addition, the antioxidant potential of quercetin glycosides is determined by the site where glucose molecule is attached. Results of an experiment by Ioku *et al.* [1995] have indicated that Q4'G and Q3G reveal weak antioxidant properties, still stronger in the case of Q3G. Such a differentiation is likely to be due to the blocking of catechol structure in the C-4' position, being of key importance to the scavenging of free radicals.

When knowledge on flavonoid metabolism has been extended, a new search has begun to answer the question: whether antioxidant properties of flavonoids demonstrated *in vitro* are at least in part maintained *in vivo*, *i.e.* since after absorption from the alimentary tract flavonoids occur mainly in the form of polar glucuronic, sulfate and methyl derivatives [Manach *et al.*, 1996; Day *et al.*, 2001; Terao, 1999]. Conjugates formed during detoxification processes possess partly-blocked free hydroxyl groups responsible for antioxidant activities of flavonoids. It should be added that increasing polarity of these compounds results in a shift of their antioxidant activity potential in biological systems towards the liquid phase. Investigations carried out by da Silva *et al.* [1998], who initiated oxidation of rat blood plasma upon flavonoid administration, have indicated an increase in plasma resistance to oxidation expressed by a slower increment of hydroperoxides of plasma cholesterol esters and by a slower loss of plasma α-tocopherol compared to control blood plasma. The results obtained imply that absorbed flavonoids strengthen the antioxidative defence system of plasma and that their metabolites can still scavenge free radicals and chelate metal ions [Terao, 1999]. On the other hand, Day *et al.* [2000] postulated that flavonoid metabolites may be indirect antioxidants due to their capability for inhibiting oxidative enzymes. The authors demonstrated that quercetin glucuronides are capable of inhibiting the activity of xanthine oxidase and lipoxygenase, still the inhibition degree depends on the position of glucuronide groups in a conjugate. Despite the fact that in general blood circulation flavonoids appear in conjugated forms only, one cannot exclude the release of their aglycone. Deglucuronidation of flavonoids can occur during inflammation. β-Glucuronidase released from stimulated neutrophils or certain injured cells can hydrolyse luteolin monoglu-

curonide to free luteolin possessing anti-inflammatory activities [Shimoi *et al.*, 2000, 2001].

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FINAL REPORT

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

Title of the individual project:
Elaboration of the *in vitro* evaluation method of availability of bioactive good compounds on the example of flavonoids.

Institution:
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Key words:
Flavonoids, humans, intake, LADME, conjugates, bioavailability, processing, antioxidants.

SYNTHESIS OF RESULTS

Usually, estimation of bioavailability is performed/done in *in vivo* studies on animals or humans which require substantial financial resources and very often are triggering protests from organisations protecting animal rights. Therefore, the objective of this project was an attempt to elaborate new approach in bioavailability estimation, bioavailability *in vitro*.

Each compound before absorption have to be solubilized in gastric or intestinal fluids. The speed and extent of its appearance in general blood circulation depends on its release from the diet. It may happen that despite a comparable content of the bioactive compound in similar food products the impact of food matrix or the form of the compound (in the case of quercetin, its aglycone or glucoside) can strongly influence its release and subsequent absorption. Therefore bioavailability *in vitro* could become a new indicator of food quality which could be determined in a model system and after placing on a label of a food product could inform the consumer on which part of bioactive/beneficial component present in the product could be absorbed by the consumer organism.

Conducted research was focused on quercetin, a bioactive compound from the group of flavonoids abundant in the diet to which a number of positive effects are attributed as indicated by epidemiological studies. Since in the Polish diet the richest dietary source of quercetin are onions, they were the basic material in the performed study.

For determination of all forms of quercetin in plant material HPLC-UV-MS method was developed and optimized with the application of advanced chemometric methods. Next, for quantitative calculations, non-commercially available standard compounds of quercetin (Q) glucosides (G), (Q3,4'G and Q4'G) were isolated from lyophilized onion on Sephadex LH-20 column, purified on HPLC semi-preparative RP18 column. The identity of the obtained standards was confirmed by checking the UV spectrum and mass of molecular ion on HPLC-MS.

The idea of a project was to model parameters of *in vitro* method in a way that bioavailability estimated *in vitro* would be in the best way correlated with *in vivo* results. Therefore, experiments *in vivo* were carried out first as these results were the bases for modelling.

Before nutritional experiments *in vivo*, standard procedure for determination of absorbed quercetin in plasma was introduced. Metabolites, after enzymatic release from conjugated forms (glucuronides/sulfates/methylated) were extracted from plasma and measured with HPLC method with electrochemical detection. Determination of *in vivo* bioavailability of quercetin was done on rats and humans with the approval of respective ethical committees, Local Ethical Committee for Experiments on Animals and Bioethics Committee of Warmia-Mazury Chamber of Physicians.

The overall aim of this study was to compare: first, bioavailability of quercetin aglycone *vs*. its β-glucosides; second, to compare the impact of different food matrixes, *i.e.* raw onion and cooked onion on quercetin bioavailability. Earlier published papers on similar a subject reported better bioavailability of β-glucosides. However, in those studies quercetin was offered in capsules as a pure crystalline substance which is very poorly soluble in water while the source of quercetin β-glucosides was onion. It means that bioavailability of quercetin and its glucosides was compared from two different sources. From the nutritional point of view, the application of only dietary sources for this comparison would be more relevant.

Analysis of quercetin distribution within the onion bulb showed that the richest in quercetin is the dry skin of onion where the predominant from of quercetin is aglycone. Moreover, it showed also that after peeling most of quercetin present in onion bulb is concentrated in the first 2–3 outer scales (rings) and contrary to onion dry skin, quercetin is present almost exclusively in the form of β-glucosides. This very high content of quercetin in the outer parts of onion bulb make them very useful in nutritional

experiments, first onion outer fleshy scales as the source of quercetin glucosides and onion dry peels as the source of quercetin aglycone.

Comparison of bioavailability of quercetin aglycone *vs*. its β-glucosides from the dietary sources was done on rats. Animals were orally administrated with three doses of onion preparations delivering 0.5 mg, 2 mg or 7 mg of quercetin per kg of animal body weight (the does expressed as quercetin aglycones). Similar experiment was carried out on humans. The group of nine healthy volunteers (4 men an 5 women) was offered shallot dry skin and fresh shallot providing 1.37 mg of quercetin per kg of body weight.

To eliminate the impact of food matrix on quercetin absorption in experiment with rats, onion dry skin and onion fleshy scales were freeze-dried and powdered and these quercetin rich onion preparations were homogenized in water prior to administration by direct stomach intubation to animals. In the experiment on humans, immediately before consumption fresh shallot was homogenised and offered with water as shallot dry skin was. From the absorption profiles describing changes of blood plasma quercetin concentration in the function of time basic pharmacokinetic parameters were calculated: elimination constant (Ke), absorption constant (Ka), the area under the plasma concentration-time curve Area Under the plasma concentration-time Curve ($A.U.C._{(t=0-24)}$) along with residual area extended to infinity ($A.U.C._{t=24-\infty}$), biological half life ($T_{1/2}$), the time to reach the peak plasma level (T_{max}) and the quercetin peak plasma level (C_{max}). Taking into consideration the A.U.C. value which is the bioavailability marker, quercetin from onion dry peels was better bioavailable than from fleshy scales, and observed differences were statistically significant ($p \leq 0.05$). Assuming that the influence of food matrix was reduced, the obtained results did not confirmed the previous reports and indicate that quercetin aglycone is better bioavailable than quercetin glucosides.

In human nutrition onion is consumed raw or cooked, and process of cooking is changing the food matrix. During cooking of onion in water, a significant part of onion material, including quercetin glucosides, is dissolving and transferred into the cooking water. Mass and quercetin transfer to cooking water was estimated to be 54% and 59%, respectively. Since dissolution of compound is prerequisite for its absorption, process of onion cooking may influence quercetin bioavailability. Therefore, the second target of the nutritional part of the project was to check the impact of food matrix on quercetin bioavailability.

Again, the group of nine healthy volunteers (4 men an 5 women), in the cross over study, was offered cooked onion with cooking water (soup) or raw onion with the water in the amount as used for cooking each time delivering the dose of 1.37 mg of quercetin per kg of body weight and pharmacokinetic parameters of quercetin were calculated. It was observed that quercetin was absorbed much faster from cooked onion, which is most likely due to disruption of the onion structure and washing out of quercetin glucosides into the cooking water, whereas the release of quercetin from raw onion was limited by digestion process. While both preparations gave different absorption profiles, *i.e.* T_{max} and C_{max} were significantly different, their A.U.C. values did not significantly differ, which suggests that bioavailability of quercetin from cooked and raw onion was the same.

Because of methodological aspects and existing standard procedures in pharmaceutical sciences for measurement of active substances realise from drugs, in a part of project focused on estimating *in vitro* bioavailability of quercetin from food, similar approach was applied. After setting up of initial conditions, like solubility of quercetin in different media, for determination of quercetin release from food matrixes different environments were checked: water, phosphate buffer pH 6.8, 0.1 N hydrochloric acid and artificial gastric juice.

Examinations of release rate of quercetin and its derivatives from food matrix were carried out on lyophilisate of and edible part and dry skin of onion, the same which were used in feeding experiments on rats. Measurements were performed with an apparatus whose construction was based on a USP2 shaft apparatus, applied as a standard in the studies into the release of therapeutic substances from drugs, thermostated on a water bath at a temperature of 37°C. Hydrodynamic properties of the reaction medium were modelled with the use of an electronic stirrer. Finally, to maximally imitate the physiological conditions, the release measurements were conducted in the medium of artificial gastric juice. Based on experimentally-plotted curves concentration-time (C – t), describing the extent and rate of the releasing process of the flavonoids from food matrix, for each analysis basic pharmacological parameters, including A.U.C. for concentration *vs*. time, and AUC for its derivative (dC/dt), were calculated with the use of Kinetica™, ver. 4.3 software, InnaPhase Corporation, USA.

An analysis of data obtained in the *in vitro* experiment indicated a strong correlation between flavonoid dose and A.U.C. value determined under the C-t curve as well as the value of the area under the dC/dt-t curve. A similar strong correlation between a dose and AUC size was observed in the case of *in vivo* results.

Next, a search was begun for a correlation between bioavailability parameters determined *in vivo* (in animals), and parameters of flavonoid release obtained in the *in vitro* experiment (STATISTICA, ver. 6 StatSoft, Inc.). Analyses of data obtained *in vitro* with pharmacological and chemometric methods pointed to a clear correlation between the parameters obtained with *in vitro* and *in vivo* methods. The research indicated that while determining the bioavailability of some food components, experiments on animals could be reduced and perhaps substituted in the future, and that the bioavailability of bioactive compounds determined *in vitro* may constitute a new quality attribute for functional food.