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Flavonoids, the Role and the Importance in Modern Investigations

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Abstract: The flavonoids are aromatic secondary plant metabolites, which belong to the class of plant polyphenolics. Structurally they are heterocyclic π-electron systems built upon a C_6H_5(A)-C_3-C_6H_5(B) flavone skeleton in which oxygen is the heteroatom. A group of flavonoids is differentiated in several classes according to the degrees of oxidation and unsaturation of the heterocyclic C ring.

Because of the remarkable array of biological and pharmacological activities, physiolological effects, complexity of the biosynthesis and metabolism, possible industrial applications and constantly rising commercial interest and relevance in the processes involved in nutrition, flavours, and aromas flavonoids, especially anthocyanins and flavones, are receiving renewed attention of many researchers during the last decade.

A considerable number of plants which contain flavonoids are reported to exert a wide range of positive health attributes, antimutagenic, antibacterial, anti-inflammatory, antiallergic, antiviral, anti-thrombotic, and vasodilatory actions, mainly arising from their antioxidant ability. Anthocyanidins and their glycosylated forms, anthocyanins, as the most widely spread class of the flavonoids, are the most intensively colored substances responsible for the existence of the most red, blue and purple colours in flowers and fruits. Increasing public concern about the safety of synthetic food colorants influenced the greater prominence of these molecules. Anthocyanins have a very long history as a part of a human diet resulting in appreciation of good food quality, flavour identification and taste thresholds influencing food preference and acceptability. The fact that these molecules are one of the thirteen pigment classes, derived from natural sources, permitted in Europe emphasise the importance of their study.

Key words: flavoniodes, biological, physiological, antioxidant activities

Introduction

Flavonoids and Flavonoid Compounds

Together with chlorophyll and carotenoids flavonoids represent the main pigments in the plant world (1-4). The term "flavonoids" was introduced by Geissman and Hinreiner in the middle of twentieth century with the aim to unite the class of heterocyclic compounds with the basic structural unit, 2-phenylchromone (flavone) (lat. "*flavus*"-yellow) (Fig. 1). The basic flavonoid structure consists of 15 carbon atoms arranged in three rings, two benzene rings joined with the γ-pyrone ring (C_6H_5(A)-C_3-C_6H_5(B)). A group of flavonoids is differentiated in several classes according to the degrees of oxidation and unsaturation of the heterocyclic C ring. The major flavonoid classes are: catechins, dihydroxychalcones, chalcons, flavanones, flavanols, flavones, isoflavones, anthocyanidols, aurones and flavonols (Fig. 2). Catechins represent reduced structural forms while the flavonols are oxidized forms. The number and the position of functional groups, mainly hydroxyl and methoxyl, introduced on the phenyl rings, distinguish structurally different compounds of the individual flavonoid classes.

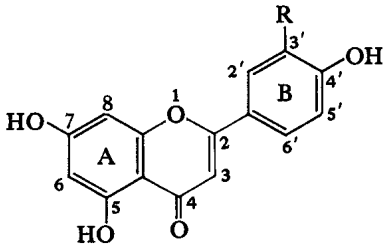

Fig. 1. Structural formulae of 2-phenylchromone (flavone)

Under *in vivo* conditions flavonoids generally occure in glycosylated forms (3, 4). Glycosydification proceeds via semiactel bond formation between hydroxyl groups of the pigment molecules and one or more suger molecules. Glycosydification enchenses the solubility and the stability of the molecules, especially of those which are more hydroxylated. Sugar molecules involved in glycosydification are simple hexsoses and penhtoses as monosacharides and di- and tri- sacharides as biosides and triosides.

Anthocyanidins represent one of the most important and the most widely spread classes of the flavonoids. Structurally, anthocyanidins aree hydroxylated and metoxylated derivates of flavylium, 2-phenyl-1-benzopyrilium, salts (1-4). Depending upon the nature of the C3 substituent flavylium salts are devided into: anhydroxy, 3-deoxy and 3-hydroxy flavylium salts. In nature there are six common, 3-hydroxy, flavylium salts, antocyanidins: pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin. The main antocyanidin structural feature is 3, 5, 7, 4'-tetrahydroxy flavylium cation. As other classes of flavonoids anthocyanidins in nature occure in glycosylated forms known as anhocyanins (Fig. 3).

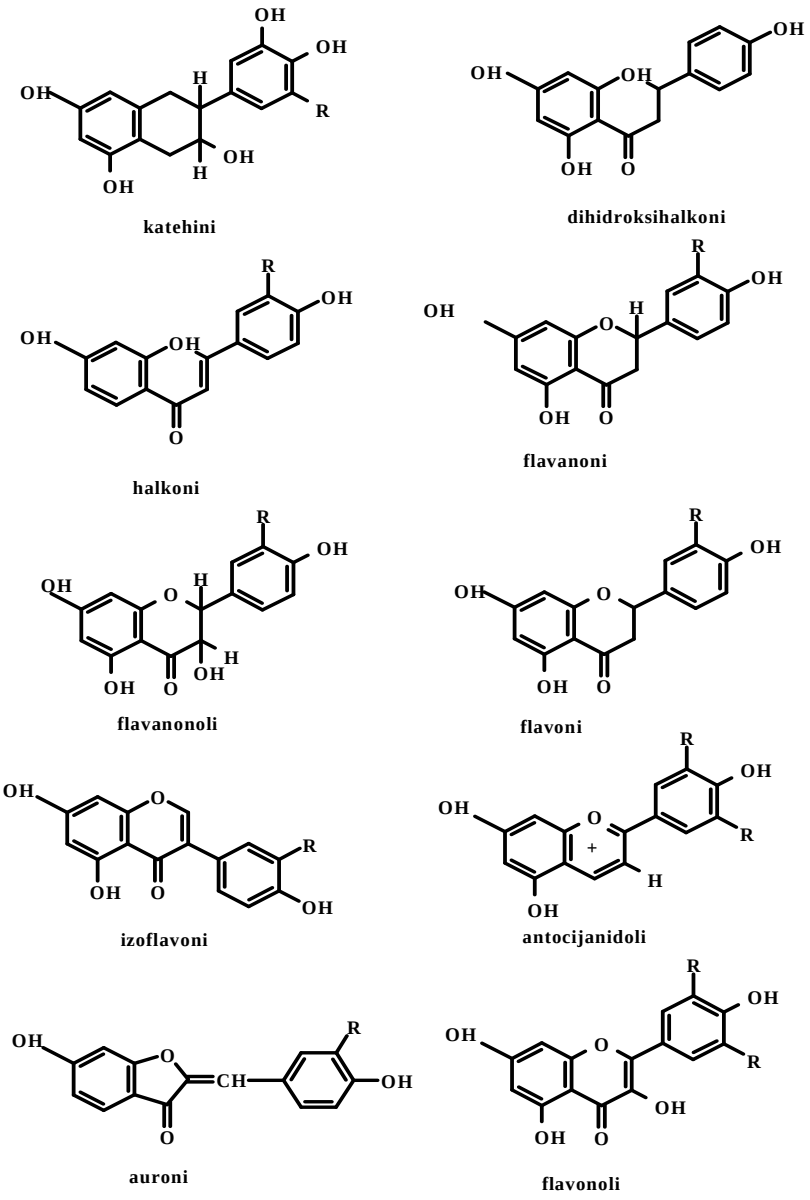

Fig. 2. General structural formulae of the compounds from the flavonoid class

The word anthocyanin has been introduced by Marquart in 1835 (1). The word has Greek origin meaning *"kyanos"- blue* and *"anthos"* – flower. These pigments as the most intensively colored are responsible for the existence of the most red, blue and purple colours in flowers and fruits. Their structure is built upon a 15 C

carbon skeleton in which oxygen is a heteroatom. Oxygen atom as positively charged causes the basic reaction of the whole molecular structure which is also called "oxygen plant base". This characteristic of the anthocyanin structure is associated with their behaviour as cations in acidic environment and as anions in basic environment. The general anthocyanin structure in acidic medium is usually presented as $[C_{15}H_{11-n}O\ (OH)_n]^{+}\ X^{-}$ where X^{-} is the anion of some of the strong inorganic acids (ClO_4^-, Cl^-, J^-).

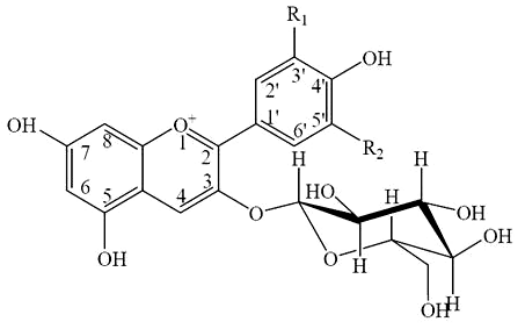

Fig. 3 General structural formulae of the one 3-glycoside

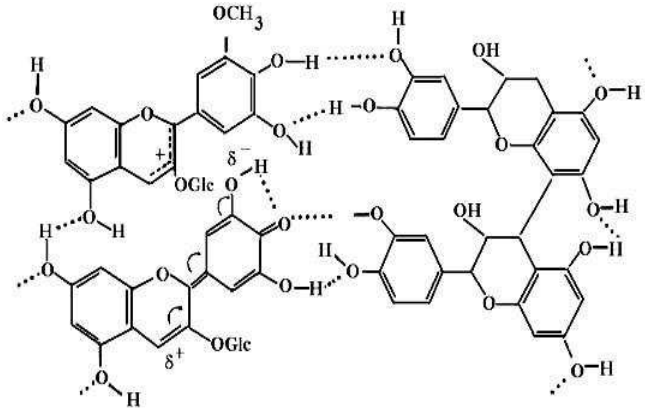

Fig. 4 Intramolecular copigmentation model between flavylium chromophore and dimer form of the flavane molecule present in red wine

The natural environments of anthocyanin pigments are cell vacuoles that are essentially aqueous, slightly acidic or neutral. Except in strongly acidic medium, which is not characteristic to natural environment, flavylium chromophore, responsible for the colour of these molecules, is highly unstable and poorly coloured. The stability of the structure of these molecules *in vivo*, conditioning the stability of their colour, is influenced by the number and the

nature of the substituents, pH value of the medium, soil composition and climate conditions. Their stability is also partially improved by glycosydification and acylation but also very dependent upon possible metal complexation, copigmentation and self-association reactions (5-8).

Biological and Physiological Activities of Flavonoids

The flavonoids are found ubiquitously in plants (1-3). They play different roles in the ecology of plants. To some extent flavonoid action in plants may be related to primary metabolism. Due to their brilliant colors, coming primarily from anthocyanins, they act as attractors for pollinating insects. They also act as catalysts in the light phase of the photosynthesis and as regulators of iron channels associated with phosphorylation. The numerous investigations provided some circumstantial evidence that flavonoids are involved in the UV-protection of plants, protection of plants against microbial invasion, protection from insects and mammalian herbivory. (9)

Flavonoids are also very important part of human diet. The consumption of fruits and vegetables play a preventive role, which is due to a variety of constituents, including minerals, vitamins, fiber and numerous phytochemicals among which flavonoids are very important (9).

The early results obtained in the first half of the nineteenth century pointed out the possible physiological activity of the flavonoids on humans. The investigation of the hemostatic activity of the naturally occurring and synthetic vitamin C emphasized the greater activity of the naturally occurring vitamin C from the citrus fruit. Active compound, firstly named vitamin P, was later identified as a flavonoid one (2, 3). Flavonoids exhibit various physiological activities including anti-allergic, anti-mutagenic, anti-hypertensive, anti-inflammatory, anti-viral, anti-arthritic, inhibitory and other activities (9). Among the variety of flavonoid activities inhibitory activity on some enzymatic systems is the most pronounced one (10-12). The inhibition of the NADH-oxidase is characteristic to flavonoids which possess keto group in the C4 position, double C2-C3 bond and 3', 4', 5'-trihydroxy system in the "B" ring. Anti-inflammatory activity is manifested in their ability to inhibit certain enzymes like cyclo-oxygenase and 5-lypoxygenase which in their metabolic cycles cause edema, dermatitis, arthritis, gout and other pathological states (13). The anthocyanins of tart cherries are assayed for their anti-inflammatory activity. It was observed that the fruit consumption, especially cherries consumption, alleviate arthritic pain and gout pain (13). Also some other simple flavonoids, as quercetin and apigenin exibit anti-inflammatory activity manifested through inhibition of fibroblast growth even at very low concentrations. This activity is very beneficial for the treatment of skin injury, in granulation and scar tissue formation (14). Some of the flavonoids such as quercetagetin 3,6,7,4'- tetramethylether and quercetagetin, may act as antispasmolytic agents causing relaxing of the smooth muscles during respiratory complications. The use of quercitrin (quercetin 3-rhamnoside) in traditional medicine for the treatment of diarrhea and dysentery is also known for many years.

Overwhelmingly, biological and physiological activities of flavonoids are generally related to their antioxidant activity which arises from their ability to scavenge free radicals (15).

Antioxidant activity of flavonoids

Reduction of atmospheric oxygen is one of the ways in which its free radicals can be formed (superoxide anion, hydroxyl-, peroxy-, alkoxyl-, nitric oxide). They are highly reactive oxidative species that are able to chemically react with a great number of organic compounds in biological and chemical systems.

Free-radical reactions are implicated in the ethiologies of many diseases as well as in aging process itself. Besides only a few "good" free radical reactions such as photosynthesis, rib nucleoside reduction and phagocitoses the majority of free radical reactions are associated with pathological states causing cell damages and enhancing mutagenic and carcinogenic processes (10). Since chemical reactions that involve free radicals can also affect physical, chemical, and biological properties of food, pharmaceutical, cosmetic, and other products research into the possibilities of their inhibition is of high importance.

It is a known fact today that many natural products whose structure is characterized by the existence of a phenolic or catecholic functional group show activity in oxidative processes with air oxygen, which gives them antioxidant properties (16, 17). The possibility of inactivating air oxygen also posses enzymatic systems, tockopherol (vitamin E), ascorbic acid (vitamin C), flavonoids, carotenoids and other naturally occurring compounds (9, 10). Antioxidant compounds, besides the free radicals scavenging, also eliminate singlet oxygen, reduce certain compounds, terminate the propagation phase in which hydro-peroxy lipids are formed and efficiently protect important bio molecules (lipids, proteins, nucleic acids) from oxidation (9, 10).

As pointed out earlier many studies suggested that flavonoids exhibit biological activities, including anti-allergic, anti-viral, anti-inflammatory and vasodilatory actions. However, most interest has been devoted to their antioxidant actvity which arises from their ability to reduce and scavenge free radicals. Among all classes of flavonoids those of the particular interest are flavones, isoflavones, flavanones, flavonols, anthocyanidins and their glycosidized forms, anthocyanins (16).

It has been shown that the structural features are very important for high antioxidant activity of flavonoids (17). The obtained results provide clear evidance that the radical scavenging activity depends on the structure and the substituents of the hetreocyclic B ring. It is known that *ortho* substituents in the B ring, especially those with electron donating capabilities, reduce the redox potentials of the molecules and enhance the free radical quenching. Among major determinants are also the presence of the carbonyl group at C4, a double bond between C2 and C3 conjugated with the 4-oxo group enabling higher electron delocalization and C3 hydroxyl group present in flavonols. It is generally observed that the glycosylation of the hydroxyl groups, especially at C3 position, due to steric hindrance effects, greatly reduces antioxidant capacity of the certain classes of the flavonoids.

As powerful antioxidants flavonoids play important physiological role not only in plants but also in humans (10). A number of studies have revealed that flavonoids act as antioxidants by scavenging reactive oxygen species. In

human organisms, in chain free radical reactions, the highly reactive oxygen radicals are produced in self oxidation reactions of fatty acids causing various types of abnormalities on the cellular level. The protective role of flavonoids is manifested in their capability to "sacrifice" first in the oxidative processes transforming free radicals into stable, deprotonated, forms. Antioxidant activity of flavonoids also correlates well with their capacity to diminish the oxidative stress which is known to participate in the initial process of atherosclerosis leading to coronary heart disease and other patho-physiological states.

The association between flavonoid consumption in human diet and cancer protection is also present but, at some instance, not very strong (18-20). Some studies have shown that the flavonoid consumption is directly correlated with the inhibition of the proteolytic urokinaze enzyme which invades cells and form metastases. The regular flavonoid consumption is also correlated with stopping cancer growth and angiogenesis. There is also increasing level of research in plant flavonoids for treatment and controlling AIDS virus, early stages of Alzheimer's disease, memory impairment and vascular dementia.

The antioxidative activity of flavonoids, besides the direct free radical scavenging, include also copigmetation and trace metals chelation reactions (21-25). Those reactions play important and multiple roles in biological systems. They are very sensitive and powerful colour stabilization mechanism developed in higher plants and also very important for normal physiological functioning of biological systems. The formation of flavonoid-DNA copigment complexes prevents the oxidative damage of the DNA molecule. It is also known that flavonoid-ascorbic acid copigment complexes formation prevent oxidative degradation of the ascorbates, which have multiple functions in the organisms. A great number of flavonoids can efficiently chelate trace metals like Fe(II), Fe(III), Cu(II) or Zn(II). By chelating metal ions flavonoids generally prevent the metal-catalyzed free radical generation and their reactions and accordingly can protect the very important biologically active molecules from oxidative stress (23-25). Some investigations show that complexed flavonoid molecules exibit more effective radical scavenging action than uncomplexed molecules.

Anthocyanins of grapes and wines

Grapes are edible fruits within the Vitaceae family of the buckthorn order. Grapes are an ancient food present in human diet more than 3000 years, dating back to the Bronze Age in Europe. Today grapes are cultivated seeds, commercially cultivated around the world, predominately in Western Europe, South Africa, the Balkans, California, Australia and parts of South America. The major species currently consumed belongs to Vitis vinifera family (26).

Phenolic compounds are secondary cell metabolites concentrated in the grape′s skins and the seeds which do not have nutritive value but protective role and also very important role influencing organoleptic characteristics of grapes, wines and juices. A variety of phenolic compounds are present in grapes (26, 27). They impart a colour to grapes, contribute to undesirable enzymatic browning reactions and add astringency and bitterness. The certain number of factors are

conditioning the specific amounts and types of phenolics compounds present in grapes. They are: variety, seasonal conditions, maturity, storage, and processing.

Generally there are four major types of phenolic compounds found naturally in grapes and throughout the plant kingdom. They are: hydroxycinnamic acids, phenolic acids, flavonoids and tannins. Gallic acid is the major phenolic acid in grapes and its amount is greater in red than in white wines. The major hydroxylcinnamic acids are: caffeic acid, ferulic acid, p-coumaric acid and chlorogenic acid as quinic glycoside of caffeic acid. The most abundant phenol components present in grapes are flavonoids, especially flavonols (kaempherol, quercetin and myrecitin), catechins and epycatechins. Anthocyanins are also present in grapes and those molecules sets up the major differences in colour and the composition between red and white species. The major anthocyanins are different cyanidin, malvidin and peonidin glycosides In addition, grapes contain also different acylated anthocyanins (27, 28).

The grapes quality is conditioned by the anhocyanin content which accumulates in the grape's skin during its maturation. The mild temperature (up to 20^0 C), light and fertilizers with low content of nitrogen are the major factors influencing the anthocyanin biosynthesis. The technological mode of the extraction, called maceration, which occurs at the same time with the alcoholic fermentation, also influences the anhocyanin content in grapes (26).

Compared to tannins anthocyanins are more easily extractable from plant tissue but at the same time they are more sensitive to oxidation which affects their stability i.e. their colour. During the first months following vinification all the phenolic compounds undergo numerous transformations, many of which are oxidative in nature. During the "aging" of the wine the anthocyanin content decreases while the tannin content increases causing a harder taste and the colour modification. The major loss in colour is associated with fermentation process, which results in the progressive degradation of the pigment complexes formed between associated phenolics, primarily anthocyanins, and other phenolic compound (Fig. 4) s. The colour stability and the anthocyanin content in wines are also associated with wine macromolecules, proteins and polysaccharide, which form colloidal fraction of the colouring matter. The colloidal fraction of the colouring matter is very sensitive to temperature and ion concentration. These coloured colloids precipitate as organic salts by increasing the temperature. In this course wine goes under clarification and filtration process accomplished by adding gelatine to the wine in order to flocculate the colloidal matter.

The wine consumption has multiple health benefits. Investigations of biological and physiological effects of grape's constituents, especially polyphenolic compounds, are receiving renewed attention today. Phenolic compounds present in grapes have been shown to elicit positive biological effects with most of them arising from their antioxidant activity.

Among all potential positive health affects of flavonoid compounds found in grapes and wines the most striking is their ability to reduce one's risk for coronary heart disease (10). A number of studies have revealed the ability of flavonoids to inhibit tyrosine kinase enzyme, an enzymatic group which is responsible for platelet aggregation. The flavonoids have the ability to increase the level of α-tockopherol, an antioxidant component from the vitamin E family.

The flavonoids prevent artheriosklerosys, which causes the reduction of the oxygen flow into the heart and also reduce allergic inflammation.

Within the past decade, epidemiologists have described what has been called the "French Paradox". This phenomenon is related to the lack of a positive correlation between a high intake of saturated fats and the coronary heart disease occurrence. Despite the fact that the French typically consume a high fat diet, they have low rates of coronary heart disease. Many studies attributed this phenomenon to antioxidative phenolic compounds found in red wine, which is frequently consumed in France. The effect was associated with the ability of the flavonoids to inhibit copper catalysed oxidation of the LDL protein, which, when oxidised, represent the major factor causing heart dysfunctions (28, 29).

However, health benefits and positive physiological effects of wine's components implicate only its resonable consumption. Besides the negative effects of the alcohol, which is present in wine, polyphenolic compounds are also asocciated with some negative health effects.

One of the major negative effects is mutagenicity, associated with the polyphnolic compounds present in both commercially available and fresh squeezed white grape juice (30). Interestingly, the degree of mutagenicity has been associated with the same structural features which were associated with the antioxidant activity. The data indicated that the damage is caused through the oxidation of phenolic compounds. However, also some specific flavonoid compounds like quercetin and kaempherol have been found to be mutagenic by the Ames test with Salmonella typhimurium type TA98 and TA100 (31).

Only within the past 25 years the scientists around the world have begun to realize the potential positive and negative heath affects of phenolic, especially flavonoid, compounds found in grapes. The coming years certainly should bring further clarification of the effects and mechanisms of their action.

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FLAVONOIDI, ULOGA I ZNAČAJ U SAVREMENIM ISTRAŽIVANJIMA

- pregledni rad -

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Rezime

Flavonoidi su sekundarni ćelijski metaboliti koji predstavljaju najveću grupu biljnih polifenola. Po strukturi to su heterociklični π-elektronski sistemi, opšte strukturne formule C_6H_5(A)–C_3–C_6H_5(B) sa atomom kiseonika kao heteroatomom. U zavisnosti od stepena oksidacije centralnog prstena C flavonoidi se mogu podeliti na više, strukturno različitih, klasa jedinjenja.

Iz razloga velike biološke i fiziološke aktivnosti, kompleksnosti hemijskih promena, složenosti biosinteze i metabolizma kojima podležu u uslovima *in vivo* i sve većih mogućnosti primena u raznim oblastima industrije flavonoidi, posebno flavoni i antocijani, se već duži niz godina nalaze u vrhu aktuelnosti fundamentalnih i primenjenih istraživanja.

Danas je poznat znatan broj biljnih vrsta koje sardže flavonoide za koje je poznato da imaju pozitivno dejstvo na ljudsko zdravlje. Pored veoma važne antioksidativne aktivnosti flavonoidi pokazuju i antimutagenu, antibakterijsku, antiinflamatornu, antielergijsku, aniviralnu, vaskularnu i dr. aktivnosti. Iz klase flavonoida posebno se izdvajaju antocijanidini odnosno njihovi glikozidifikovani oblici, antocijani. Pored toga što daju neizmerno bogatstvo boja i nijansi cvetova i plodova u prirodi antocijani su sastavni deo naše ishrane i kao takvi veoma važni za nas. Antocijani se kao netoksična jedinjenja sve više koriste u prehrambenoj industriji, u ulozi nosioca i konzervatora obojenja sokova, vina i drugih proizvoda kao i u očuvanju opštih fizičkih i hemijskih karakteristika hrane. Činjenica da antocijani predstavljaju jednu od 13 klasa prirodnih pigmenata čija je upotreba dozvoljena u Evropi naznačava važnost ispitivanja ovih jedinjenja.