Flavonoid C-Glycosides in Diets

F. Bucar, J. B. Xiao, and S. Ochensberger

Contents

F. Bucar (✉) · S. Ochensberger
Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Graz, Graz, Austria
e-mail: franz.bucar@uni-graz.at; sandra.ochensberger@uni-graz.at

J. B. Xiao
State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Taipa, Macao
e-mail: jianboxiao@yahoo.com

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Abstract

Flavonoids are one of the most widely occurring secondary plant constituents and are rich in vegetable and fruit diets as well as beverages of plant origin. In flavonoid glycosides, the sugars can either be linked to the aglycone via an ether bond (*O*-glycosides) or via a C–C- bond resulting in flavonoid C-glycosides. Their occurrence in food plants, their role in bioactivity of food, and their catabolism are covered in this chapter. The major class of C-glycosylflavonoids in food plants is represented by flavones, in addition dihydrochalcones and C-glycosylisoflavones can be found. Citrus fruits can be considered as a major source of C-glycosylflavones, whereas in most cases relatively low amounts have been found in cereals. A rich source of C-glycosylated dihydrochalcones are tomatoes, as well as rooibos and honeybush herbal teas, and the most common C-glycosylisoflavone puerarin is mainly consumed via kudzu roots. Due to their higher chemical stability in terms of hydrolysis during cooking and also after ingestion, they can be considered as a specific group within flavonoids. Their metabolic fate is clearly different from *O*-glycosidic flavonoids with absorption of intact glycosides, followed by phase II metabolization. However, also deglycosylation by gut microbiota and degradation of aglycones to compounds like (hydroxy)phenylpropionic acids have been recognized. Only limited data on the actual daily intake of C-glycosylflavonoids including information on content in fresh and processed food are available.

Keywords

C-Glycosylflavonoids · Flavonoid C-glycosides · Citrus fruit flavonoids · Cereal flavonoids · Flavonoid metabolisms · Flavonoid bioactivities · Flavones · Dihydrochalcones · Isoflavonoids

Abbreviations

ABTS	2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt
CGF	C-glycosylflavonoids
CGT	C-glycosyltransferase
COX	Cyclooxygenase
CYP	Cytochrome P450 enzymes
DPPH	2,2-diphenyl-1-picrylhydrazyl
ESI	Electrospray ionization
IC_{50}	Half maximal inhibitory concentration
IL	Interleukin
MCF	Human breast cancer cell line
MIC	Minimal inhibitory concentration

mRNA	Messenger RNA
OGF	*O*-glycosylflavonoids
PGE-2	Prostaglandin E2
P-gp	P-glycoprotein
PXR	Pregnane-X- receptor
RAW	Mouse macrophage cell line
TE	Trolox equivalents
TNF-α	Tumor necrosis factor α
Vero	African green monkey kidney cells

1 Introduction

Flavonoids are one of the most widely occurring secondary plant constituents and are rich in vegetable and fruit diets as well as beverages of plant origin. In plant food, flavonoids mostly occur as glycosides, i.e., bound to one or more monosaccharide moieties. Major structural diversity of flavonoids arises from hydroxylation, methoxylation, and glycosidation. The sugars can be attached to the aglycone via an ether bond (*O*-glycosides) or being directly linked to the aglycone via a C–C bond resulting in flavonoid C-glycosides (C-glycosylflavonoids, CGF). This type of glycosidation results in distinct features compared to *O*-glycosides concerning their resistance to hydrolysis. Hence, C-glycosylflavonoids represent a specific group of plant phenolics which is often underestimated. Their occurrence in food plants, their role in bioactivity of food, and their catabolism will be covered in this chapter.

2 Bioactive Constituents

2.1 Chemistry of Flavonoids with Emphasis on C-Glycosylflavonoids

Chemically, their basic structure can be designated as 2-phenylbenzopyrane which is deriving major flavonoid classes, i.e., flavones, flavonols, flavanones, flavanols (which are related to proanthocyanidins), chalcones including dihydrochalcones and α- and β-OH-dihydrochalcones, and anthocyanidins. Migration of the aromatic ring from C-2 to C-3 position of the basic skeleton leads to isoflavonoids. The nomenclature of flavonoid classes follows the oxidation pattern of the C-ring. Figure 1 illustrates the nomenclature of flavonoids and shows the basic structures of most important plant flavonoid classes.

In C-glycosylflavonoids, glucose is the predominant sugar moiety, although also xylose, arabinose, rhamnose, galactose, and apiose were identified as glycosidic moieties. Additionally, C-glycosylflavonoids can be acylated with acetyl, p-coumaroyl, sinapoyl, feruloyl, and/or vanilloyl residues. By far the majority of C-glycosylflavonoids in plant derived food can be found among flavones, more rarely

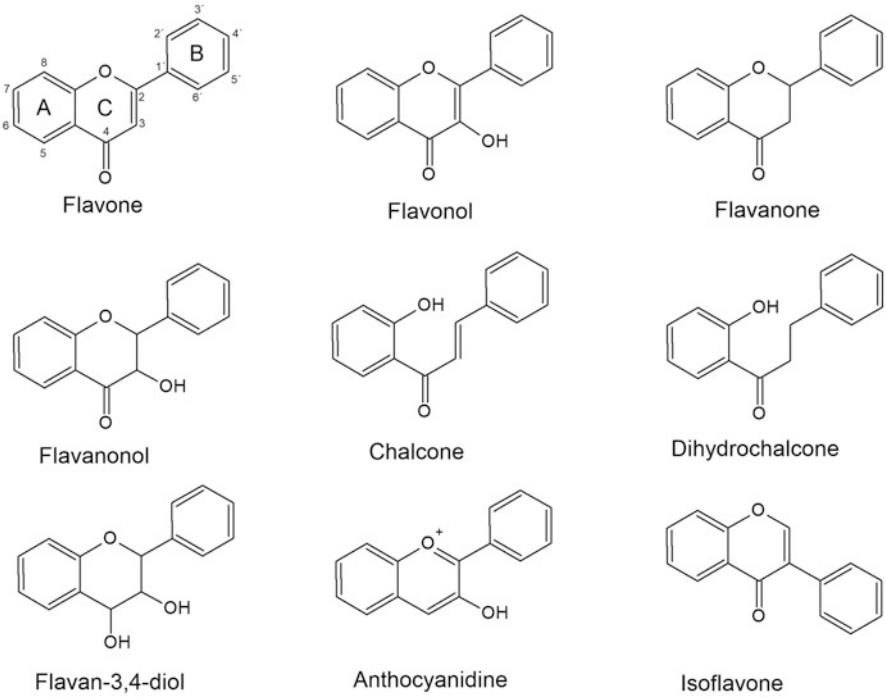


Fig. 1 Major flavonoid classes and common numbering of flavonoids

in flavanols, flavonols, flavanones, dihydrochalcones, isoflavones, and isoflavanones. In C-glycosylflavones, glycosylation can occur either as mono-C-glycosylflavones (positions C-6- or C-8-), di-C-glycosylflavones (positions C-6,8-, 3,6-, 3,8-), as mixed C-/*O*-glycosides, as well as acylated glycosides, resulting in a remarkable complexity of C-glycosyl patterns. In addition, the interconversion of 6-C- and 8-C-glycosylflavones via Wessely-Moser rearrangement which can already occur during boiling has to be considered (Courts and Williamson 2015).

2.1.1 Analysis of C-Glycosylflavonoids

Analysis of CGF in food sources is above all accomplished by HPLC coupled to photodiode array detection and mass spectrometry usually applying electrospray ionization (ESI). The C-glycosidic linkage obviously has higher stability in mass spectrometric fragmentation processes leading in the first step not to the aglycone as in case of *O*-glycosylflavonoids by cleavage of the hemi-acetal C–O bonds, but to fragmentation of the sugar moiety when medium fragmentation energy is applied.

Intraglycosidic cleavages (Fig. 2) and losses of water can be observed. For instance, as outlined by Vukics and Guttman (2009), in product ion spectra of protonated vitexin mainly losses of 1–3 water molecules as well as $^{0,2}X^{+}$, $^{0,2}X^{+}-H_2O$, $^{0,4}X^{+}-2\ H_2O$, and $^{2,3}X^{+}-2\ H_2O$ fragments could be found, whereas in negative ion mode $^{0,2}X^{-}$ was the major fragment.

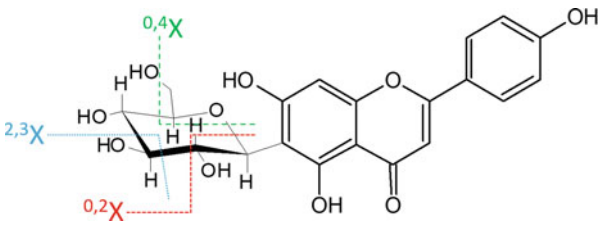


Fig. 2 Most frequently occurring intraglycosidic cleavages observed in mass spectral analysis of C-glycosyl flavones. ^{a,b}X: a, b indicate the broken sugar bonds; X indicates fragments where the charge is kept on the aglycone

Significant simultaneous losses of −90 and −120 mass units in case of hexoses and −60 and −90 u in case of pentoses can be used by applying neutral loss scans to detect CGF in complex food matrix. Also, in case of di-C-glycosyl flavones with different monosaccharides attached to the aglycone LC-ESI-MS with stepwise fragmentation will help in identification. Further, it has been shown that due to hydrogen bonding in case of a 6,8-di-C-glycosylflavone, the loss of a fragment −60 u if the pentose is attached at position C-6 is higher compared to a pentose attached to position C-8. However, it has to be considered that relative abundances of fragments depend on type of instrumentation and sound conclusions can only be drawn when spectra of C-6- and C-8-pentosylglycosides can be compared under identical instrumental settings (Waridel et al. 2001; Ferreres et al. 2003; Vukics and Guttman 2009).

2.1.2 Biosynthesis of C-Glycosylflavones

The biosynthetic pathway of C-glucosylflavones in cereals has been studied in detail by Brazier-Hicks et al. (2009). The flavanone precursor is converted to a 2-hydroxyflavanone and its corresponding open-chain form. A C-glucosyltransferase (CGT) forms 2-hydroxyflavanone C-glucosides by attaching a glucose moiety either at position C-6 or C-8. Through dehydration the flavone-6-C-glucoside and flavone-8-C-glucoside is finally formed, respectively. Most of the CGF identified so far in plants are members of the UGT708 subfamily which are able to C-glycosylate 2,4,6-trihydroxyacetophenone-like structures (e.g., 2-hydroxyflavanones). In a number of food plants, such CGF were found, such as rize, maize, buckwheat, citrus fruits, kudzu, and soy bean. However, also direct formation of 6-C-glycosylflavones or isoflavones by CGTs belonging to different UGT (UDP glycosyltransferase) subfamilies, i.e., UGT84 and UGT71, has been described for gentian (*Gentiana triflora*), kudzu and wasabi (*Eutrema japonicum*) (Mashima et al. 2019).

Table 1 Food sources of C-glycosylflavonoids

Food source	Plant	Flavonoid class	C-Glycosylflavonoids	References
Fruits and seeds				
Cereals				
Wheat	*Triticum sps.*	C-Glycosylflavones	Apigenin-6-C-, 8-C-, 6,8-di-C-glycosides (including acylated derivatives)	(Courts and Williamson 2015; Wijaya and Mares 2012)
Barley	*Hordeum vulgare*	C-Glycosylflavones	Apigenin-6,8-di-C-glycosides	(Courts and Williamson 2015; Xiao et al. 2016)
Oat	*Avena sativa*	C-Glycosylflavones	Luteolin-6-C-, and 6,8-di-C-glycosides	(Popovici and Weissenboeck 1976)
Rye	*Secale cereale*	C-Glycosylflavones	Apigenin-6,8-di-C-glycosides	(Courts and Williamson 2015)
Spelt	*Triticum aestivum* subsp. *spelta*	C-Glycosylflavones	Apigenin-6,8-di-C-glycosides	(Hostetler et al. 2017)
Buckwheat	*Fagopyrum esculentum*	C-Glycosylflavones	Apigenin- and luteolin-6- and 8-C-glycosides	(Brazier-Hicks et al. 2009; Courts and Williamson 2015; Hostetler et al. 2017)
Rice plant	*Oryza sativa*	C-Glycosylflavones	Apigenin-6-C- and 6,8-di-C-glycosides, chrysoeriol-6-C-glycosides (including acylated derivatives), luteolin-6,8-di-C-glycosides	(Courts and Williamson 2015; Xiao et al. 2016; Hostetler et al. 2017)
Rice fruit	*Oryza sativa*	C-Glycosylflavones	Apigenin- and luteolin-6,8-di-C-glycosides	(Pereira-Caro et al. 2013; Hostetler et al. 2017)
Maize	*Zea mays*	C-Glycosylflavones	Maysin; maysin analogues; derhamnosyl-maysin	(Elliger et al. 1980; Xiao et al. 2016)
Millet	*Sorghum bicolor*	C-Glycosylflavones	Apigenin-6-C-and 8-C-glycosides; luteolin-8-C-glycosides	(Courts and Williamson 2015)
Pearl millet	*Pennisetum glaucum (syn.*	C-Glycosylflavones	Apigenin- and luteolin-8-C-glycosides	(Boncompagni et al. 2018; Courts and Williamson 2015)

	Pennisetum americanum)			
Citrus fruits				
Bergamot	*Citrus bergamia*	C-Glycosylflavones	Apigenin-6,8-di-C-glucoside, diosmetin-6,8-di-C-glucoside	(Caristi et al. 2005; Abad-Garcia et al. 2012)
Grapefruits	*Citrus paradisi*	C-Glycosylflavones	Apigenin-6-C- and 6,8-di-C-glycosides	(Abad-Garcia et al. 2012; Wang et al. 2017)
Kumquats	*Fortunella japonica*	C-Glycosylflavones, C-glycosyl-dihydrochalcones	Acacetin-6-C- and 8-C-glycosides, apigenin-8-C-neohesperidoside, phloretin-3′,5′-di-C-glucoside	(Lou et al. 2016; Xiao et al. 2016)
Lemons	*Citrus limon*	C-Glycosylflavones	Apigenin-6-C- and 6,8-di-C-glycosides, chrysoeriol-6,8-di-C-glycosides, diosmetin-6-C-, 8-C- and 6,8-di-C-glycosides	(Abad-Garcia et al. 2012; Courts and Williamson 2015; Wang et al. 2017)
Limes	*Citrus aurantiifolia*	C-Glycosylflavones	Diosmetin-6-C- and 6,8-di-C-glucoside	(Caristi et al. 2005; Courts and Williamson 2015)
Mandarins, tangerines	*Citrus reticulata*	C-Glycosylflavones	Apigenin-6,8-di-C-glucoside, diosmetin-6,8-di-C-glucoside	(Hostetler et al. 2017)
Oranges (sweet oranges, blood oranges)	*Citrus sinensis*	C-Glycosylflavones	Apigenin-6-C-,8-C- and 6,8-di-C-glycosides, chrysoeriol-8-C-glucoside, chrysoeriol-6,8-di-C-glucoside (stellarin-2), diosmetin-6,8-di-C-glucoside	(Caristi et al. 2005; Abad-Garcia et al. 2012; Courts and Williamson 2015; Barreca et al. 2016; Hostetler et al. 2017; Wang et al. 2017)
Oranges (sour oranges)	*Citrus aurantium*	C-Glycosylflavones	Apigenin- and luteolin-6,8-di-C-glucoside (lucenin-2, vicenin-2), diosmetin-6,8-di-C-glucoside	(Barreca et al. 2010)
Pummelo	*Citrus grandis*	C-Glycosylflavones	Apigenin 6-C- and 6,8-di-C-glycosides (including acylated derivatives), diosmetin-6.C- and 6,8-	(Abad-Garcia et al. 2012; Zhang et al. 2014; Wang et al. 2017)

(*continued*)

Table 1 (continued)

Food source	Plant	Flavonoid class	C-Glycosylflavonoids	References
			di-C-glycosides, luteolin-6,8-di-C-glucoside	
Tangelo	*Citrus reticulata X C. paradisi*	C-Glycosylflavones	Apigenin- and luteolin-6,8-di-C-glucoside	(Barreca et al. 2013)
Legumes				
Peas	*Pisum sativum*	C-Glycosylflavones	Apigenin- and luteolin-C-glycosides	(Courts and Williamson 2015; Hostetler et al. 2017)
Fava beans	*Vicia faba*	C-Glycosylflavones	Apigenin-C-glycosides	(Hostetler et al. 2017)
Chick peas	*Cicer arientinum*	C-Glycosylflavones	Luteolin C-glycosides	(Hostetler et al. 2017)
Mung beans	*Vigna radiata*	C-Glycosylflavones	Apigenin-6-C- and 8-C-glycosides	(Xiao et al. 2016)
Other fruits and seeds				
Passion fruits (pericarp)	*Passiflora sps.*	C-Glycosylflavones	Apigenin-6-C-, 8-C- and 6,8-di-C-glycosides, luteolin-6-C-, 8-C-glycosides, chrysin-6,8-di-C-glucoside	(Zucolotto et al. 2012)
Cayenne pepper	*Capsicum annuum*	C-Glycosylflavones	Apigenin-6-C-glycoside, luteolin-6-C- and 6,8-di-C-glycosides	(Materska 2015)
Dates	*Phoenix dactylifera*	C-Glycosylflavones	Apigenin-di-C-hexoside	(Xiao et al. 2016)
Tomatoes	*Solanum lycopersicum*	C-Glycosyl-dihydrochalcones	Phloretin-3′,5′-di-C-glucoside	(Courts and Williamson 2015)
Chayote	*Sechium edule*	C-Glycosylflavones	Apigenin-6-C- and 6,8-di-C-glycosides	(Siciliano et al. 2004)
Cucumber	*Cucumis sativus*	C-Glycosylflavones	Apigenin- and luteolin-6-C- and 8-C-glycosides, cucumerin A, B (phytoalexins)	(McNally et al. 2003)

Carob seed germ flour	*Ceratonia siliqua*	C-Glycosylflavones	Apigenin 6-C-, 8-C- and 6,8-di-C-glycosides (including acylated derivatives)	(Picariello et al. 2017)
Carambola fruit	*Averrhoa carambola*	C-Glycosyl-dihydrochalcones; C-glycosylflavones	Apigenin-6-C-glycosides (carambolaflavones)	(Wang et al. 2018)
Fenugreek (seed germs)	*Trigonella foenum-graecum*	C-Glycosylflavones	Apigenin- and luteolin-6-C-, 8-C- and 6,8-di-C-glycosides	(Xiao et al. 2016)
Mesquite (South American algarrobo)	*Prosopis spp.*	C-Glycosylflavones	Apigenin-6,8-di-C-glycosides (including feruloylate derivatives)	(Picariello et al. 2017)
Quince (seeds)	*Cydonia oblonga*	C-Glycosylflavones	Apigenin-, chrysoeriol- and luteolin-6-C-, 8-C- and 6,8-di-C-glycosides	(Ferreres et al. 2003)
Cocoa (processed)	*Theobroma cacao*	C-Glycosylflavan-3-ols	Catechin- and epicatechin-C-glycosides	(Stark and Hofmann 2006; Courts and Williamson 2015)
Figs (skin)	*Ficus carica*	C-Glycosylflavones	Luteolin-6,8-di-C-glycoside	(Vallejo et al. 2012)
Leaves and stems				
Bamboo shoots (leaves)	*Different species*	C-Glycosylflavones	Apigenin-C-glycosides, luteolin-8-C-glycosides	(Zhang et al. 2007)
Beet root and leaves	*Beta vulgaris*	C-Glycosylflavones	Apigenin-8-C-glycosides	(Courts and Williamson 2015; Ninfali et al. 2017)
Honeybush herbal tea	*Cyclopia sps.*	C-Glycosylflavones, C-glycosyldiydrochalcones	Phloretin-3′,5′-di-C-glucoside, vicenin-2, 3-hydroxyphloretin-3′,5′-di-C-hexoside	(Schulze et al. 2015)
Lemongrass leaves	*Cymbopogon citratus*	C-Glycosylflavones	Apigenin-6-C-, 8-C- and 6.8-di-C-glycosides, luteolin-6-C-, 8-C- and 6.8-di-C-glycosides	(Figueirinha et al. 2008)

(*continued*)

Table 1 (continued)

Food source	Plant	Flavonoid class	C-Glycosylflavonoids	References
Lotus (different plant parts)	*Nelumbo nucifera*	C-Glycosylflavones	Apigenin- and luteolin-6-C- and 8-C-glycosides; especially rich in Lotus plumules (all other parts contain less CGFs)	(Li et al. 2014)
Palm products (palm leaves etc.)	*Different species*	C-Glycosylflavones	Apigenin-6- and 8-C-glycosides, luteolin-6-C- and 8-C-glycosides	(Williams et al. 1973)
Pigeon pea	*Cajanus cajan*	C-Glycosylflavones	Apigenin-8-C-glycosides, luteolin-8-C-glycosides	(Nix et al. 2015)
Rooibos herbal tea	*Aspalathus sp.*	C-Glycosyl-dihydrochalcones, C-glycosylflavanones, C-glycosylflavones	Aspalathin, nothofagin, eriodictyol-C-glycosides, apigenin- and luteolin-6-C- and 8-C-glucosides	(Courts and Williamson 2015; Hostetler et al. 2017)
Sugar cane	*Saccharum officinarum*	C-Glycosylflavones	Apigenin-6-C-, 8-C- and 6,8-di-C-glycosides, luteolin-8-C-glycosides, 4′,5′-dimethyl-luteolin-8-C-glycoside	(Courts and Williamson 2015)
Taro (leaves)	*Colocasia esculenta*	C-Glycosylflavones	Apigenin-, chrysoeriol-, diosmetin-, luteolin-6-C-, 8-C-, 6,8-di-C-glycosides	(Ferreres et al. 2012)
Tea (green, oolong, white, black)	*Camellia sinensis*	C-Glycosylflavones	Apigenin C-glycosides, luteolin C-glycosides	(Hostetler et al. 2017)
Rhubarb	*Rheum sp.*	C-Glycosylflavones	Apigenin-6-C- and 6,8-diC-glycosides	(Courts and Williamson 2015)
Roots				
Beet root and leaves	*Beta vulgaris*	C-Glycosylflavones	Apigenin-8-C-glycosides	(Courts and Williamson 2015; Ninfali et al. 2017)
Kudzu	*Pueraria montana*	C-Glycosylisoflavones	Daidzein 8-C-glucoside (puerarin), 3′-OH-puerarin, 3′-OMe-puerarin, 5-OH-puerarin	(Wu et al. 2011)

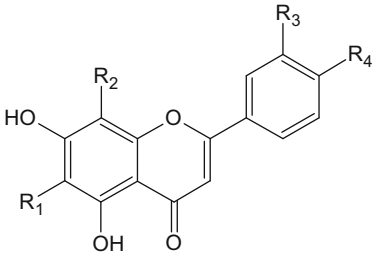
2.2 Food Sources of C-Glycosyl Flavonoids

A condensed summary of C-glycosylflavonoids detected in plant food sources can be depicted from Table 1. The most common structures of CGF in food are presented in Table 2 and Fig. 3.

As shown in Table 1, by for the majority of CGF in food plants are present as C-glycosylflavones, followed by C-glycosyldihydrochalcones, main food resources of this type of compounds are tomatoes (phloretin-3′, 5′-di-C-glucoside) and rooibos tea (aspalathin, nothofagin). Fermentation (heating and/or sundrying) of rooibos tea forms C-glycosylflavanones (eriodictyol glycosides), and further oxidation leads to C-glycosylflavones. Similar as in rooibos tea, C-glycosylflavan-3-ols in cocoa seeds are resulting from the fermentation process (Courts and Williamson 2015).

In order to evaluate the relevance of CGF as compounds in food and their possible impact on human health, of course their amounts in the diet have to be known. However, data concerning quantities, especially in processed food, are scarce. The following data refer to reported quantities summarized by Hostetler et al. (2017). Citrus fruits represent a major source of C-glycosylflavones (Fig. 4), with total quantities of 1.6–9.2 mg/100 g fresh weight (corresponding to 6–23 mg per serving) in orange juice, 8.6–15.4 mg/100 g fresh weight in fruit juices of bergamot, 2.9–3.5 mg/100 g in mandarin juice, and 6.7–7.6 mg/100 g fresh weight in lemon

Table 2 Structures of most common C-glycosylflavones and aglycones



Compound	R_1 (C-6)	R_2 (C-8)	R_3 (C-3′)	R_4 (C-4′)
Apigenin	H	H	H	OH
Luteolin	H	H	OH	OH
Chrysoeriol	H	H	OCH_3	OH
Diosmetin	H	H	OH	OCH_3
Vitexin	H	β-D-gluc	H	OH
Isovitexin	β-D-gluc	H	H	OH
Orientin	H	β-D-gluc	OH	OH
Isoorientin	β-D-gluc	H	OH	OH
Vicenin-2	β-D-gluc	β-D-gluc	H	OH
Lucenin-2	β-D-gluc	β-D-gluc	OH	OH
Schaftoside	α-L-ara(p)	β-D-gluc	H	OH
Isoschaftoside	β-D-gluc	α-L-ara(p)	H	OH

β-D-gluc = C-β-D-glucosyl-; α-L-ara(p) = C-α-L-arabinopyranosyl-

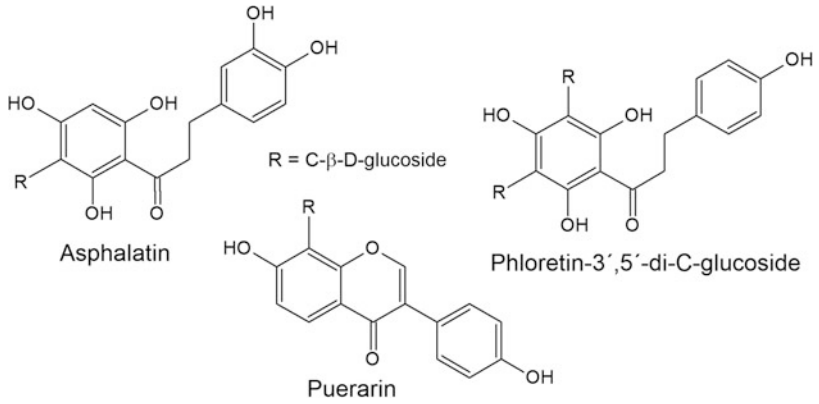


Fig. 3 C-Glycosylflavonoids other than flavones: asphalatin and phloretin-3′,5′-di-C-glucoside as examples of C-glycosyldihydrochalcones, puerarin representing a C-glycosylisoflavone

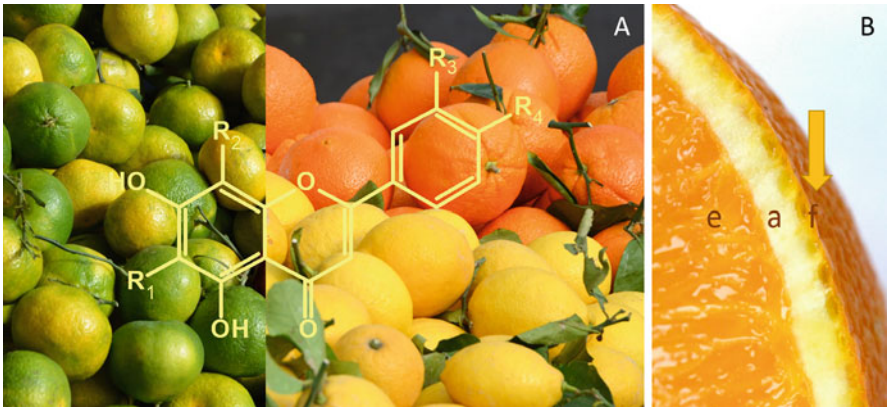


Fig. 4 (**A**) Citrus fruits like oranges, lemons, and mandarins are a major food source of C-glycosyl flavones. (**B**) C-Glycosylflavones can mainly be found in the outer flavedo layer (f) whereas lower amounts are present in the albedo layer (a) and endocarp (e)

juice. Investigations of commercials juices of pink grapefruit (*C. paradisi*) and oranges (*C. sinensis*) showed quantities of vicenin-2 (apigenin-6,8-di-C-glucoside) of 29.3–45.3 mg/L (Bucar, unpublished data). Investigations of accumulation patterns of C-glycosylflavones in various tissues and *Citrus* species revealed the highest proportions in the flavedo layer (colored outer layer of the pericarp) and significantly less in the albedo layer (inner whiteish layer of the pericarp), segment membranes, and fruit juice (Wang et al. 2017), see also Fig. 4.

In a wheat grain-based meal, the estimated amount of C-glycosylflavones is relatively low ranging between <1–5 mg per serving (28 g dry grain). Our own exploratory analysis of apigenin-6,8-di-C-glycosides in spelt (*Triticum aestivum* subsp. *spelta*) pointed towards preferable intake of wholemeal flour (66 mg/kg) compared to plain flour (32 mg/kg), and amounts in wheat (*Triticum aestivum*)

semolina were comparably low (6 mg/kg) (Bucar, unpublished results). According to Hostetler et al. (2017), this is also true for different types of rice (amounts of apigenin and luteolin-C-glycosides below <1 mg per serving (28 g) and dehulled, roasted, as well as whole buckwheat (<1 mg per serving). Similar low amounts are reported for legumes like field peas (*Pisum sativum*), i.e., <1–1 mg per 28 g serving, and chick peas (<1 mg per serving). However, unexpectedly high amounts of vitexin (apigenin-8-C-glucoside) equivalents were detected in flour of millet seeds with 77–275 mg/100 g (Courts and Williamson 2015), however determined by a spectrophotometric method without further confirmation after chromatographic separation. A study of Boncompagni et al. (2018) on grains of 96 pearl millet lines from a panel of inbred lines covering a large genetic diversity revealed also highly divers quantities of C-glycosylflavones. Ranges of 0 to 283.4, 272.7, and 261.1 mg/kg (mean values of 71.9, 28.1, and 40.0) were found for glucosylvitexin, orientin, and vitexin, respectively. Extremely high amounts of different apigenin-C-glycosides were reported for carob seed germ flour summing up to more than 8.3 g/kg in total (Picariello et al. 2017).

Although high quantities of apigenin- and luteolin-C-glycosides (up to 246.6 mg/100 g dry weight) have been found in different processed teas (green, black, oolong teas of *Camelia sinensis*), in the final infusion only <1–4 mg per serving (corresponding to 2 g dry material) can be expected. Flavonoids and phenolics in tea are covered in more detail in chapters on "► Tea Catechins" (Daglia and Baldi) and "► Procyanidins in Food" (Sieniawska et al.).

Tomatoes and infusions of rooibos herbal tea (*Aspalathus linearis*) are the main food source of C-glycosyldihydrochalcones (Courts and Williamson 2015). In investigated tomato cultivars, phloretin-3′,5′-di-C-glucoside contributed by 5–14% to the total flavonoid content which varied from 4 to 26 mg per 100 g fresh weight. Further relevant sources of this C-glycosyldihydrochalcone are the fruit peels of mature kumquats (*Fortunella japonica*) showing highly remarkable 1.35 g per 100 g dry weight (Lou et al. 2016), as well as honeybush herbal tea (*Cyclopia spp.*) with 0.77–13.3 mg/L hot water infusion (Schulze et al. 2015).

In rooibos (leaves and stems), a significant difference in unprocessed and fermented herbs can be observed. Whereas unfermented freeze-dried rooibos tea contains about 1.5 g aspalathin and 0.43 g nothofagin per 100 g, due to oxidative ring cyclisation to eriodictyol-C-glycosides, the amount of both dihydrochalcones declines to 102 mg and 35 mg per 100 g, respectively, in fermented rooibos tea. In addition, the C-glycosylflavones luteolin-6-C-glucoside (isoorientin) and luteolin-8-C-glucoside (orientin) have been extracted to aqueous infusions of fermented rooibos tea at quantities of 100 and 80 mg per 100 g, whereas the corresponding apigenin glucosides were only present in lower amounts of 33 and 27 mg per 100 g, respectively (Courts and Williamson 2015; Hostetler et al. 2017).

As most prominent source of C-glycosylisoflavonoids (mainly puerarin), kudzu (Japanese arrowroot, *Pueraria montana var. lobata*) has to be mentioned. According to Wu et al. (2011), its dried roots contained 1.97% of puerarin, whereas other puerarin derivatives were present only in low quantities (0.024–0.16%). Structures of most common flavone C-glycosides are shown in Table 2, and C-glycosylflavonoids other than flavones are presented in Fig. 3.

3 Bioavailability

CGF have a higher stability compared to their *O*-glycosylflavonoids (OGF) in regard to their hydrolysis rate. Human studies reveal that flavonoid C-monoglycosides (such as vitexin, isovitexin, orientin, isoorientin, and puerarin, for structures see Table 2 and Fig. 3) are only absorbed to a minor extent. Metabolism of CGF differs mainly from that of *O*-glycosylated flavonoids which are hydrolyzed easier by acid and enzymes compared to CGF. Therefore, CGF are not affected to the extent as seen for OGF by hepatic and gastrointestinal hydrolysis obvious due to the appearance of intact CGF in urine. No mammalian enzymes which are capable of cleaving the C-glycosidic linkage are known; therefore, resident bacteria in the human colon are the only metabolism step experienced by CGF. With a bamboo extract (containing vitexin, isovitexin, orientin, isoorientin) administered to rats, it was found that these CGF are poorly absorbed from the gastrointestinal tract and are therefore passed to colon which were then excreted by feces within 24 h. After examination of rats after 12 h of administration, no CGF were found in liver and brain. Microbial degradation gave rise to metabolites like phloroglucinol, hydrocaffeic, and phloretic acid. In contrast, C-multiglycosides of flavonoids are absorbed from the small intestine in unmodified nature and distributed to the liver. From there, CGF are either infiltrated into the system circulation and distributed to various tissues or directed to urinary excretion (Xiao et al. 2016). Hence, deglycosylation does not seem to be essential for CGF absorption (Courts and Williamson 2015).

Bioactivity of such phenolic compounds is strongly dependent on the release rate from the matrix (bioaccessibility), alterations during the passage of gastrointestinal tract, and the principal metabolism in vivo. Bioaccessibilty of dietary flavonoids is again dependent on many factors such as matrix, filling of stomach, liquid consumption during eating, pH value of gastrointestinal tract, peristalsis, and blood and lymph flow. On the other hand, it is dependent on the nature of the flavonoid – nature and scale of glycosidation, type of sugar, and how it is linked to the aglycone. The interaction of flavonoids and proteins is feasible after what they are less likely to be ready for digestion. The main reactions going on in the liver include methylation, sulfatation, and glucuronidation (Czubinski et al. 2019).

Glycosides can be seen as prodrugs enhancing the solubility of their aglycones. Only a small extent of flavonoid glycosides is absorbed in the small intestine – the majority is transported to the large intestine and there deglycosylated by colonic bacteria takes place. Once they are absorbed, they are metabolized by phase II enzymes and then conveyed into the liver. Hydroxylation, reduction, or methylation take place to release the aglycones which in turn get further metabolized (sulfated/glucuronidated) to the typical flavonoid metabolites (with improved solubility and molecular weight) which circulate in the body.

Especially the enzymes of UDP-glucuronosyltransferases 1 and 2 family are responsible for the glucuronidation of flavonoids. Local release of aglycones from glucuronidated species has been observed in inflammatory cells, such as macrophages which release lysosomal enzymes, including β-glucuronidase, which can lead to deconjugation in the tissues and uptake of aglycones by the cells to a much

higher rate compared to the glucuronide conjugates. The in situ deconjugation has been pointed as an absolute requirement to the flavonoid bioactivity by delivering the free aglycone to the tissues, which is the final effector (Xiao et al. 2016; Xiao 2017) (Fig. 4).

More than 400 bacterial species can habitat a human body and therefore the metabolization rate differs from one person to another. Little is known for the deglycosidation processes for CGF although some bacterial strains were reported to be involved in the cleavage of C-glycosides; among them are strains from *Enterococcaceae*, *Lachnospiraceae*, and *Streptococcaceae*. The specific strain responsible for the cleavage of C-coupled sugar is noted in the sections below assigned to the affected compound. It is further reported that most bacteria able to cleave the C-linkage are also able to act on *O*-glycosides. Bacteria could be responsible for one step in the metabolization or can perform the whole cascade to degradation products (Braune and Blaut 2016). Degradation of isoorientin by human intestinal bacteria was studied by Hattori et al. (1998).

The binding of ingested compounds to serum albumin is an important factor how pursuing distribution, excretion, and metabolism is carried out in human body. Serum albumin is known for its function as carrier and storage place for various compounds of endogenous and exogenous nature (such as fatty and amino acids, steroid hormones, and drugs) and therefore is of critical importance for bioavailability and duration of drug action. A study conducted by Cao et al. examined the affinity of various flavonoids to human serum albumin by using high performance affinity chromatography. For this reason, 63 structurally different flavonoids were tested for their human serum albumin binding affinity among others 4 CGF. Apigenin-6-C-glycoside, puerarin, vitexin, and vitexin-2″-rhamnoside were tested with protein binding rates of 59.6%, 30.6%, 47.7%, and 19.1%, respectively. When drugs are bound to proteins, reversible complexes are formed from which drugs cannot diffuse through plasma membranes and no metabolization is possible. On the other hand, drugs can diffuse from these reversible complexes when drug plasma concentration is low and can therefore maintain an equilibrium. In this study, various substitution on the flavonoid basic structure was performed which allow interpretation of structural relationships. Glycosylation of flavonoids leads to a decrease of protein binding rate by 30–50%. This effect was mediated by steric hindrance and the higher polarity due to glycosylation. Compounds are bound to human serum albumin by a hydrophobic pocket which in turn is decreased by a lower lipophilicity. A high protein binding rate stands for a longer half-life and a slow elimination into plasma of these drugs (Cao et al. 2019).

3.1 Vitexin and Isovitexin

Vitexin was found to be deglycosylated to its aglycone apigenin by a bacterium found in human feces, *Lachnospiraceae* CG19-1 (Courts and Williamson 2015), whereas isovitexin was also deglycosylated by *Eubacterium cellulosolvens* to apigenin and 3-(4-hydroxyphenyl)propionic acid (Xiao et al. 2016) (see Fig. 5). The excretion ways

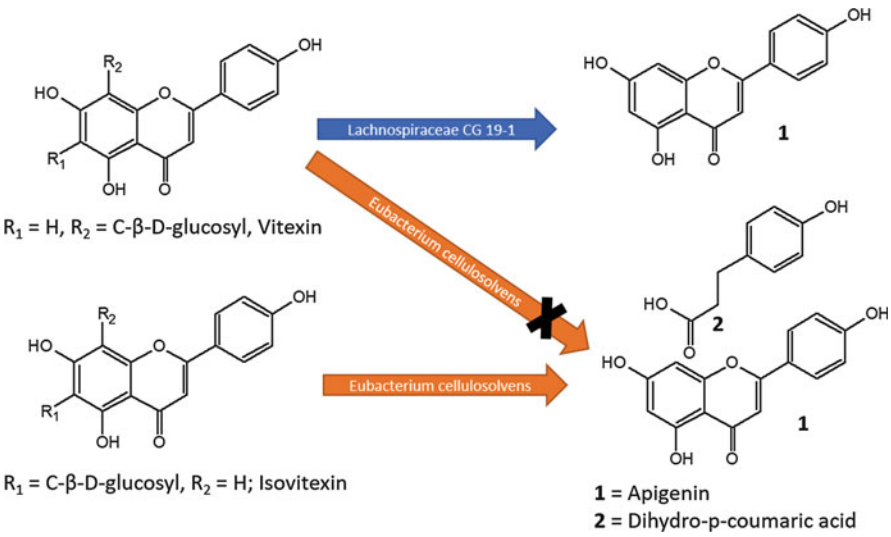


Fig. 5 Deglycosylation of vitexin and isovitexin by selected gut bacteria. Whereas Lachnospiraceae strain CG 19-1 deglycosilated vitexin to apigenin (Braune and Blaut 2016), *Eubacterium cellulosolvens* degraded only isovitexin to apigenin and dihydro-p-coumaric acid, but not vitexin (Xiao et al. 2016)

of vitexin were studied with the substance vitexin-4″-*O*-glucoside by Cai et al. For this reason, mice were given 30 mg/kg vitexin-4″-*O*-glycoside either orally or intravenously and samples were collected at certain time points. In an oral dosing experiment after 24 h, original substance was recovered from urine with 17.9% and in feces with 6.3%. Intravenous administration led to a recovery rate of 4.7% (urine) and 0.8% (feces). These low recovery rates showed that vitexin-4″-*O*-glucoside have to be metabolized also by other means. The authors conclude that this glycoside has entered enterohepatic recirculation (elimination from the bile to the intestine and simultaneously reabsorption into the blood flow) and was then eliminated. More than four-fold higher elimination was detected after oral administration, whereas plasma concentrations were higher after i.v. administration. It can be concluded the renal way is the major excretion way of vitexin-4″-*O*-glucoside from mice (Cai et al. 2013).

A further experiment was performed with vitexin-2″-*O*-xyloside in order to gain insight into the bioavailability of CGF. The compound was administered (8.15 μmol) directly in the caecum of rats and blood samples were taken at various time points. The CGF gave rise to two peaks in the blood – on the one hand the unchanged vitexin-2″-*O*-xyloside and, on the other hand a vitexin glucuronide. This glucuronide was detectable as early as 30 s after administration. Unmetabolized drug enters enterohepatic recirculation and therefore gets reabsorbed in the intestine partly in conjugated form. The caecum was observed, showing a retarded amount of vitexin-2″-*O*-xyloside of around 25% and no conjugates were detected – therefore metabolization products by gut bacteria or enterocytes are thought to be immediately released into the blood (Xiao et al. 2016).

3.2 Schaftoside, Isoschaftoside, Vicenin-2

With a human gastrointestinal model, the metabolization of schaftoside and isoschaftoside was shown resulting in the information that these CGF are stable through their passage of gastrointestinal dialyze model. The same data were obtained for vicenin-2 and vitexin. During this model, only *O*-glycosidic linkages of vitexin-*O*-glycosides were broken by colon bacteria. A search was started for specific degradation products of vitexin, but it can be considered as stable due to the fact that no metabolites (such as 3-(4-hydroxyphenyl) propionic acid) occurred over time. Data regarding active absorption and enzymatic influence is missing in this model (van Dooren et al. 2018). In an in vivo model using rats it was shown that schaftoside, isoschaftoside, and vicenin-2 were rapidly absorbed into the blood circulation. The plasma curves led to the conclusion that there are possibly two absorption sites in the intestine. The distribution of these three CGF was mainly in the kidneys followed by liver, lungs, heart, and spleen (for vicenin-2 and isoschaftoside) (Xiong et al. 2015).

Lupin seeds (*Lupinus angustifolius*) are widely used in nutrition. Isolation gave rise to the free aglycone apigenin and three CGF could be isolated from this plant whereas apigenin 6,8-di-*C*-glucoside (vicenin-2) and apigenin 7-*O*-apiosyl-6,8-di-*C*-glucoside as the two main CGF. It was found that these CGF are able to bind to proteins and during digestion can be released if these proteins are broken down. When digesting these seeds in an in vitro model, the total phenolic content decreased by 57% (at stomach level) and an additional 5% when measured at small intestine level. No qualitative change in CGF composition was found after digesting the seeds, but quantitative changes were found (a lesser amount was documented). In other *Lupinus* species a change was found in the CGF composition after digestion. In *L. luteus* no hexoside of apigenin feruloyl-7-apiosyl-6,8-di-C-glucoside was detectable and in *L. albus* no aglycone apigenin was found post digestion. In case of *L. luteus* new *O*-glycosides appear in after digestion measurements. On the example of *L. angustifolius*, it was demonstrated that 83% of apigenin 7-*O*-apiosyl-6,8-di-*C*-glucoside were released at stomach digestion and additionally 9% at intestine level. Very similar results were obtained for apigenin 6,8-di-*C*-glucoside with 86% in stomach and + 7% in the intestine. These results show that CGF are bioaccessible in a high extent. An additional experiment of protein digestion was conducted and showed that 54% of lupin protein was digested during the in vitro model. The higher the total phenolic content, the lower was the protein digestion rate. The decrease could be explained by complex formation or the inhibitory potential of phenolic compounds on proteolytic enzymes (Czubinski et al. 2019).

3.3 Orientin and Isoorientin

Isoorientin was found to be deglycosylated by a bacterium found in human feces, *Lachnospiraceae* CG19-1 (Courts and Williamson 2015). In a study using human intestinal bacteria, isoorientin was metabolized to phloroglucinol, luteolin, (+)-eriodictyol, 6-C-glucosyleriodictyol, and 3,4-dihydroxyphenylpropionic acid. This metabolic deglycosylation were also reported to happen together with orientin in

sheep and cows mediated by *Eubacterium cellulosolvens* (Xiao et al. 2016). Orientin and isoorientin were metabolized to luteolin by *Lachnospiraceae* CG19-1 and for isoorientin a further bacterial deglycosylation way was described with *Eubacterium cellulosolvens* (Braune and Blaut 2016).

When CGF are not absorbed in the upper intestinal tract, they get degraded by intestinal bacteria to breakdown products. In this context, isoorientin is metabolized to (±)-eriodictyol-6-C-β-glucoside which in turn is reduced to luteolin and (±)-eriodictyol. The latter substance is further degraded to phloroglucin, 3-(3,4-dihydroxyphenyl)-propionic acid and 3-(3-hydroxyphenyl)-propionic acid (Webster and Wood 2016). A newly found human bacterial strain *Enterococcus* sp. 45 (affiliated with *Enterococcus casseliflavus*) was able to cleave orientin to luteolin. Furthermore, it was noted that these bacteria are able to acetylate and deoxygenate orientin. These effects were carried out by several other bacterial strains too. Furthermore, the original substance orientin could be detected (Xu et al. 2014).

3.4 Puerarin (Daidzein-8-C-Glucoside)

A specific bacterial *strain PUE* (affiliated with *Dorea longicatena*, *Lachnospiraceae*) was able to cleave puerarin to daidzein. This strain is regularly found in human gut microbiota. This reaction was also reported for *Lachnospiraceae* CG19-1, *Lactococcus* sp. MRG-IFC-1, and *Enterococcus* sp. MRG-IFC-1 (Braune and Blaut 2016). Furthermore, human microbiota is not only able to metabolize puerarin to daidzein and glucose but also to (3S)-equol (Xiao et al. 2016).

The CGF puerarin was found to have a 138-times lower binding rate to human serum albumin compared to the corresponding aglycone. In contrast, the binding to bovine hemoglobin was found to be 2.45 times higher compared to daidzein. And in the case of plasma proteins of type II diabetes, the CGF and the aglycone had similar affinities. When looking at the pharmacokinetics, a 7-*O*-glucoside of puerarin showed a higher plasma concentration and was longer detectable in the blood as puerarin itself (Xiao 2017).

3.5 Aspalathin

This is the best examined CGF so far in human and porcine studies. In these studies, methylated metabolites of aspalathin were found after oral administration. No intact aspalathin was found in the plasma of human and pig. However, in urine this substance was detected showing limitation of plasma extraction methods (eventually binding to serum albumin) and therefore, surveillance of plasma concentrations of CGF is challenging due to insensitive methods. In a bioavailability study with aspalathin, there were glucuronide and sulfate conjugates found in vivo. Furthermore, it was shown that the catechol-*O*-methyl-transferase takes action in this metabolic process by methylating the catechol moiety of aspalathin giving rise to a 3-*O*-methylaspalathin which was then detected in urinary samples. With Caco-2

cells, the passive transport by diffusion of aspalathin into the intestinal epithelium was demonstrated. During this transport, aspalathin was not deglycosylated. The uptake into enterocytes occurred only in a small manner probably by the non-selective transport of *O*-glycosides by enterocyte glucose carrier proteins (such as SGLT-1). Aspalathin has only a poor bioavailability (Courts and Williamson 2015).

After administration of fermented and unfermented rooibos tea (*Aspalathus linearis*) to human, volunteers' urine and blood samples were collected over 24 h and evaluated. The main metabolite found after fermented tea consumption was eriodicytol-*O*-sulfate and *O*-methyl-aspalathin-*O*-glucuronide in case of unfermented tea. Further metabolites found in both teas include aspalathin-*O*-sulfate, *O*-methyl-asphalathin-sulfate, and aspalathin-*O*-glucuronides. The authors suggest the absorption of eriodicytol-*O*-sulfate in the large intestine due to the excretion via urine after 5–12 h, whereas most aspalathin metabolites were found in urine within 5 h (uptake from small intestine). No metabolites in plasma were found throughout this study (Xiao et al. 2016).

4 Bioactivities

In food plants, as in ethnomedically applied plants, a plethora of compounds can be responsible for the observed effects, it is not clearly possible to distinct whether a plant is solely active due to the high CGF content. Therefore, here only plants are linked to an activity which are especially rich in CGF. Betimes, only in vitro data are available.

4.1 Antidiabetic Activity

Due to the stable C-glycoside linkage, it is thought that this substance group is able to modulate the blood glucose level. In an in vitro experiment, model pancreatic β-cells (RIN-5F cells) were incubated with and without aspalathin (100 μM). Within 3 h, it was found that insulin secretion has risen by 30% along with no increase of cytotoxicity. The potential of this substance is thwarted by its poor bioavailability. In another study using L6 rat myotubes aspalathin was able to increase glucose uptake by 24% when used in 1 μM and by 64% in 10 μm concentration. Isovitexin was able to show in vivo an insulin secretion increase by 58% after 1 h. With this, also the glycogen values were upregulated (27%) in the muscles of these rats (Courts and Williamson 2015).

The leaves of *Microctis paniculata*, a common ingredient in Chinese herbal teas, were studied for their CGF content. Two substances, vitexin and isovitexin, were isolated from these leaves and tested for their activity to inhibit α-glucosidase. As both compounds are also frequently found in food plants, the results of this study should be presented here. The methanolic extract was able to inhibit α-glucosidase with a half maximal inhibitory concentration (IC_{50}) of 61.3 μg/ml in a concentration-dependent manner. For vitexin (see Fig. 6) and isovitexin, an IC_{50} concentration of

96.9 μg/ml (244.0 μM) and 115.1 μg/ml (266.2 μM) was obtained in the α-glucosidase assay (positive control: acarbose with 1007 μM). Therefore, the two CGF are emphasized as possible antidiabetes drug candidates (Xiao et al. 2016). The same two substances were tested in the α-glucosidase assay by another working group according to Xiao et al. (2016). They reported an in vitro IC_{50} of 4.1 μg/ml for vitexin and 6.7 μg/ml for isovitexin, which are both lower than that of acarbose. Both CGF were isolated from *Ficus deltoidea*. In addition, an in vivo experiment using normoglycemic mice and diabetic rats induced by streptozotocin was performed. Both CGF were found to lower the postprandial glucose level when measured at 30 min. Vitexin when orally administered at 1 mg/kg to normoglycemic mice significantly reduced the blood glucose level by 24.7%, and at higher concentrations of 3 mg/kg and 15 mg/kg the reduction was further enhanced to 26.5% and 31.3%, respectively. After 1 h, the glucose level returned to normal in normoglycemic mice. All three doses were equivalent to acarbose (3 mg/kg), the positive control. Extrapolation of 1 mg/kg in mice leads to an equivalent dosage of 0.08 mg/kg in humans. Isovitexin was able to lower the postprandial glucose level in normoglycemic mice at 60 min when used at 3 mg/kg and 15 mg/kg, whereas 1 mg/kg isovitexin was comparable with acarbose. In diabetic-induced rats higher concentrations of corresponding CGF were employed. The peak of postprandial glucose level in these rats was found to be at 60 min with an additional increase of 20.8%. The highest reduction (19.7%) in diabetic rats was achieved with 200 mg/kg vitexin and 100 mg/kg isovitexin, respectively. An already significant result was obtained with 50 mg/kg vitexin (human equivalent dose of 8.1 mg/kg) and 20 mg/kg isovitexin

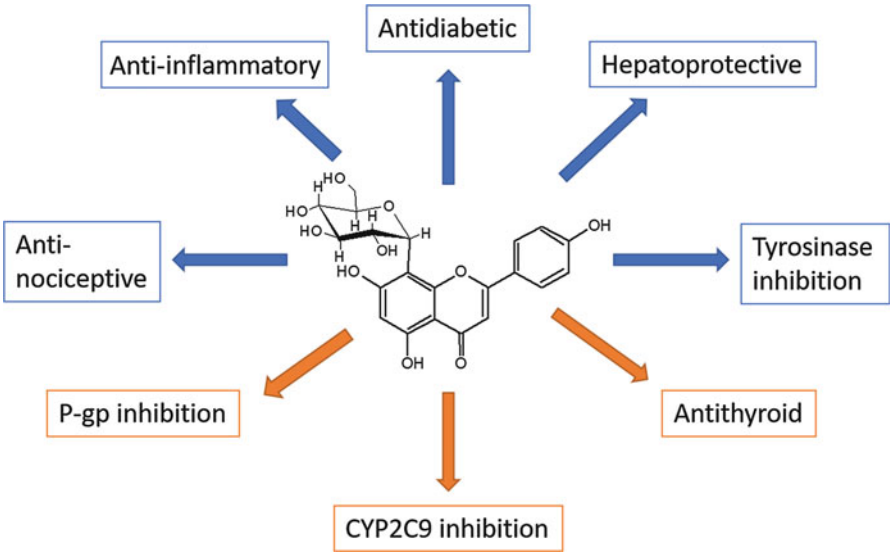


Fig. 6 Biological activities of vitexin. Activities which could cause undesired interactions with other compounds or lead to adverse effects are indicated in orange

(human equivalent dose of 3.3 mg/kg). Similar effects were obtained with 5 mg/kg acarbose. Furthermore, the toxicity of oral administration of 2 g/kg vitexin and isovitexin was measured with no signs of toxicity (mortality and weight change) after 24 h and 14 days. The dried leaves of *F. deltoidea* only contain 0.34% vitexin and 0.13% isovitexin; therefore, an infusion can only be active in patients with mild diabetes (Xiao et al. 2016). The formation of advanced glycation endproducts (AGE) is feared as a driving force in diabetic pathogenicity. It is associated with cataract, neuro-, nephro-, and retinopathy in diabetic patients. A hydroethanolic extract of mung beans (*Vigna radiata*) was found to be a potential inhibitor of this AGE product formation. 80.4% inhibition was achieved with a concentration of 500 ppm in the bovine serum albumin glucose test. In the subsequent analysis, it was found that mung beans are rich in phenolic compounds – especially vitexin and isovitexin. These two CGF were tested in the same test model at a concentration of 100 μM and were found to be slightly lower inhibitory active than rutin, an already known excellent AGE inhibitor. Researchers believe that the anti-glycation activity can be attributed to the free radical scavenging ability of these CGF (Xiao et al. 2016). Sprouts are part of a modern diet; therefore, it was documented in an experiment that fenugreek (*Trigonella foenum graecum*) and barley sprouts (*Hordeum vulgare*) could be of potential use in diabetes management. Both sprout juices were able to reduce the fasting blood glucose level compared to untreated diabetic rats. An additional effect on the lipid profile of rats was observed, whereas barley sprout juice performed best, nearly improving the lipid profile of diabetic rats to normal status (total cholesterol – 27.9%, triglycerides – 16,9 and LDL – 51.4% compared to untreated diabetic rats). Both juices were also able to reduce lipid peroxidation and oxidative stress measured by malondialdehyde levels and catalase activity (Mohamed et al. 2019). The leaves of *Centaurea alexanderina* were extracted with methanol and then screened for their constituents resulting in three reported CGF: vicenin-2, vitexin, and isovitexin, all of them can also be found in citrus fruits. With this extract and oral glucose tolerance test with normoglycemic rats was conducted. The dose of 600 mg/kg extract was able to lower the blood glucose levels by 9.4 and 10.5% when tested after 1 and 2 h, respectively. These results were just over the reference substance glibenclamide. In diabetic rats, the treatment with the extract over 30 days led to a significant decrease of blood glucose levels by 3.8% for 600 mg/kg, whereas the maximum was reached after 60 days with 4.9% (Kubacey et al. 2012).

4.2 Anticancer Activity

Citrus CGF were found to alter key enzymes responsible for cell activation and receptor binding and simultaneously be of low toxicity tested in animals (Xiao et al. 2016). In an experiment using isoorientin, it was documented that this CGF was able to increase apoptosis and autophagy and inhibit proliferation in human hepatoblastoma cancer cells. The induction of apoptosis was mediated by mitochondrial dysfunction, the inhibition of signals which trigger apoptosis and autophagy

(such as p53 and NFκB) and also Fas-pathway was activated. Isoorientin was tested against human liver cells and no toxicity was found for this CGF using concentrations up to 80 μM (Xiao et al. 2016). P-glycoprotein (p-gp) is a membrane protein known for its efflux capacity and therefore of importance in the resistance development of cancer cells to anticancer treatment. 75 flavonoids were screened for their ability to inhibit p-gp amongst others also three CGF: orientin, puerarin, and vitexin (see Fig. 6). In a first step it was found that all three CGF were of nontoxic nature when tested in 100 μM against MDR1-MDCKII cells. Vitexin was the best CGF to inhibit p-gp with 34.3%, followed by orientin with 20.8% and puerarin with 18.5% respectively (Bai et al. 2019). Furthermore, it was shown with esophageal cancer cells (EC-109) that orientin and vitexin were able to induce apoptosis and therefore inhibit cell growth. The oncogene expression of p53 was significantly increased after 48 h incubation in a concentration-dependent manner (up to 80 μM), whereas the effect of orientin was found to be stronger (An et al. 2015). An extract rich in vitexin and isovitexin (and chlorogenic acid) from *Trigonella foenum graecum* was tested active against human breast cancer cell line (MCF-7) and African green monkey kidney (Vero) cells with an IC_{50} of 2.5 μg/ml for each strain (Kadaikunnan et al. 2015). A mixture of CGF (vitexin, isovitexin, and orientin) was detected in a CO_2 extract of the leaves of *Cajanus cajan*. This extract was tested against three cell lines in order to evaluate the cytotoxicity: mouse macrophage cell line RAW 264.7 (68.9 μg/ml), Vero (62.5 μg/ml), and baby hamster kidney-21 cells (64.1 μg/ml). For the MCF-7 an IC_{50} value of 55.7 μg/ml was determined (Zu et al. 2010).

4.3 Antioxidant Activity

In general, CGF show higher antidiabetic and antioxidant activity as their equivalent O-glycosides and aglycones. CGF, mainly vitexin (see Fig. 6) and orientin, isolated from pigeon pea leaves (*Cajanus cajan*) were found to be active in DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging experiment and was therefore successfully tested as stabilizer for blueberry juice (Xiao et al. 2016). From *Ficus deltoidea*, a traditional Malaysian tea, 16 CGF were extracted, whereas vicenin-2 (apigenin-6,8-*C*-diglucoside) was found to contribute with 9.1% antioxidative activity to the over-all effect of this tea. Other CGF do not elicit a notable antioxidant effect (Xiao et al. 2016). CGF of cayenne pepper (*Capsicum annuum*), widely used as spice, were found to act as antioxidant by superoxide radical scavenging and further were a strong inhibitor of xanthine oxidase. From these fruits, luteolin-6-*C*-glucoside, luteolin-6,8-*C*-diglucoside, and apigenin-6-*C*-glucoside-8-*C*-arabinoside were extracted. Luteolin-6-*C*-glucoside revealed the strongest antiradical-activity when tested in nonenzymatic β-nicotinamide adenine dinucleotide/phenazine methyl sulfate assay with an IC_{50} of 207.8 μM. In the xanthine oxidase test, apigenin-6-*C*-glucoside-8-*C*-arabinoside was able to elicit the strongest response with IC_{50} of 51.4 μM. In a DPPH model, only luteolin derivatives were active, whereas the apigenin derivatives were found to have no impact which can be traced back to the missing 3,4-dihydroxy group in the B-ring (Materska 2015). Barreca et al. found

the juice of sour oranges (*Citrus aurantium*) rich in CGF like lucenin-2, lucenin-2 4′-methyl ether, and vicenin-2 and was able to show a high antioxidative power mediated by reducing free radicals of DPPH by 48% and ABTS (2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt) by 75%. The authors conclude that lucenin-2 is the most important CGF in consideration of total antioxidant effect due to the catechol moiety which confers stabilization to formed radicals (Barreca et al. 2010). The same working group screened various *Citrus* varieties in order to gain insight into the CGF content distribution. In this study, it was detected that the main CGF of lemon and citron (*Citrus limon* and *Citrus medica*) was lucenin-2 4'-*O*-methyl ether. In case of orange (*C. aurantium*), clementine (*C. deliciosa*), and tangerine (*C. reticulata*), vicenin-2 was found as the most abundant substance. Kumquats (*C. japonica*) showed a totally different profile with phloretin-3′,5′-di-C-glucoside as main component. The flavonoids of one juice were extracted and tested for their ability to quench free radicals. The overall flavonoid fraction was able to reduce DPPH radicals by 50% and ABTS radicals by 80%. It was found out that the fraction containing lucenin-2 and vicenin-2 was co-responsible for this excellent antioxidant effect by bearing free and highly active phenolic hydroxyl groups and the essential double bond in the pyrone C-ring (Barreca et al. 2014). The seeds of lotus (*Nelumbo nucifera*) are used in China to cook soup and are used as herbal tea. An ethanolic fraction was successfully tested as antioxidant against DPPH radicals (1476.2 μM TE (trolox equivalents)/g) and ABTS (2974.0 μM TE/g) and exerted also an anti-inflammatory activity (see below) (Chen et al. 2019). Finger millet (*Eleusine coracana*) is considered as staple in developing countries and was found to include some CGF, in particular five apigenin derivates. Variations of this grain were tested for its ability to reduce radicals of DPPH in a range of 14.2–21.5 μM TE/g and ABTS from 19.0 to 26.8 μM TE/g. No declaration of CGF to a specific antioxidant activity was drawn (Xiang et al. 2019). Another food ingredient, mung beans (*Vigna radiata*) were found to possess antioxidant activity. The best result in the DPPH radical scavenging capacity assay was found to be 16.79 μmol TE/g when extracted with acetone-water. Further antioxidant assays were performed: ferric reducing ability of plasma assay (20.43 μmol TE/g), hydroxyl radical scavenging capacity (95.59 μmol TE/g), and oxygen radical absorbance capability with 184.14 μmol TE/g. Therefore, mung beans can be considered as natural antioxidant and furthermore anti-inflammatory activities were observed by this working group (see chapter ▶ "Anti-inflammatory"). A correlation was found between antioxidant activity and the anti-inflammatory activities (Xiao et al. 2016). From the seeds and sprouts of fenugreek (*Trigonella foenum graecum*), a popular spice used worldwide two well-known CGF were isolated, namely, vitexin and isovitexin. The antioxidant power of the ethanolic extract of the seeds was found to be 41.23 μg/ml (IC_{50}) in the nitric oxide radical inhibition assay. In the β-carotene assay, 50% inhibition was achieved with an extract concentration of 22.75 μg/ml. With this assay it can be shown that the compounds found in the extract are able to reduce the by-products of lipid peroxidation. Various antioxidative assays were conducted: Further results (anticancer and antibacterial effects) were obtained for fenugreek and can be found in the respective section of this chapter (Kadaikunnan et al. 2015).

4.4 Hepatoprotective Activity

Isoschaftoside was found to be present in green tea leaves. In an animal testing model, 0.1% of isoschaftoside was given to rats as a dietary supplementation and no change in growth and food intake was measured. It was found that the liver injury induced by D-galactosamine was partly reversed by this CGF due to a suppression of increase of plasma alanine aminotransferase and asparatate aminotransferase (Xiao et al. 2016). The leaves of beetroots (*Beta vulgaris*) host two flavonoids which are *O*-substituted C-glycosides – namely, vitexin-7-*O*-β-D-glucopyranoside and vitexin-2″-*O*-β-D-glucopyranoside. Rat liver cells were injured with carbon tetrachloride and the hepatoprotective effect of these two CGF was measured by looking at the release rate of glutamic pyruvic transaminase. The first CGF was found to be active at 65.8% and the latter one with 56.1% when tested at a concentration of 100 μM. This effect was comparable with silibinin, a substance known for its hepatoprotective activity. It was further noticed that vitexin has an inhibitory activity on cell death in hepatocytes induced by tumor necrosis factor α (TNF-α) (Xiao et al. 2016).

4.5 Anti-inflammatory Activity

The abovementioned fraction of lotus (see Antioxidant activity) was further tested against RAW 264.7 cells in a concentration of 12.5–50 μg/ml and was able to significantly reduce the production of NO radicals which are part in the development of inflammation. Furthermore, the flavonoids of lotus were able to reduce the production of proinflammatory cytokines such as interleukin (IL)-1β, IL-6, prostaglandin E2 (PGE-2), and TNF-α. Using affinity ultrafiltration LC/MS, the authors were able to identify potential cyclooxygenase (COX)-2 ligands. In the subclass of CGF vitexin was found to be the most promising COX-2 ligand (Chen et al. 2019). The working group of Kwon et al. determined the influence of glycosidation of flavonoids on nitric oxide production after lipopolysaccharide stimulation in BV2 microglial cells. It was found that most CGF were able to reduce the amount of NO produced. In relation to our topic, kaempferol-6-C-glucoside (present in lotus, figs and citrus juice) and luteolin-8-C-glucoside were still able to significantly inhibit the LPS-induced NO production. Especially the C-glycosidation in 6 and 8 position proved as superior (Xiao et al. 2016). A further aspect in inflammation is the vascular dysfunction. For this it was studied that orientin (and to a smaller extent also isoorientin) was able to inhibit the barrier disruption mediated by LPS and further blocked the leukocyte migration which leads to vascular permeability. These findings were also confirmed in an in vivo model with mice verifying the enhanced barrier integrity and reduced migration by a reduction of LPS-induced mortality. By various tests it was found out that orientin and isoorientin are able to inhibit MAPK and NFκB pathways. This study was able to draw structure-activity relationships: the glucoside position 8 (orientin) seems to be superior regarding the anti-inflammatory activity compared to the glucoside in position 6 as can be found in isoorientin.

Vitexin and isovitexin were also tested in this setting but did not elicit a positive effect due to the missing OH-group in position 3 (Xiao et al. 2016). In a study exploring the anti-inflammatory effect of the leaves of lemongrass (*Cymbopogon citratus*), a new luteolin-C,*O*-glycoside was described as cassiaoccidentalin B, and was found together with isoorientin in this plant. The glycosides were found to be less cytotoxic (up to 100 μM) compared to the aglycon luteolin. The CGF were found to possess no anti-inflammatory activity due to the decrease of activity by C-glycosylation. The effect on the activity of O-glycosides was less pronounced. It is also noted by the authors that macrophages release enzymes such as β-glucuronidase which are able to catalyze the deconjugation of flavonoids in the peripheral tissues of inflammation (Xiao et al. 2016). Various mungbean samples (*Vigna radiata*) were evaluated for their anti-inflammatory potential. For this, the production of various inflammatory markers was evaluated, namely, IL-1β, IL-6, and COX-2 in RAW 264.7 mouse macrophages. In the case of IL-1β, half of the samples of mung beans were able to inhibit the LPS-induced messenger RNA(mRNA) expression, whereas the highest inhibition ranged at 60%. A similar picture emerged when looking at the expression levels of IL-6. For COX-2, all samples were able to significantly inhibit the expression. The expression levels of IL-1β and IL-6 correlated with the anti-oxidant activity also reported from this study (see Antioxidant activity). Pure CGF vitexin and isovitexin and a physiological mixture of both as found in the beans were tested for their capacity to inhibit cytokine expression. It was shown that vitexin is of great importance for inhibiting the mRNA expression of COX-2, for both other cytokines no effect was shown and therefore other pure compounds such as phenolic acids are responsible for this phenomenon. Furthermore, no synergistic effect was achieved by the combination of both CGF (Xiao et al. 2016). A QSAR study performed with CGF and their corresponding anti-inflammatory activity showed various correlations. In general, C-glycosylation was preferable to O-glycosylation and the anti-inflammatory activity is increased by a glycosylation of ring A. In return, the activity is reduced by methoxylation of the hydroxyl groups in the flavone and the higher the polarity of C-2″-moiety is (Xiao et al. 2016), see Fig. 6.

An ethanolic extract of two varieties of muskmelon (*Cucumis melo*) was found to possess in vivo anti-inflammatory activities. By an UPLC-MS/MS setup among other flavones and flavonols, CGF such as isovitexin and isovitexin-2″-*O*-rhamnoside were found. Animals were orally pretreated with 25 and 50 mg/kg ethanolic extracts of peels and pulps of *Cucumis melo* var. *cantalupensis* and *Cucumis melo* var. *reticulatus* and inflammation was induced with carrageenan in the hind paw. All extracts were able to significantly inhibit the formation of edema after 3 h. The best result was obtained with the pulp of *Cucumis melo* var. *reticulatus* (50 mg/kg) reaching an inhibition of 69.41%. After this time point, the results of edema volume reduction were comparable with indomethacin for the pulp of *Cucumis melo* var. *reticulatus* (50 mg/kg) and the pulp of *Cucumis melo* var. *cantalupensis* (25 and 50 mg/kg). The extracts were also found be able to reduce pro-inflammatory cytokines like IL-1β, IL-6, PGE-2, and TNF-α in various extents (Ezzat et al. 2019). Another study conducted by Borghiet al. focused on the effect of the pure compound vitexin. Various assays were performed to evaluate the anti-

inflammatory and antinociceptive (for further details see section below) mechanisms of vitexin. In the carrageenan assay, this CGF was able to reduce the production of pro-inflammatory cytokines (IL-1β, IL-6, IL-33, and TNF-α) and in the same time to induce IL-10 which is known as anti-inflammatory marker. Therefore, it can be concluded that vitexin is able to reduce inflammatory pain (Borghi et al. 2013). An extract rich in vicenin-2, schaftoside and isoschaftoside was administered to rats in a carrageenan paw swelling model in a concentration of 25–100 mg/kg. This treatment with the extract significantly reduced the nonspecific inflammation, whereas the highest swelling was maintained after 4 h. In the highest concentration (100 mg/kg), the extract was superior compared to the well-known anti-inflammatory drug aspirin, which was used as control substance (Xiong et al. 2015).

4.6 Antimicrobial/Antiprotozoal Activity

From the seeds and sprouts of fenugreek (*Trigonella foenum graecum*), a popular spice used worldwide, two well-known CGF were isolated, namely, vitexin and isovitexin. Gram-positive bacteria were susceptible for this extract and showed good activity whereas gram-negative bacteria were of low activity. Highest inhibition zones were achieved by the highest concentration tested (1000 μg of extract). Therefore, especially *Bacillus subtilis*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* were susceptible to the fenugreek extract. In a smaller extent, the extract was also active against *Salmonella typhi* and *Escherichia coli*. Furthermore, various fungi were tested and it appeared that *Candida albicans*, *Penicillium notatum*, and *Aspergillus flavus* were vulnerable by these extracts (Kadaikunnan et al. 2015). Various flavonoids were tested against *Trypanosoma* and *Leishmania* strains. Vitexin was only moderately active against *T. brucei rhodesiense* with an IC_{50} of 55.7 μg/ml, whereas the aglycone apigenin was active against all three tested strains with lower IC_{50} values (Xiao 2017). In the leaves of the Brazilian plant *Serjania erecta*, two CGF were identified – vitexin and isovitexin. An ethanolic extract was produced and tested against various bacterial strains. For subsequent bacterial strains (minimal inhibitory concentration (MIC)) values of $\leq$15 μg/ml were detected: *Pseudomonas aeruginosa* (performed the best with MIC of 5 μg/ml), *Escherichia coli*, *Saccharomyces cerevisiae*, and *Staphylococcus aureus*. No activity was found for *Mycobacterium tuberculosis* (MIC of 128 μg/ml) (Cardoso et al. 2013). The leaves of *Centaurea alexanderina* were extracted with methanol and then screened for their constituents resulting in three reported CGF: vicenin-2, vitexin, and isovitexin. Various extracts of this plant were screened against a variety of bacterial strains, whereas only *Pseudomonas aeruginosa* was susceptible against the methanolic extract with an inhibition zone of 40 mm (Kubacey et al. 2012). An extract from *Carissa opaca,* which was found to be rich in vitexin, isovitexin, and orientin, was tested against various bacterial strains. *Bacillus subtilis*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Staphylococcus aureus* were susceptible to a varying extent with a MIC of $\leq$5 mg/ml. No breakdown of components to a distinct activity was done in this study.

Furthermore, the inhibition of various fungi, i.e., *Aspergillus flavus*, *A. fumigatus*, and *A. niger* was successfully tested for this extract (Sahreen et al. 2013). Another extract with the same compounds, namely, a mixture of vitexin, isovitexin, and orientin, was detected in an ethanolic and a CO_2 extract of the leaves of *Cajanus cajan*. The CO_2 extract (MIC $\leq$2.5 mg/ml) showed much lower MIC values compared to the ethanolic extract. Bacterial strains such as *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* were found to be vulnerable by this plant extract. Fungi species such as *Aspergillus niger* and *Candida albicans* were also susceptible. The CO_2 extract showed a higher activity against gram-positive bacteria than gram-negative bacteria. With *S. aureus* a time-kill assay was conducted with the CO_2 extract in order to evaluate the death rate in relation to drug and time. For this, a concentration of the respective MIC value and ½ MIC was tested over 4 h. Bacterial cells were able to survive the ½ MIC concentration, but with MIC concentration (39 μg/ml) cells were dead within these 4 h. An in vivo experiment with mice was conducted where animals were infected with *S. aureus* and either administered penicillin or the CO_2 extract (30 or 60 mg/kg intragastrically). After 4 weeks the mice were sacrificed and liver and spleen weights were significantly higher as in the control group. This could be traced back to the inflammation going on in these organs. Other organs were in the same range as the control group. It is mentionable that the group which received the CO_2 extract had reduced inflammation rates, even at the low dose. Therefore, it can be concluded that this extract works in vivo and no direct damage is originating from the extract (Zu et al. 2010).

4.7 Antinociceptive Activity

Urtica circularis, a herb cultivated in Argentina, is added to food due to its high content of minerals and vitamins. An ethanolic extract was produced from the aerial parts and a HPLC measurement showed the presence of mainly vicenin-2 but also a small amount of vitexin in the extract. In an experiment where pain was induced by formalin and the licking time of mice was observed, the extract was administered intraperitoneally at 30 mg/kg and 100 mg/kg doses and an additional 500 mg/kg by oral route. The licking time, checked between 15 and 30 min after exposure, was markedly reduced by 78.5%, 91.0% and 88.5% respectively. For this, an ED_{50} of 15.8 mg/kg was calculated. When active in the late phase, substances are attributed to act as antinociceptives by peripheral mechanisms (no involvement of the opioid system). By testing the extract in combination with atropine, an involvement of the cholinergic system could be documented. The writhing test, where pain was induced by acetic acid, was also performed and prior testing the mice were administered i.p. with 10–300 mg/kg *Urtica* extract. The extract dose-dependently inhibited the writhing of mice, whereas the maximum of 80.6% inhibition was achieved with the 300 mg/kg dose surpassing the effect of 10 mg/kg indomethacin i.p. (ED_{50}: 72.2 mg/kg). The 500 mg/kg p.o. dose inhibited the writhing by 48.4%. The experiments showed that the combination of *Urtica* extracts in not effective

concentrations (50 and 100 mg/kg p.o.) with indomethacin (3 mg/kg p.o., also no effect) gave rise to a promising antinociceptive effect. Furthermore, pure compounds found in the extract were tested as control. 10 mg/kg i.p. of vitexin and vicenin-2 were administered and resulted in an inhibition of 91% (again superior compared to indomethacin 10 mg/kg) and 41%, respectively. In contrast, apigenin did not elicit any effect. As gastrointestinal side effects often occur with antinociceptive drugs, the effect of the extract was observed on the stomach were only mice administered with 300 mg/kg and indomethacin 10 mg/kg were tested positive for lesions in the stomach (extract: 71% vs. indomethacin: 100%). When administered with 100 mg/kg *Urtica* extract, no difference was observed to control group which were fed only with water. Furthermore, the acute toxicity of the *Urtica circularis* extract was tested in oral concentrations up to 3000 mg/kg and no signs of toxicity were observed (Gorzalczany et al. 2011). This effect of vitexin was confirmed by another working group. Mice were pretreated with 0.3–10 mg/kg of vitexin i.p. and 30 min afterwards writhing was induced by acetic acid which led to a dose-dependent inhibition of writhing score. Another assay with phenyl-p-benzoquinone was performed where also the writhing was inhibited by vitexin. For this reason, a participation of nociceptive mechanisms which are attributed in both assays such as prostanoids, IL-33, and endothelin-1, is feasible (Borghi et al. 2013). The leaves of *Centaurea alexanderina* were extracted with methanol and then screened for their constituents resulting in three reported CGF: vicenin-2, vitexin, and isovitexin. Writhing was induced with acetic acid and mice were pretreated with doses of 300 and 600 mg/kg leaves extract. These doses significantly reduced the writhing by 35% and 49%, respectively, whereas aspirin (100 mg/kg) as reference compound reached 72%. An additional experiment where a nociceptive irritation was induced by formalin was conducted. Especially the second pain phase involving inflammation was significantly reduced by 47% (300 mg/kg) and 58% (600 mg/kg), respectively. Aspirin again inhibited paw licking by 71%. In the tail flick test, only the higher concentration of 600 mg/kg extract was able to inhibit the mechanism significantly. In the hot plate test, the extract was able in both concentrations to delay the thermal irritation but was less pronounced as with tramadol as positive control substance. One last test was performed inducing edema with carrageenan. 600 mg/kg of extract was able to protect 41% of the edema production after 4 h and was therefore significant. As positive control indomethacin was used in this experiment showing a protection of 48%. An inhibition of the prostaglandin production is discussed as the analgesic effect of this plant. With all these five tests, it can be concluded that *C. alexanderina* is able to peripherally (writhing and paw licking) and centrally (tail flick and hot plate) inhibit the emergence of pain (Kubacey et al. 2012).

4.8 Various

Vitexin and isovitexin, isolated from mung beans (*Vigna radiata*), were tested for their ability to inhibit tyrosinase (see Fig. 6). Tyrosinase is an essential enzyme in the melanin synthesis and therefore applicable in cosmetic industry for

hyperpigmentation and as whitening agent. In an in vitro assay vitexin and isovitexin were found to inhibit tyrosinase with an IC_{50} of 6.3 and 5.6 mg/ml, respectively (Xiao 2017). The sprouts of buckwheat (*Fagopyrum esculentum*) showed promising results in an in vivo study against stress in mice. The sprouts of this grain are rich in rutin, orientin, isoorientin, vitexin, and isovitexin. Mice were intragastrically given a 100 mg/kg buckwheat sprout solution and then exposed to stress (limited food and immobilization). The control group (no buckwheat in the solution) showed elevated corticosterone, glucose blood level, hyperlipidemic and thiobarbituric acid reactive substance values. The last substance is assayed in order to gain insight into the oxidative damage effect of stress. All these factors were downregulated by buckwheat flavonoids and therefore are a promising candidate for antistress treatment. Furthermore, plasma concentrations were determined for each CGF in buckwheat sprouts and it was found that 2 h after oral administration all CGF were intact (Xiao 2017). Isovitexin and vitexin were isolated from various plants of Argentina and tested for their ability to reduce oxidative stress (ROS) which was induced by gentamicin. Both CGF were able to inhibit the reactive oxygen species production in mononuclear leucocytes with 22.4% and 34.3% when 10 μM of the flavonoid was administered. In an additional experiment, IC_{50} values of ROS production were determined resulting values for isovitexin and vitexin of 75.36 and 22.7 μM, respectively. As reference substance vitamin C was tested and lead to an IC_{50} of 1.06 μM. The actual antistress experiment was only conducted with luteolin which was able to show a significant decrease of ROS compared to the control group. Luteolin was also able to reduce lipid peroxidation in this context. Furthermore, it was able to enhance the susceptibility of *Escherichia coli* and *Staphylococcus aureus* in combination with gentamicin (Bustos et al. 2018).

5 Benefits (Human Studies)

Increasing interest in flavonoids lead to an increasing number of research studies which step-by-step shed light on the metabolism and activities of this class of substances. Since not all flavonoids from one class behave in the same way in the body the clarification and testing of various flavonoids of one class are needed. Due to the fact that in vivo studies with humans are very limited, general conclusions are difficult to make. Especially the family of C-glycosylated flavonoids need a lot more attention. From the literature, it can be concluded that C-glycosylation is beneficial for the antioxidant and antidiabetic activity. In general, flavonoid glycosides are able to create higher plasma levels than their corresponding aglycones. The influence of the attached sugar has to be investigated very carefully in order to predict absorption and metabolism in vivo (Xiao 2017).

The bioactivities of CGF can be seen in subchapter 4 (► "Bioactivities") which presents some promising effects such as antidiabetic and anti-inflammatory action. However, until the application in humans against diabetes is feasible, a lot more research has to be done.

In plant derived food, CGF do not occur as singular phenolic compounds, but are embedded in a complex mixture including also other types of flavonoids except CGF.

There are some human studies of dietary flavonoids which have been comprehensively reviewed by Crozier et al. (2010) and Del Rio et al. (2013). Unfortunately, no CGF were specifically examined in these studies. Some data is available for CGF which only show the metabolization of CGF by intestinal bacteria. They are able to cleave the sugar moiety and release the pure aglycone. For instance, it was found that the *Lachnospiraceae* CG19-1 strain is able to metabolize the CGF vitexin to its aglycone apigenin. Also, the binding affinities of flavonoids to human serum albumin are documented in literature. For the bioactivity, human cell lines were utilized but no in vivo data for humans is available until now for CGF.

One aspect in literature has to be taken into account when dietary flavonoids are administered for boosting health. It is reported that flavonoids could be substrates for p-gp which leads to an efflux of these compounds back into the lumen of the gut. Therefore, no effect is exhibited by these administered flavonoids. Another aspect is the importance of p-gp in cancer chemotherapy. Overexpression of p-gp plays an important role in the development of cancer cell resistance. The working group around Di Pietro et al. tested various flavonoids as potential efflux pump inhibitors against various efflux transporters. The in silico predicted binding affinity for vitexin was so low that no further experiments were determined. But in the overall experiment it was shown that glycosylation of flavonoids is detrimental for the binding affinity to efflux transporters (Di Pietro et al. 2002).

6 Application in Food

Data on alterations of CGF in plant derived food during processing are quite limited. In rooibos (leaves and stems) a significant difference in unprocessed and fermented herbs can be observed. Fermentation leads to a severe loss of aspalathin which undergoes oxidative cyclisation to the corresponding flavanone C-glycosides. Further oxidation results in flavone C-glycosides (Courts and Williamson 2015; Hostetler et al. 2017). Oxidation is also a prerequisite in processed cocoa seeds to form C-glycosylated flavan-3-ols nonenzymatically. This process leads to a less bitter product as the puckering astringent taste of epicatechin and catechin is converted to a smooth astringent sensation without exhibiting a bitter taste (Stark and Hofmann 2006; Courts and Williamson 2015). High temperature applied for sterilization and preparation of food products indicate a high probability of such nonenzymatically generated C-glycosides, which could be present in the human diet. Furthermore, undergoing a Wessely-Moser rearrangement isomerization concerning C-6 or C-8 position of bound monosaccharides under cooking conditions could take place (Courts and Williamson 2015). For kudzu roots extracts, containing significant amounts of the isoflavonoid C-glycoside puerarin, cooking in beef patties did not lead to a relevant loss of puerarin content, indicating stability of this isoflavonoid C-glycoside during cooking (Kumari et al. 2015). Of course, as with water soluble glycosides cooking will leach out these compounds from the food material, however,

generally a higher stability compared to their O-glycosidic counterparts can be expected.

7 Safety: Toxicity and Side Effects

Studies on antinutritional effects have been performed for pearl millet (*Pennisetum glaucum*) (Gaitan et al. 1995; Boncompagni et al. 2018). Aside from the most relevant antinutrient compound, phytate, CGF were reported to have unwanted goitrogenic effects. The compounds in question are vitexin, glucosyl vitexin, and glucosyl orientin. Epidemiologic studies indicate that endemic goiter in rural areas of Africa and Asia might be related to a diet based on pearl millet as staple food (Boncompagni et al. 2018). In vivo antithyroid activity by purified vitexin could be shown in rats at 80 μM test concentration, 20 μM was not active (Gaitan et al. 1995), supporting this assumption.

In a study of Fantoukh et al. (2019), the influence of a methanolic extract of unfermented rooibos tea and selected chemicals of this plant material including the C-glycosyldihydrochalcones aspalathin and nothofagin as well as the C-glycosylflavones vitexin, isovitexin, isoorientin, and the C-glycosylflavanone eriodictyol-6-C-glucoside on interaction with cytochrome P450 enzymes (CYP), pregnane-X-receptor (PXR) and p-gp were investigated. Whereas the results obtained with the methanolic extract are only of limited relevance for infusions made for food purposes, the activities of pure compounds could give some indications of potential interactions with simultaneously taken pharmaceutical drugs or other food phytochemicals. Most prominent inhibitory effects were found for isovitexin on CYP3A4 (IC_{50} 3.4 μM) and for vitexin on CYP2C9 (IC_{50} 8.0 μM). Aspalathin and nothofagin exhibited only a moderate activation of PXR, associated with an increased mRNA expression of CYP3A4 and CYP1A2.

8 Marketed Products

There are no food products on the market specifically due to CGF content. Compared to food products made from plain flour, higher contents of CGF can be found in corresponding food products prepared from whole meal flour (Bucar, unpublished data; Hirawan and Beta 2011). However, these products are generally richer in plant phenolics. Rooibos herbal teas might serve as an example which focuses on a CGF (aspalathin) as active ingredient (Courts and Williamson 2015; Hostetler et al. 2017).

9 Patents

One Chinese patent describes the preparation of a C-glycosylflavones extract and a preparation method thereof, among others bamboo leaves are mentioned as source as well as blueberry without defined species (CN 103159750 A 20130619). Several

patents have been filed for rooibos and aspalathin, i.e., an antidiabetic extract from rooibos (US 8877717 B2 20141104), an aspalathin-like dihydrochalcone, extracts from unfermented rooibos and the treatment of the central nervous system (EP 2053050 A1 20090429), an antidiabetic extract of rooibos (WO 2008110551 A1 20080918), related to that a product which contains aspalathin and acts as a pancreatic β cell insulin secretion and tissue glucose promoting agent (JP 2008214274 A 20080918), an anticancer agent containing aspalathin (JP 2007197409 A 20070809), and a rooibos extract with increased aspalathin content (WO 2006081989 A1 20060810). Numerous patents have been filed for puerarin containing plant materials, and this is true also for vitexin and vitexin containing materials. To mention a few, a process to extract passion fruit skin for a phenolic rich extract contain CGF like vitexin and orientin was described (BR 102016030133 A2 20180717), similarly taro bean leaves (CN 107281258 A 20171024) as well as common buckwheat husk (CN 105859701 A 20160817) or peeled mung bean (KR 2009045964 A 20090511) can serve as source for CGF.

10 Perspectives

The major class of C-glycosylflavonoids in food plants is represented by flavones, in addition dihydrochalcones and some selected examples from C-glycosylisoflavones can be found. Citrus fruits can be considered as a major source of C-glycosylflavones, whereas relatively low amounts have been found in cereals with exception of millet seeds and carob seed germ flour. A rich source of C-glycosylated dihydrochalcones are tomatoes, as well as rooibos and honeybush herbal teas, and the most common C-glycosylisoflavone puerarin is mainly consumed via kudzu roots.

Due to their higher chemical stability in terms of hydrolysis during cooking and also after ingestion, they can be considered as a specific group within flavonoids. Their metabolic fate is clearly different from O-glycosidic flavonoids with absorption of intact glycosides, followed by phase II metabolization. However, also deglycosylation by gut microbiota and degradation of aglycones to compounds like (hydroxy) phenylpropionic acids have been recognized. The catabolic potential of gut microbiota for C-glycosylflavonoids seems to be still underestimated with insufficient data on microbial community – catabolic activity correlations. It can also be recognized that only limited data on the actual daily intake of C-glycosylflavonoids including information on content in fresh and processed food are available. This is also important for considering the appropriate doses for in vitro and in vivo studies which have shown a wide range of bioactivities of C-glycosylflavonoids.

11 Cross-References

► Biflavonoids and Oligomeric Flavonoids from Food
► Chalcones in Diets

▶ Citrus Flavanones
▶ Dietary Flavones and O-Glycosides
▶ Dietary Flavonols and O-Glycosides
▶ O-Glycosides
▶ Prenylated Flavonoids in Food
▶ Soy Isoflavones
▶ Tea Catechins
▶ Theaflavins, Thearubigins and Theasinensins

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