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Biological Activities and Effects of Food Processing on Flavonoids as Phenolic Antioxidants

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1. Introduction

Plants produce a great variety of organic compounds as a response to environmental stresses like microbial attack, insect/animal predation and ultraviolet radiations. The role of these metabolites is to increase plants resistance to these stresses. They can be classified into three major groups according to their biosynthetic route and structural features: terpenoids, alkaloids, and phenolic compounds. Phenolic compounds, which are mainly synthesized from phenylalanine produced by the shikimic acid pathway, are the most widely distributed in the plant kingdom. Plant tissues may contain up to several grams per kilogram of polyphenols. External stimuli such as microbial infections, ultraviolet radiation, low temperature, water and nutrition stresses induce their synthesis. The chemical structure of plant phenolics varies from simple to highly polymerized compounds as lignin and tannins. Phenolics have been categorized in different classes according to their basic carbon skeleton: the most relevant phenolic groups for nutritional health value are phenolic acids (C6-C1), phenylpropanoids (C6-C3) from which derivates the lignin polymer (C6-C3)n, coumarins (cyclized C6-C3) and flavonoids (C6-C3-C6). These compounds have a role in the visual appearance (peel and flesh pigmentation, browning), taste (astringency and bitterness), and health-promoting properties (free radical scavengers). This chapter focuses mainly on the biological activities of flavonoïds and on the effect of processes on the evolution of flavonoids and phenolic compounds during food transformations.

2. Flavonoids: Classification and biological activities

Flavonoids except chalcones, aurones and isoflavones share the same basic skeleton, a flavanone nucleus containing two hexacarbonic aromatic rings formed by fifteen atoms of carbon (A and B) interconnected with an heterocyle C composed of three carbon atoms and one oxygen atom (Figure 1).

This nucleus can undergo many modifications such as hydroxylation, alkylation or glycosylation. Depending on these modifications, the flavonoids are classified into 9 groups (chalcones, aurones, flavanones, dihydroflavanols, flavones, isoflavones, anthocyans, flavonols, flavanols). Compounds belonging to the same group differ between them by the degree and the position of hydroxylation, the presence of substitute on the nucleus and the state of their polymerization (Table 1).

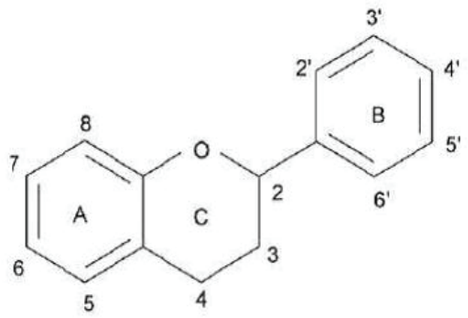
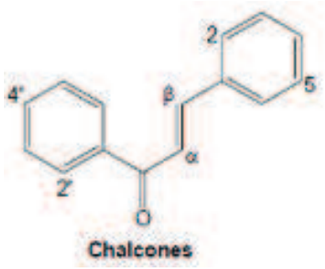


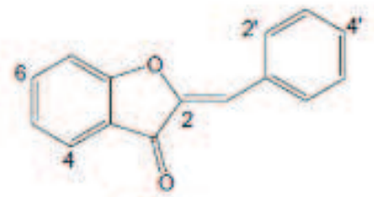
Fig. 1. The flavanone nucleus.

Besides conferring coloration to plants, flavonoids can act as enzyme and microbial inhibitors, chelating agents, protection against UV, against free radicals. Moreover, flavonoids believed to have health properties such as analgesic, anti-inflammatory, anti-allergic and protectors against cardiovascular diseases. These properties are attributed mainly to their antioxidant activity and are variable depending to their structure.



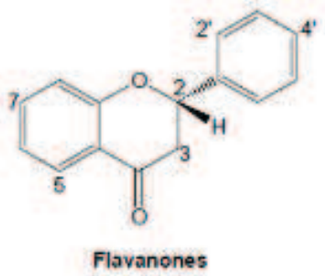
Chalcones

Flavonoid	Substitution					
	2′	3′	4′	5′	6′	4
Davidigeni	OH		OH			OH
Asebogenin	OH		OMe		OH	OH



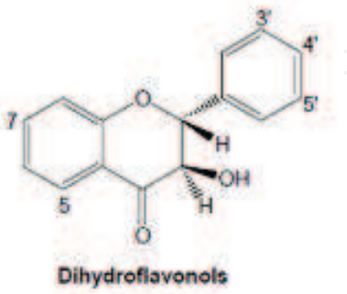
Aurones

Flavonoid	Substitution					
	4	6	7	3′	4′	5
Leptosodin		OH	OMe	OH	OH	
Maritimetin		OH	OH	OH	OH	



Flavanones

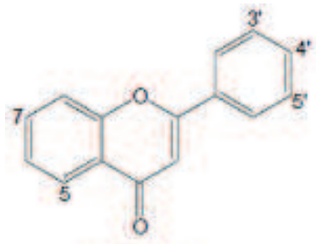
Flavonoid	Substitution					
	5	6	7	3′	4′	5′
Eriodictyol	OH		OH	OH	OH	
Hesperitin	OH		OH	OH	OMe	
Naringenin	OH		OH		OH	



Dihydroflavonols

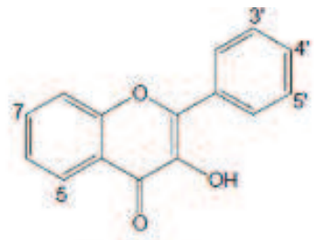
Flavonoid	Substitution					
	5	6	7	3′	4′	5′
Taxifolin	OH		OH	OH	OH	
Fusecin			OH	OH	OH	

Table 1. Different classes of flavonoids and their structures.



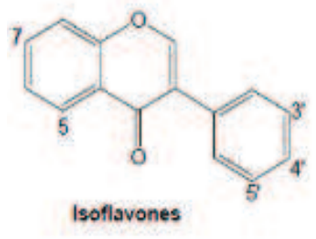
Flavones

Flavonoid	Substitution					
	5	6	7	3′	4′	5′
Apigenin	OH		OH		OH	
Chyrisin			OH			
Luteolin	OH		OH	OH	OH	



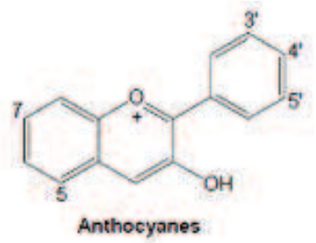
Flavonols

Flavonoid	Substitution					
	5	6	7	3′	4′	5′
Kampherol	OH		OH		OH	
Myricetin	OH		OH	OH	OH	OH
Quercetin	OH		OH	OH	OH	



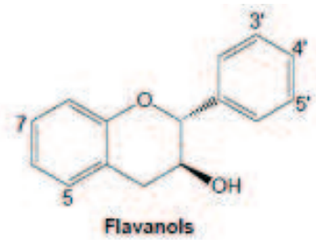
Isoflavones

Flavonoid	Substitution					
	5	6	7	3′	4′	5′
Daidzein			OH		OH	
Genistein	OH		OH		OH	



Anthocyanes

Flavonoid	Substitution					
	3	5	7	3′	4′	5′
Pelargonidin	OH	OH	OH		OH	
Cyanidin	OH	OH	OH	OH	OH	
Delphinidin	OH	OH	OH	OH	OH	OH



Flavanols

Flavonoid	Substitution					
	5	6	7	3′	4′	5′
Catechin	OH		OH	OH	OH	
Gallocatechin	OH		OH	OH	OH	OH

Table 1. Different classes of flavonoids and their structures. (Continuation)

2.1 Anti-oxidant and anti-free radical activities of flavonoids

The most described property of flavonoids is their capacity to protect the organism against free radicals and oxygenated reactive species (ORS) produced during the metabolism of oxygen (Grace, 1994). The cellular damage by the free radicals causes a change of the net charge of cells, thus modifying their osmotic pressure and inducing their swelling and their death. The free radicals act also on the mediators of the

inflammatory diseases, and accelerate the tissue damage. Moreover, cells lesions lead to an increase in the production of the ORS which induces the consumption and the depletion of the endogenous chelating agents. To protect against oxygenated reactive species, the organism and living cells have developed several mechanisms (Halliwell, 1995) including enzymes like the superoxyde dismutase, the catalase and the glutathion peroxidase, and also non-enzymatic homologues such as the glutathion, the ascorbic acid and l'α-tocopherol.

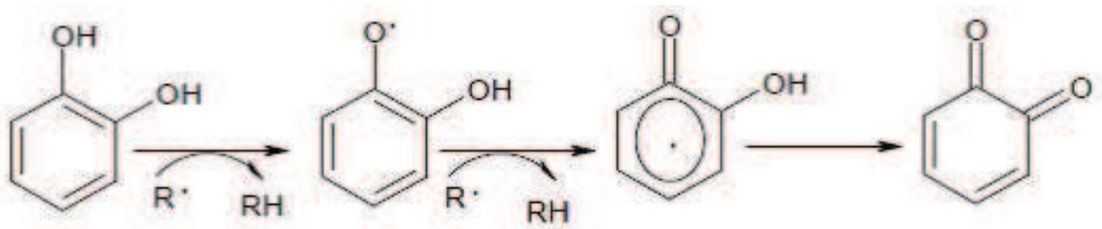
The protective effect of flavonoids is due to several mechanisms such as free radicals trapping, enzymes inhibition and metallic ions chelation. These properties depend on the structure of the flavonoids and the degree of substitution and saturation (Table 1).

2.1.1 Free radicals trapping

The flavonoids can prevent the damage caused by the free radicals according to various ways; one of them is the direct trapping of the radicals. In this case, the flavonoids are oxidized by the radicals (R•) leading to less reactive and more stable species according to the following mechanisms (Halliwell, 1995):

$$\text{Flavonoid}\,(\text{OH}) + \text{R}\bullet \rightarrow \text{Flavonoid}\,(\text{O}\bullet) + \text{RH}$$

The formed flavonoxy radical (flavonoid (O•)) is stabilized by resonance. The non-paired electron can be delocalized on the whole of the aromatic cycle. But, it can continue to evolve according to several processes (dimerisation, dismutation, recombination with other radicals, oxidation in quinon) either while reacting with radicals or other antioxidants, or with biomolecules. The flavonoxy (FL-O•) radical can react with another radical to form stable quinine as follows:



The flavonoxy radical can interact with oxygen to give a quinone and a superoxide anion. This reaction is responsible for an undesirable prooxidant effect of flavonoids. So the capacity of flavonoids to act as antioxidant depends not only on the redox potential of the couple Flavonoid (O•)/ Flavonoid (OH), but also on the reactivity of generated flavonoxy radical (Van Acker et al., 1995).

2.1.2 Effect on the mediator of nitric oxide synthesis

Several flavonoids reduce the cellular lesions related to ischaemia, by interfering with the activity of nitric oxide synthase. The nitric oxide is produced by various types of cells such as the endothelium cells and the macrophages. The nitric oxide is produced through the constitutive activity of nitric oxide synthase. It plays a role for the maintenance of the

dilation of the blood-vessels, the relaxation of the smooth muscles, the signal of transduction and the inflammation (Parihar et al., 2008; Valko et al., 2007). However, at high concentrations it induces an irreversible oxidative damage on cellular walls; because the activated macrophages increase their simultaneous productions of nitric oxide and the superoxide anions. The nitric oxide reacts with the free radicals producing peroxynitrite anion (ONOO-), a more reactive species:

$$H^+ + O_2^{\cdot-} + NO^{\cdot} \rightarrow ONOO^- + H^+ \leftrightarrow ONOOH$$

When the flavonoids are used as antioxidants, the free radicals are trapped thus reducing the conversion of nitric oxide into peroxynitrite (Shutenko et al., 1999). Flavonoids can also react with nitric oxide directly (Van Acker et al., 1995). Therefore, it was speculated that the trapping of nitric oxide by the flavonoids is in the origin of their protective effect of the cardiovascular system.

2.1.3 Inhibition of the enzymes activities

It is well known that flavonoids are able to inhibit the activities of several enzymes implicated in radical's generation. Among these enzymes, the xanthine oxidase, lipoxygenase, cyclo-oxygenase, peroxidase and tyrosin kinase are the most studied.

The xanthine dehydrogenase and the xanthine oxydase are implied in the metabolism of the xanthine into uric acid. The xanthine deshydrogenase is the configuration available under the normal physiological conditions, but it changes into xanthine oxydase during cells reperfusion (reoxygenation) and reacts with molecular oxygen to release the superoxyde radical ($O_2^{\cdot-}$). The flavonoids act as a strong inhibitor of the xanthine oxydase and as a trapper of the superoxide radical (Sanhueza et al., 1992).

The flavonoids have also the capacity in one hand, to inhibit the metabolism of the acid arachidonic (Ferrandiz & Alcaraz, 1991) by inhibiting the lipooxygenase and thus preventing the production of the chimiotactic compounds from this acid. This characteristic gives to the flavonoids the anti-inflammatory and anti-thrombogenic properties. In the other hand, flavonoids have also the capacity to reduce the release of peroxidases and proteolytic enzymes and thus the production of the ROS (Middleton & Kandaswami, 1992).

The activity of the tyrosin kinase is affected by the presence of the flavonoids (Nijveldt et al., 2001). This enzyme is implied in several cellular functions such as the enzymatic catalysis, the transport through the membrane, the transduction of the signals for hormones or growth factors and the transfer of energy in the synthesis of ATP. So, the inhibition of this enzyme by the flavonoids interferes with the way of transduction of the signals controlling the cellular proliferation.

2.1.4 Chelation of the metal ions

The ions of iron (Fe^{2+}) and copper (Cu^{+}), are essential for certain physiological functions of living cells (Van Acker et al., 1995). They can be, either as components of hemoproteins, or

of cofactors of various enzymes implicated in antioxidant defense system of cells. Besides their beneficial role, they are also responsible for the production of the hydroxyl radical by the reduction of hydrogen peroxide (·OH) according to the following reaction:

$$H_2O_2 + Fe^{2+}(Cu^{+}) \rightarrow \cdot OH + \text{-}OH + Fe^{3+}(Cu^{+})$$

The flavonoids form a stable complex with transition metals (Fe^{3+}, Al^{3+}, Cu^{2+}, Zn^{2+}); the stoichiometry of the complex and the site of chelation depend on the nature of the flavonoid mainly the presence of the catechol part (Le Nest et al., 2004) and the pH (Cornard & Merlin, 2002[a,b]). Moreover, this phenomenon of chelation is accompanied sometimes by the oxidation of the flavonoid (Cu^{2+}, Fe^{3+}). The chelation is occurred generally on the hydroxyl groups in position 3' and 4' of the B cycle, on the position 3 of hydroxyl group of A cycle and on the positions 3 and 4 of carbonyl group of C cycle (Figure 2).

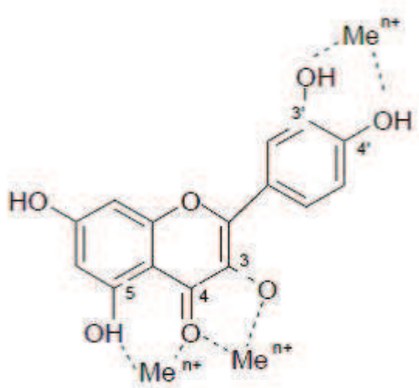


Fig. 2. Chelation of flavonoids.

When the flavonoids have several chelating metal sites, they can be polymerized. The copolymerization of the flavonoids and iron is responsible for anemia disease observed in large consumers of tea (Damas et al. 1985). The capacity of the flavonoids to complex metals is probably at the origin of the inhibition of many enzymes whose active site contains metals.

2.2 Pharmacologic effects of flavonoids

A general presentation of the hypothetical links between the mechanism of action and clinical effects of the consumption of flavonoids is summarized in Table 2 and Figure 3. The various clinical effects of the flavonoids are described in details in the following paragraphs.

Biological activities of flavonoids (anti-cancer, anti-inflammatory, antioxidant) depend on the presence of several functional groups on the backbone of these compounds (Limem et al., 2008). The most important are the hydroxyl groups and their positions (3 and 4' on C and B cycles respectively or 3' and 4' on the cycle B), the double bond between carbon 2 and 3 and 4 oxo function of the cycle C. The absence or the substitution of these groups leads to a significant reduction of biological activities of the flavonoids. The relation between flavonoids structure and biological activities is well summarized (Figure 4).

Consumption	Effect	References
Quercetin –rich fruits and vegetables (during 15 years)	Decrease of apoplexy incidence	Imai et al., 1997
Green tea (during 10-11 years)	Decrease of cancers risk (lung, liver, colon) Retard cancers progression Decrease of breast cancer risk	Nakachi et al., 1996/1998
Dadzein, genistein and coumestrol -rich food	Decrease of prostate cancer risk	Strom et al., 1999
375mg of curcumin (3 times a day for 12 weeks)	Effective treatment of anterior uveitis Increase of blood levels of glutathione peroxidase	Lal et al., 1999
55mg/day of isoflavonoids for 8 weeks	No effect on lipid peroxidation	Hodgson et al., 1999
Extract of red vine leaf (360 or 720 mg/day for 12 weeks)	Small effect on chronic venous insufficiency	Kieswetter et al., 2000
Green tea extract + linoleic acid (3g/day for 4weeks)	Decrease of blood levels of malondialdehyde No effect on other markers of oxidative stress or production of nitric oxide	Freese et al., 1999
Red vine phenolic compounds (3time 660mg/day for 2 weeks)	Increase in serum antioxidant capacity	Carbonneau et al., 1997
Blackcurrant and apple juices (750 up 1500ml/day for one week)	Decrease of blood levels of malondialdehyde Increase in glutathione peroxidase (no other changes in antioxidant status)	Young et al., 1999
Green or red tea (2g/day for 2 days)	Transient increase in blood antioxidant parameters (radical scavenging)	Serafini et al., 1996
113ml of alcohol-free red or white wine for 3 weeks	Transient increase in blood antioxidant parameters (radical scavenging)	Muzes et al., 1990
1L/day of soy milk for 4 weeks	No effect on blood cholesterol Decrease in oxidative damage of DNA bases	Mitchell & Collins, 1999
Flavonones, vitamin C	Decrease of prostate cancer risk	Rossi et al., 2007
Lyophilized grape powder (flavans, anthocyanins, qercetin, myricetin, kaempferol, resveratrol)	Reduction in plasma triglyceride concentration, cholesterol (LDL), apolipoproteins B, E and TNF α	Zern et al., 2005
Quercetin	Reduced risk of mortality due to ischemic heart disease Reduced risk of lung cancer	Knekt et al., 2002
Quercetin, naringenin, hesperitin	Reduced risk of breast cancer	
Quercetin, myricetin	Reduced risk of asthma	
Kaempferol, naringin, hesperitin	Reduced risk of type 2 diabetes	
Myricetin	Reduced risk of cerebrovascular diseases	

Table 2. Effects of flavonoids consumption on biological activities.

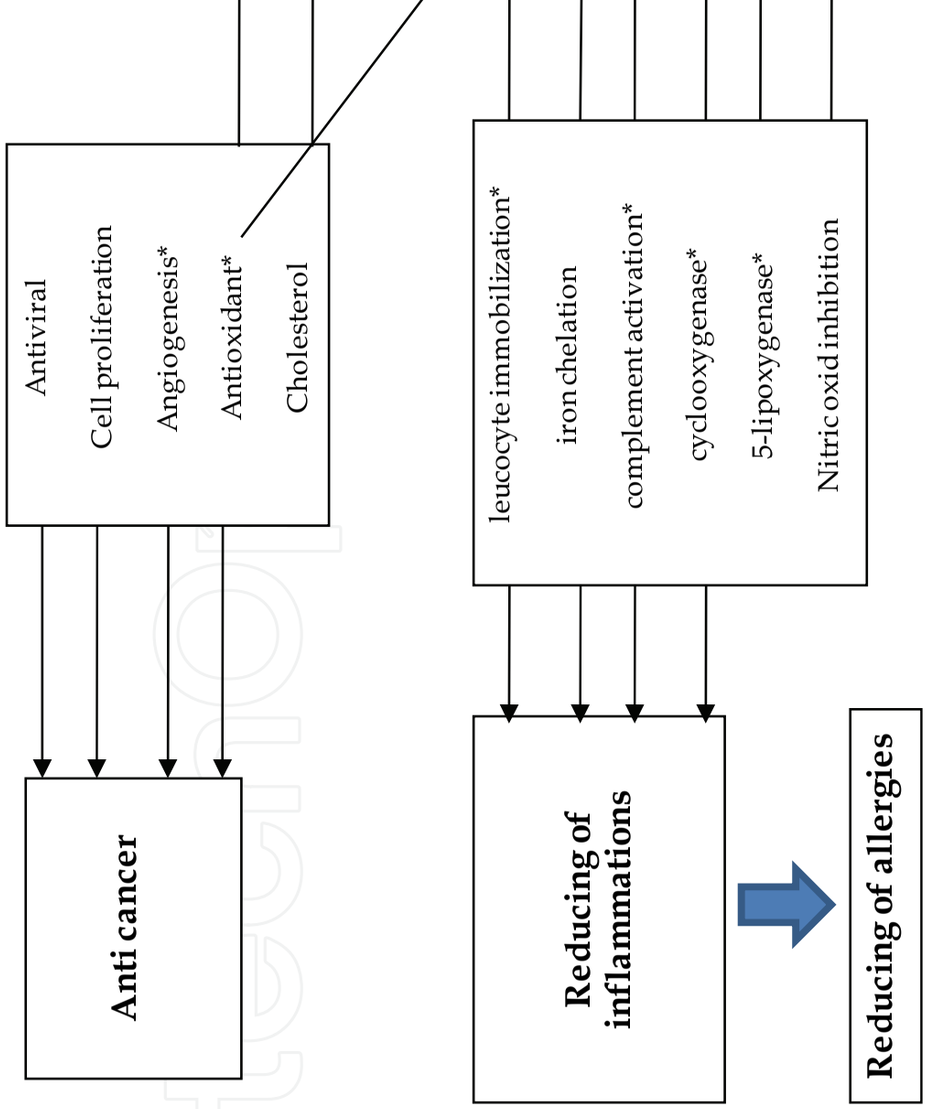


Fig. 3. Assumption of the links between the mechanisms of action of flavonoids and their effects on diseases (Nijveldt et al., 2001). * indicates the reduction of the enzyme activity or the function indicated.

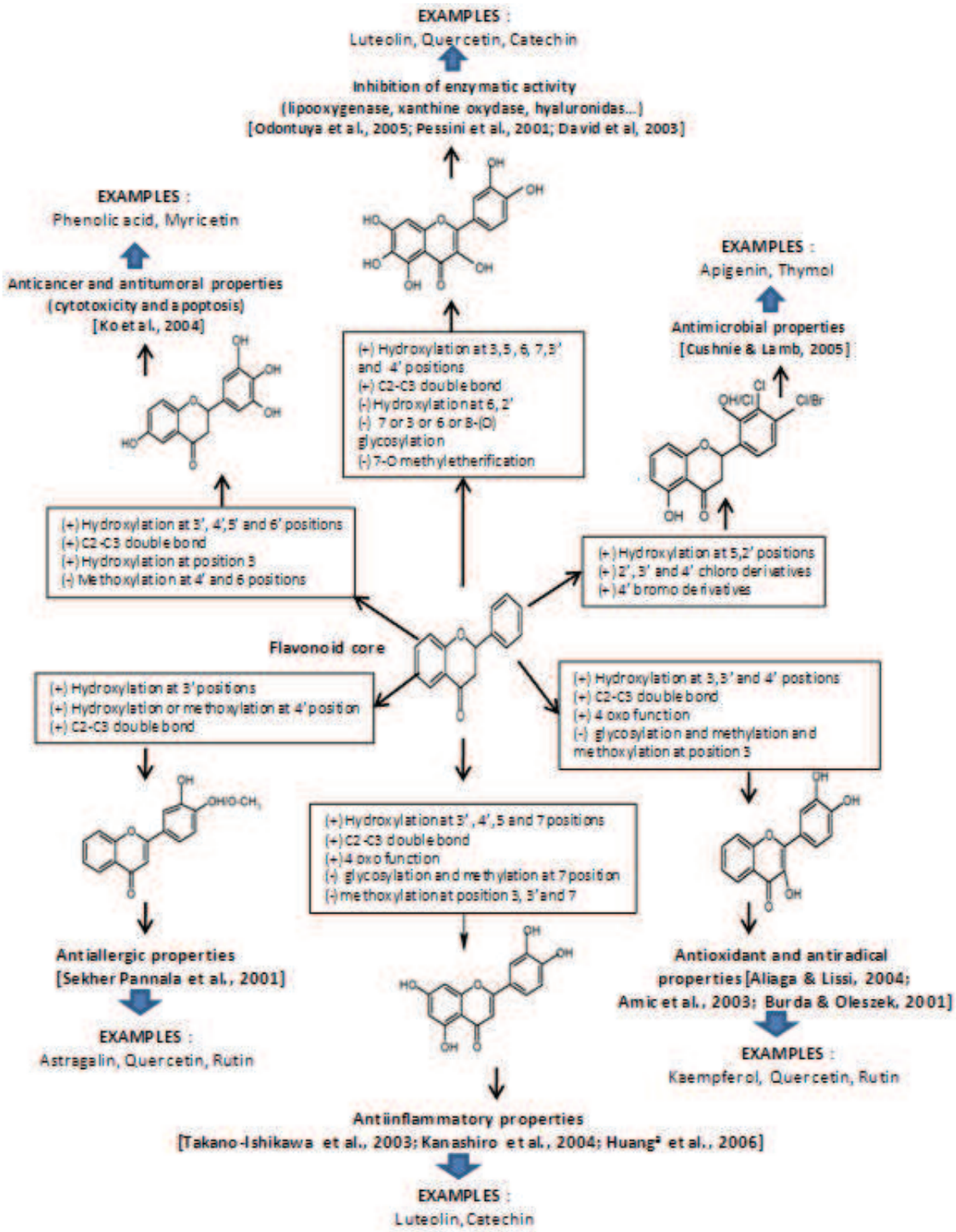


Fig. 4. Flavonoid structural elements necessary for biological activity: (+), the presence of structural elements promotes the cited activity; (-), the presence of structural elements reduces the cited activity.

2.3 Extraction of flavonoids

Extraction is the most important step in the development of analytical methods for plant extracts analysis. A summary of experimental conditions of the extraction methods is reported in Table 3. Basis unit operations of extraction is often the plant drying and its milling to obtain an homogenous powder and improve the extraction kinetic of the molecules. Methods as sonication, heating under reflux, extraction with Soxhlet apparatus are the most used (Ong, 2004). However, these methods are often long and need large volumes of organic solvents, with low extraction rates. Molecules we want to extract can be polar, non-polar or heat sensitive; thus the extraction method must take all these parameters into account.

To reduce the use of organic solvents and to improve the extraction rate, other methods such as extraction assisted by microwave, supercritical extraction, accelerated extraction by solvents, the pressurized liquid extraction, the pressurized extraction by hot water and the pressurized extraction by hot water associated to surfactants were introduced to the phenol extraction from plants. These different techniques were summarized in Table 3.

Extraction method	Solvents	Temperature (°C)	Pressure	Time
Sonication	Methanol, Ethanol, Mix alcohol/water	Can be heated		1 h
Soxhlet extraction	Methanol, Ethanol, Mix alcohol/water	Depending on the solvent used		3-18 h
Microwave extraction	Methanol, Ethanol, Mix alcohol/water	80-150	Depending on the extraction container	10-40
Extraction by supercritical fluid	Carbon dioxid, Mix carbon dioxid/Methanol	40-100	250-450 bar	30-100 min
Extraction by accelerated solvent	Methanol	80-200	100 bar	20-40 min
Extraction by pressurized liquid	Methanol	80-200	10-20 bar	20-40 min
Pressurized extraction by hot water	Water, water with 10-30% ethanol	80-300	10-50 bar	40-50 min
Pressurized extraction by hot water with surfactant	Water with surfactant (triton X100 ou SDS)	80-200	10-20 bar	40-50 min

Table 3. Experimental conditions for the phenol extraction.

2.4 Flavonoid occurrence in foods

Since several decades, many studies dealt with the analysis of foods to determine its composition in flavonoids. Many reviews were published, where the main flavonoids in foods are gathered. Tomás-Barberan et al. (2000) focused on fruits and vegetables. In 2009, INRA

(French National Institute Of Agricultural Research) developed a database on flavonoids in foods (http://www.phenol-explorer.eu). Table 4 was built according to data collected on the database of INRA; it presents some examples of foods containing flavonoids cited. Flavonoids chosen are the main found in foods, their quantity is specified into brackets.

Flavonoids	Foods (flavonoid content in mg/100g or 100ml)
Flavanons:	
- **Naringenin**	Red wine (0.05), Grapefruit (1.56), Mexican oregano (372), Almond (0.02)
- **Hesperidin**	Grape fruit juice from concentrate (1.55), Lemon juice from concentrate (24.99), Orange juice from concentrate (51.68), Peppermint dried (480.65)
Flavons:	
- **Luteolin**	Olive oil extra virgin (0.36), Thyme fresh (39.50), Olive black (3.43), Artichoke heads (42.10)
- **Apigenin**	Olive oil extra virgin (1.17), Italian oregano (3.50), Marjoram dried (4.40).
Flavonols:	
- **Kaempferol**	Red wine (0.23), Red raspberry pure juice (0.04), Tea black bottled (0.13), Capers (104.29), Cumin (38.60).
- **Quercetin**	Red wine (0.83), Buckwheat whole grain flour (0.11), Chocolate dark (25), Black elderberry (42), Orange pure juice (1.06), Mexican oregano (42), Onions red raw (1.29), almond (0.02)
Flavan-3-ols:	
- **Catechin**	Beer regular (0.11), Wine red (6.81), Barley whole grain flour (1.23), Cocoa powder (107.75), Grape black (5.46), Strawberry (6.36), Plum (4.60), Pistachio (3.50), Broad bean pod (16.23)
- **Epicatechin**	Red wine (3.78), Chocolate dark (70.36), Blackberry (11.48), European cranberry (4.20), Apricot (4.19), Custard apple (5.63), Tea green infusion (7.93)
Anthocyanins:	
- **Petunidin 3-O-glucoside**	Red wine (1.40), Highbush blueberry (6.09), Black grape (2.76), Black common bean (0.80)
- **Malvidin 3-O-glucoside**	Red wine (9.97), White wine (0.04), Black grape (39.23), Red raspberry (0.62)

Table 4. Examples of composition in flavonoids of certain foods.

According to Table 4, foods containing great quantity of flavonoids are fruit and vegetables; the processing of these raw foods modify the flavonoid content according to the process conditions. For example, in olive oil extra virgin, there is 1.17 mg of apigenin for 100g, but if this oil is refined the apigenin content decrease to 0.03 mg/100 g. Thus processes induce some consequences on flavonoid composition in foods.

3. Effect of food processing

Processes used in food engineering are numerous. We focus on the effect of unit operations on the degradation of the phenolic compounds as flavonoids and their antioxidant activity.

Among unit operations, we distinguish different categories: (i) the thermal processes such as pasteurization, baking, cooling, freezing, (ii) the non-thermal processes such as high pressure, pulsed electric fields, filtration, (iii) the mechanical processes such as peeling, cutting or mixing and (iv) the domestic processes that is to say processes by means of preparation of the convenience foods at consumers home.

3.1 Thermal processes

Thermal processes have a large influence in flavonoid availability in foods which depends on their magnitude and duration. Different heating methods (drying, microwaving, heating by an autoclave, roasting, water immersion, pasteurization, pressured-steam heating, blanching) were used and their effects were analyzed (Table 5). On this table, are gathered examples of significant studies to show the effect of thermal processes on the degradation of phenolic compounds.

As shown in Table 5, most of thermal processes lead to a degradation of phenolic compounds except in some cases as the apple juice processing where an increase of temperature from 40°C to 70°C allows increasing flavonoid content (50%) (Gerard & Roberts, 2004). A roasting of 130°C, 33 min increases the phenol content of cashew nuts (Chandrasekara & Shahidi, 2011); same results were noticed for peanuts (Yu et al., 2005). In these cases, an increase of temperature improves the extraction of phenolic compounds from foods; others results showed losses of phenolic compounds in different quantities. A loss of about 22% in total flavonoids has been observed in boiled products at a temperature of 50°C during 90s (Viña & Chaves, 2008). For the roasting process at 120°C, 20 min provokes a decrease of 12% of total flavonoid content (Zhang et al., 2010) and 15.9% for 160°C, 30min (Zielinski et al., 2009). Sharma & Gujral (2011) noticed for a roasting at 280°C during 20s, a loss of 8% in phenolic content. Steam heating at 0.2 MPa during 40 min induces a decrease of 25% in flavonoid content (Huang[b] et al., 2006; Zhang, et al., 2010). Similar findings were reported with microwaving at 700W during 10 min (Zhang, et al., 2010), 900 W during 120 s (Sharma & Gujral, 2011) and autoclaving at 100°C, 15 min (Choi et al., 2006). However, one blanching per immersion in water at 100°C during 4 min does not deteriorate flavonoids (Viña et al., 2007). Drying processes lead also to flavonoids degradation. The proportion lost depends on the drying method. Freeze-drying is the less aggressive method whereas hot air drying leads to major losses. As intermediate solutions microwave and vacuum drying can be used (Dong et al., 2011; Viña & Chaves, 2008; Zainol et al., 2009; Zhang et al., 2009). Pasteurization induces losses in phenolic compounds, significant losses are noticed for tomatoes' sauce pasteurized at 115°C during 5 min (Valverdú-Queralt et al., 2011), likewise a loose of 40% for a temperature of 85 °C during 5 min is measured by Hartman et al. (2008) for strawberries.

A few studies identified phenolic compounds in foods and followed their degradation during heat treatment. They noticed that individual phenolic compounds are also subject to heat degradation. The identification and quantification of these compounds were performed with high performance liquid chromatography. Rutin in buckwheat groats is reported to be more stable to heat then vitexin, isovitexin , homoorientin and orientin during roasting at 160°C for 30 min (Zielinski, et al., 2009). However, an increase of the dehulling time (10 to 130 min) leads to greater losses of rutin in the same product grains (Dietrych-Szostak & Oleszek, 1999). Boiling including soaking (100°C/121°C) with/or without draining stages induces 1-90% losses of quercetin and kaempferol in Brazilien beans (Ranilla et al., 2009).

Thermal pasteurization treatments (90°C, 60s) for strawberry juices have no effect on quercetin and kaempferol contents (Odriozola-Serrano et al., 2008), whereas it reduces naringin, rutin, quercetin and naringenin content for grapefruit juices (Igual et al, 2011). For Fuleki & Ricardo-Da-Silva (2003), pasteurization of grape juice increased the concentration of catechins in cold-pressed juices, but it decreased concentrations in hot-pressed juices. The concentration of most procyanidins was also increased by pasteurization.

However, the above results may not be comparable, because on the one hand, the food matrix is different from one assay to another and on the other hand, the food matrix can act as a barrier to heat effect or induce the degradation. It is not easy then to dissociate the thermal processing effect from the food matrix effects. Thus, some authors studied the effects of thermal processes on model solutions of phenolic compounds; these studies are led especially on flavonoids. The data indicated that flavonoids in aqueous solutions show different sensitivity to heat treatment depending on their structures. However, whatever their structure a significant degradation is observed for temperature above 100°C. For rutin, a higher stability compared to its aglycon form (quercitin) is observed (Buchner et al., 2006; Friedman, 1997; Makris & Rossiter, 2000; Takahama, 1986). These findings are attributed to the prevention of carbanion formation because of the glycosylation of the 3-hydroxyl group in the C-ring (Buchner, et al., 2006; Friedman, 1997; Takahama, 1986). Authors reported also that Luteolin was more stable to heat than rutin and luteolin-7-glucoside when heated at 180°C for 180min (Murakami et al., 2004). The degradation of flavonoids is not only a function of temperature and magnitude of heating; it may depend also on other parameters such as pH, phytochemicals, structure and even the presence or absence of oxygen. Indeed, original flavonol concentration has no effect on the degradation of rutin and quercetin. It is suggested that the reaction pathways are not influenced by the different flavonol solutions molarities (Buchner, et al., 2006). Moreover, under weak basic (Buchner, et al., 2006; Friedman, 1997; Takahama, 1986) and neutral (Friedman, 1997; Takahama, 1986) reaction conditions, more degradation of rutin and quercetin is observed (Buchner, et al., 2006). The absence of oxygen highly reduces quercetin degradation and prevents rutin breaking up during heating. The presence of oxygen is shown to accelerate quercetin and rutin degradation due to the presence of the reactive oxygen species (Buchner, et al., 2006; Makris & Rossiter, 2000). Chlorogenic acid is observed to protect rutin against degradation when a mixture of the two substances is heated at 180°C (Murakami, et al., 2004).

Sometimes, authors dealt with the antioxidant activity of foods or solutions studied. It is difficult to summarize the evolution of the antioxidant activity according to conditions heat processes. Too numerous factors are implied in its evolution. Decreases in phenol content do not lead systematically to a decrease of the antioxidant activity. Indeed, the degradation products of phenolic compounds can also have an antioxidant activity sometimes higher than the initial phenolic compounds (Buchner, et al., 2006; Murakami, et al., 2004); for high temperatures, these products can be Maillard products. Thus, an increase of antioxidant activity is noticed in many studies using thermal processes (Chandrasekara & Shahidi, 2011; Hartman et al., 2008; Sharma & Gujral., 2011). However interactions are important phenomena which act on the antioxidant activity of molecules. Depending on this environment, synergies between antioxidant compounds and the food matrix can occur (Wang et al., 2011). In some cases, the antioxidant capacity of flavonoids in a food matrix is enhanced (Freeman et al., 2010) ; while in other cases, the antioxidant capacity is reduced (Hidalgo et al., 2010). Thus, in other studies, antioxidant activity remains constant (Leitao et al., 2011) or can be decreased (Davidov-Pardo et al., 2011).

			Food product/Flavonoid	Processing conditions	Impact on flavonoid content	References
Heat processes	Food products	Total phenol content	Nuts	Roasting (130°C, 33 min)	Increase of phenol content	Chandrasekara & Shahidi, 2011
			Eucommia ulmoides flower tea	Microwave drying (Power : 140, 240, 480, 560 and 700 W; time durations: 1, 2, 3, and 4 min)	Stability of total flavonoid content	Dong et al., 2011
			Barley	Roasting (280°C, 20s) Microwave cooking (900 W, 120s)	A 8% loss in phenol content A 49.6 % loss in phenol content	Sharma & Gujral, 2011,
			Buckwheat	Roasting 20min and 40min at 80°C and 120°C Pressurized steam-heating (0.1 MPa, 20 min ; 0.2 MPa, 40 min) Microwaving (700W, 10 min)	20-30% increases depending on the conditions 18-30% increases depending on the conditions 20 % increase in flavonoid content	Zhang et al., 2010
			Tomatoes	Pasteurization (115°, 5 min)	Losses in phenol content	Valverdú-Queralt et al., 2010
			C. asiatica leaf, root and petiole	Air-oven drying Vacuum oven drying Freeze drying	A 97% loss in flavonoid content A 87.6% loss in flavonoid content A 73% loss in flavonoid content	Zainol et al., 2009
			Buckwheat seeds Buckwheat groats	Heating at 160°C for 30 min	A 15.9% loss in flavonoid content A 12.2% loss in flavonoid content	Zielinski et al., 2009
			Strawberry	Pasteurization (85°C, 5 min)	A 40% loss in phenol content	Hartman et al, 2008
			Celery	Dry air (48°C,1h) Water immersion (50°C, 90s)	A 60% loss in flavonoid content A 22% loss in flavonoid content	Viña et Chaves, 2008
			Brussels sprouts	Blanching (50°C)	Stability of total flavonoid content	Viña et al., 2007
			Mushroom (Shiitake)	Autoclave : (100, 121°C, 10 or 30 min)	Increase of free flavonoids (64%) Decrease of bound flavonoids: 50% (100°C, 30min), 75% (121°C, 10 min), 90% (121°C, 30 min) Stability under (100°C, 10 min)	Choi et al., 2006
			Sweet potato	Steaming (40 min)	14 % increase in flavonoid content	Huang[b] et al., 2006
			Peanut	Roasting (175°C, 5min)	40% increase in total phenol content	Yu et al., 2005
			Apple juice	Heating at 40_C, 50_C, 60_C and 70_C in a	50% increase between 40°C and 70°C	Gerard & Roberts, 2004

Table 5. Effects of heat processes on phenolic content.

			Food product/Flavonoid	Processing conditions	Impact on flavonoid content	References
Heat Processes	Food products	Individual phenolic compound	Grapefruit juices	Pasteurization (95°C, 80s)	Decrease of naringin, rutin, quercetin and naringenin content	Igual et al., 2011
			Bean (Quercetin , kaempferol)	Atmospheric (100°C) and pressure boiling (121°C) with and without soaking and draining	Increases of 1-90% of quercetin and kaempferol derivatives with soaking and drainning	Ranilla et al., 2009
			Buckwheat (Vitexin, isovitexin, rutin)	Roasting at 160°C for 30 min.	Losses of 80% of vitexin, isovitexin and rutin. Disappearance of homoorientin and orientin.	Zielinski et al., 2009
			Strawberry juices (kaempferol, quercetin, myricetin, anthocyanins)	High-intensity pulsed electric fields Pasteurization (90°C, 60s ; 90°C, 30s)	Stability of kaempferol, quercetin and myricetin. 10% increase of anthocyanins content (90°C, 60s)	Odriozola-Serrano et al., 2008
			Grape juice (Catechin, procyanidin)	Flash pasteurization (85°C)	Increase of Catechins in cold-pressed juice Decrease of Catechins in hot-pressed juice Increase of Procyanidins	Fuleki & Ricardo-Silva, 2003
			Buckwheat (Rutin, isovitexin)	Heating for (10,70, 130 min) to 150°C then steaming (0.35 MPa, 20 min)	Increase of rutin and isovitexin Steaming induces more losses	Dietrych-Szostak & Oleszek, 1999
	Model solutions		Aqueous flavonol solutions (quercetin and rutin)	Heating at 100°C for 300 min under pH 5 and 8 with air or nitrogen perfusion	Quercetin is more sensitive to heat under weak basic pH The presence of oxygen accelerates the degradation of quercetin and rutin	Buchner et al, 2006
			Aqueous flavonol solutions (quercetin and rutin)	Heating at 97°C for 240min under pH 8	Quercetin is more sensitive to heat than rutin The presence of oxygen accelerates the degradation of quercetin and rutin	Makris &Rossiter, 2000
			Rutin, luteolin, luteolin-7-glucoside	Heating at 100°C for 300 or 360min Heating at 180°C for 120 or 180min	Flavonoids are generally stable at 100°C Luteolin is more stable to heat than rutin and luteolin-7-glucoside (180°C,180min)	Murakami et al., 2004
			Aqueous flavonol solutions (quercetin and rutin)	Heating at 97°C for 240min under pH 8	Quercetin is more sensitive to heat than rutin The presence of oxygen accelerates the degradation of quercetin and rutin	Makris & Rossiter, 2000

Table 5. Effects of heat processes on phenolic content. (Continuation)

3.2 Non thermal processes

Certain authors showed the capacity of innovative processes (microwave, infra-red, high-pressure processing) to less degrade the phenolic antioxidants in food as regard to thermal processes. Odriozola-Serrano et al. (2008) studied the effect of high-intensity pulsed electric fields (HIPEF) process on quercetin and kaempferol contents of strawberry juices and

Food product	Processing conditions	Impact on flavonoids content	References
Onions	Cutting	Induction of flavonol biosynthesis	Pérez-Gregorio et al., 2011
Tomatoes	Peeling, Dicing	Great losses in phenol content	Valverdú-Queralt et al., 2011
Potatoes	Cutting	Induction of flavonol biosynthesis	Tudela et al., 2002
Asparagus	Chopping	18.5% decrease of rutin content	Makris and Rossiter, 2001
Onions	Peeling, trimming	Losses of 39%	Ewald et al., 1999

Table 6. Mechanical processing effects on phenol content.

	Food product	Processing conditions	Impact on flavonoid content	References
Domestic processes	Onion bulbs Asparagus spears	Boiling (60min)	A 20.5% decrease in total flavonoid content in onion bulbs A 43.9% decrease in total flavonoid content in Asparagus spears	Makris and Rossiter, 2001
	Onions	Sautéing (5min)	Increase of quercetin conjugates and total flavonoid contents	Lombard et al., 2005
		Baking (15min, 176°C)		
		Boiling (5min)	A 18.8% decrease of total flavonoid content	
	Onions	Boiling (3min)	Boiling gave limited reduction in flavonoids content	Ewald et al., 1999
		Microwaving (650w)		
		Warm-holding (60°C, 1h, 2h)		
	Brown - skinned Onions Red skinned-Onions	Boiling (20min)	A 14.3% loss of quercetin conjugates	Price et al., 1997
			A 2 1.9% loss of quercetin conjugates	
		Frying (5min, 15min)	23-29% Losses of quercetin conjugates	
	Onions	Boiling (5min)	A 20% loss of total flavonoids	Lee et al., 2008
		Microwaving (1min, High heat)	No significant effect on total flavonoid content	
		Sautéing (3min)	No significant effect on total flavonoid content	

Table 7. Effects of domestic treatment on phenol content.

reported that such a process has no damage on these compounds. In 2009, the same study was led on tomatoes' juice; pulsed electric field has no effect on phenol content and led to a

better conservation during the storage (Odriozola-Serrano et al, 2009). The use of high pressure, instead pasteurisation, on fruit smoothies is better to keep phenolic content constant (Keenan et al., 2011). Suarez-Jacobo et al. (2011) found the same results for an apple juice, phenolic content and antioxidant activity remain constant.

Few studies deal with filtration, Pap et al. (2010) recommended for blackcurrant juice filtration an enzymatic pre-treatment instead a reverse osmosis process, since it results in a juice concentrates highest in anthocyanins and flavonols. Hartman et al. (2008) also used an enzymatic treatment for strawberry mash; no loss of phenolic compounds was noticed.

3.3 Mechanical processes

Processes studied in literature concern essentially peeling, trimming, chopping, slicing, crushing, pressing and sieving of flavonoid-rich foods (Table 6). Processing is expected to affect content, activity and availability of bioactive compounds (Nicoli et al., 1999). According to authors, major losses of flavonoids took place during the pre-processing step when parts of product was removed: onions peeling and trimming resulted in 39% flavonoids losses (Ewald, et al., 1999) and asparagus chopping yielded a 18.5% decrease of rutin content (Makris & Rossiter, 2001). Great losses are also noticed for the peeling and the dicing of tomatoes (Valverdú-Queralt et al., 2011).

Slicing significantly affected the rutin content of asparagus (Makris & Rossiter, 2001). However, cutting increased flavonol content in fresh cut-potatoes (Tudela, et al., 2002) and fresh-cut onions (Pérez-Gregorio et al., 2011). In fact, wounding enhances flavonol biosynthesis through the induction of phenylalanine ammonia-lyase enzyme which is related to the wound-healing process in order to fight pathogen attack after tissue wounding (Tudela, et al., 2002).

3.4 Domestic processes

Several studies simulated food home preparation conditions in order to investigate their effects on flavonoid degradation (Table 7). Common domestic processes such as boiling, frying, baking, sautéing, steam-cooking and microwaving were studied.

Boiling resulted in flavonoids losses which are leached in cooking water, 43.9% for asparagus spears and 20.5% for onions (Makris & Rossiter, 2001). Similar losses in onions were reported (Lee et al., 2008; Lombard et al., 2005; Price et al., 1997). Microwaving does not markedly affect flavonoid content in onions (Ewald et al., 1999; Lee, et al., 2008; Lombard, et al., 2005; Price, et al., 1997; Tudela et al., 2002). As regards sautéing operations, contradictory findings were reported. Lee et al. (2008) reported a decrease of flavonoid content at almost of 21% whereas Lombard et al. (2005) showed an increase of the total flavonoid of 25% in onions (Lombard, et al., 2005).

Frying is reported to decrease onion flavonoid content between 25 and 33% (Lee, et al., 2008; Price, et al., 1997).Steaming and baking do not significantly affect the flavonoid content of onions (Lee, et al., 2008). Conversely, baking is found to increase quercetin conjugate and total flavonol content (7%) in onions as these compounds were concentrated in the tissues, as water and other volatiles were lost during cooking (Lombard, et al., 2005).

These contradictory results can be attributed easily to the diversity of food products used and the lack of the standardization of domestic processes.

Table 8 summarizes the possible evolution of phenolic antioxidants and their antioxidant activities according to the data collected in this chapter.

Phenolic Antioxidants		**Antioxidant activity**	
Evolution	Possible Cause	Evolution	Possible Cause
Increase	- Better extraction of phenolic compounds. - A stress inducing phenol synthesis as mechanical processes.	Increase	- Degradation products have an antioxidant activity. - Increase of the total phenol content. - Positive Synergies occur between phenolic antioxidants.
Decrease	- Degradation of phenolic compounds.	Decrease	- Degradation of the phenolic antioxidants. - Negative synergies occur between phenolic antioxidants
No change	- No degradation. - Compensation of an increase and a decrease.	No change	- No degradation of the phenol antioxidants. - Compensation of an increase and a decrease.

Table 8. Possible evolutions of phenolic antioxidants content and their antioxidant activity during food transformations.

4. Conclusion

Phenolic antioxidants have a great importance in human food diet: (i) they are widely widespread in raw foods as fruit and vegetables, tea, coffee, cocoa, (ii) they gather numerous properties beneficial for human health as anti-oxidant, anti-inflammatory, anti-allergic, antimicrobial and anticancer properties and (iii) they can be preserved during food transformation by using adapted process conditions and also nonaggressive processes. However, provide to consumers enriched food products in antioxidants is not so easy; indeed, despite the number of studies on the effect of food processes on the degradation of phenolic antioxidants and their antioxidant activities, it is difficult to generalize results. Many factors influence the evolution of these parameters: (i) the kind of raw food (genotype, cultivation method), (ii) the lack of standardization of measurement methods: phenolic content, antioxidant activity by ABTS, DPPH, ORAC, (iii) the influence of the food matrix: existence of interactions between molecules and iv) the lack of standardization of processes applied (conditions, materials).

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Advances in Applied Biotechnology
Edited by Prof. Marian Petre

ISBN 978-953-307-820-5
Hard cover, 288 pages
Publisher InTech
Published online 20, January, 2012
Published in print edition January, 2012

Biotechnology is the scientific field of studying and applying the most efficient methods and techniques to get useful end-products for the human society by using viable micro-organisms, cells, and tissues of plants or animals, or even certain functional components of their organisms, that are grown in fully controlled conditions to maximize their specific metabolism inside fully automatic bioreactors. It is very important to make the specific difference between biotechnology as a distinct science of getting valuable products from molecules, cells or tissues of viable organisms, and any other applications of bioprocesses that are based on using the whole living plants or animals in different fields of human activities such as bioremediation, environmental protection, organic agriculture, or industrial exploitation of natural resources. The volume Advances in Applied Biotechnology is a scientific book containing recent advances of selected research works that are ongoing in certain biotechnological applications. Fourteen chapters divided in four sections related to the newest biotechnological achievements in environmental protection, medicine and health care, biopharmaceutical producing, molecular genetics, and tissue engineering are presented.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Ioannou Irina and Ghoul Mohamed (2012). Biological Activities and Effects of Food Processing on Flavonoids as Phenolic Antioxidants, Advances in Applied Biotechnology, Prof. Marian Petre (Ed.), ISBN: 978-953-307-820-5, InTech, Available from: http://www.intechopen.com/books/advances-in-applied-biotechnology/biological-activities-and-effects-of-food-processing-on-flavonoids-as-phenolic-antioxidants

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