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REVIEW PAPER

Health Benefits of Quercetin

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ABSTRACT

Flavonoids are natural antioxidants derived from plant pigments and commonly found in agricultural produce such as fruits, vegetables, and also in beverages like tea and wine. Quercetin is the most important flavonoid which belongs to the class of flavonol. Quercetin is a vital biologically active compound, which is present in many products, such as onion (*Allium cepa*), black tea (*Camellia sinensis*), Broccoli (*Brassica oleracea* var. *italic*), and also in red wine and green tea, It is widely used in medicine and pharmaceutics. In particular, it is used for cancer treatment; as it restrains the growth of cancer cells. Earlier some of computational investigations of this molecule were reported in literature, but they were made at low theory level. Quercetin provided many health promoting benefits, like cardiovascular properties, cancer reducing agent, Anti-inflammatory, asthma and many more. That is why the further investigation of this molecule is important. The main important of this review is to understanding of the structure of quercetin and corresponding biological properties of quercetin expressed in vitro studies, absorption is critical, but in vivo studies, better absorbed antioxidant were observed like vitamin C, further reported studies on effect of food processing, health benefits, storage effects, and evaluate its safety and dosage.

Keywords: Quercetin; Flavonoids; Health benefits; Food processing; Safety

1. INTRODUCTION

Flavonoids involve an important group of naturally occurring, bioactive polyphenolics, popular in plants of higher generation[1]. Currently, the interest and awareness on flavonoids have largely focused on two different beneficial aspects. First, for their biological activities, and second for its anti carcinogenic properties. The anti carcinogenic property of flavonoids is most frequently attributed to their anti oxidant activity.

Flavonoids are said to be highly effective as an anti-proliferative agent against lymphoid, colorectal, ovarian, and breast cancer cells. Similarly, they are identified to induce chromatin condensation and apoptosis in some cancer cells. The mechanisms of flavonoid induced cytotoxicity are not yet established, but it is said to influence the sequential occurrence of apoptosis[2].

Quercetin is a most abundant poly phenolic bioflavonoid or flavonoid, which is generally classified as a flavonol. Quercetin is also classified as water-soluble pigments, which cannot be produced by human. It is also known as a phytoestrogens. Quercetin, comprising 3 rings and 5 hydroxyl groups, has many health beneficial effects, including improvement of cardiovascular health and reducing the risk for cancer.

Quercetin, present in fruits and vegetables, is identified to occur in various forms of glycosides; although its skin is found to posses quercetin aglycone structure and are in higher concentration. It is also naturally present as glycone or carbohydrate conjugates in plants. It is one of the most profusely present dietary flavonoids that are present in apples (*Malus domestica*); black and green tea (*Camellia sinensis*) onions (*Allium cepa*) (predominantly in the outer rings); broccoli (*Brassica oleracea*).

Quercetin glycosides in the onion extracts were converted to quercetin and sugars by thermo-stable β-glucosidase enzyme. It is found to posses several beneficial biological activities, like antioxidant, anti-inflammatory, anti-cancer, and anti-viral properties. The beneficial effects of quercetin are limited due to its sparingly soluble nature in water, which makes its absorption limited.

2. CHEMISTRY OF QUERCETIN

2.1 Structure

The International Union of Pure and Applied Chemistry (IUPAC)) nomenclature for quercetin is 3,3',4',5,7-pentahydroxyflavanone (or) 3,3',4',5,7-pentahydroxy-2-phenylchromen-4-one with a molecular formula $C_{15}H_{10}O_{7}$. The presence of five hydroxyl groups, at positions 3, 5, 7, 3', and 4' in quercetin molecule as shown in Fig. 1, leads to the formation of and pentamethyl derivatives. It is also commonly termed as quercetine, sophretin, meletin[3].

Quercetin is acts as a building block for other flavonoids. Quercetin is commonly present as an aglycone in food. On hydrolysis with acid, quercitrin is converted to quercetin and rhamnose as shown in Fig. 2.

2.2 Chemical and Physical Properties of Quercetin

Quercetin with its high molecular weight (302.24),

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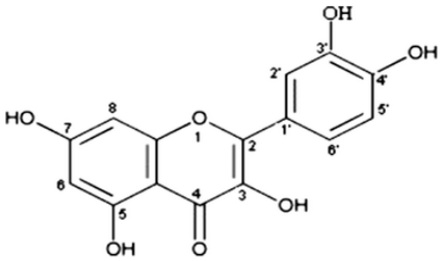

Figure 1. Structure of quercetin (pubchem).

$$C_{21}H_{20}O_{11} + H_2O \xrightarrow{HCl} C_{15}H_{10}O_7 + CH_3(CHOH)_4CHO$$

Quercetin → Quercetin + Rhamnose

Figure 2. Hydrolysis of quercetin.

melting point (316.5 °C) and poor water-solubility makes it a big challenge for being available for biologically. The presence of five hydroxyl groups in the quercetin molecule makes it lipophilic nature. The Table 1 represents the physical properties of quercetin. Quercetin derivatives can be lipophilic or hydrophilic in nature based on the type of substituents in the molecule. In general, O-methyl, C-methyl and prenyl derivatives of quercetin are lipophilic in character. They are synthesised by the glands present on the surface of leaves, flowers and fruits. They can be easily isolated by immersing the plant tissue in acetone[4]. The structural decomposition of quercetin occurs when heated at higher temperatures, wherein it emits acrid smoke and irritating fumes[5].

Table 1. Physical properties of quercetin

Boiling point	Sublimes
Melting point	316.5 °C
Solubility	Less than 1 mg/mL at 70 °C
(a) Very soluble	Ether, methanol
(b) Soluble	Ethanol, acetone pyridine, acetic acid
(c) Water	60 mg/mL at 16 °C

2.3 Absorption and Metabolism of Quercetin

Initially it was assumed that quercetin is absorbed in the small intestine following the cleavage of ß-glucoside linkage by colonic micro flora found in humans, but later on it was concluded that its absorption was improved by conjugation with glucose as shown in Fig. 3. It is proposed that quercetin-3-glucoside on reacting with bacterial enzyme results in the formation quercetin, which further reacts with colon and tissues to give 3,4-diOH-phenylacetic acid and isorhamnetin. 3,4-diOH-phenylacetic acid which is produced, further interacts with colon and tissues to give 3-OH-phenylacetic acid and

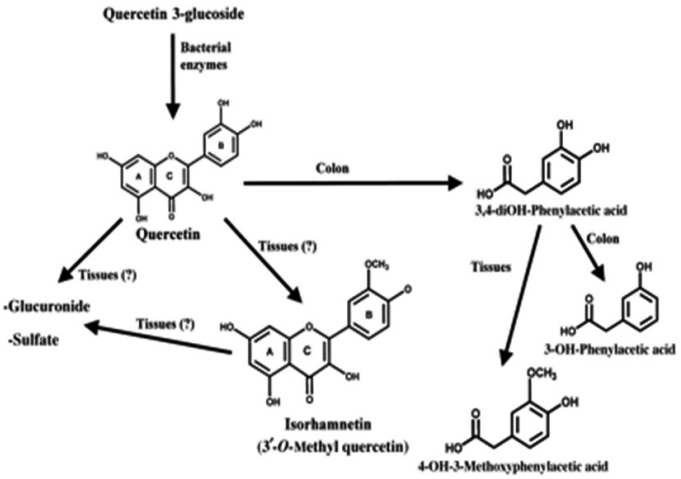

Figure 3. Proposed pathways of quercetin absorption (Source: Wu *et al*, 2002) extraction and analysis of quercetin.

4-OH-3-methoxy-phenylacetic acid; thereby finally quercetin formed is absorbed from the small intestine to colon[6].

From various studies it was found that 0.07 per cent to 17.4 per cent of the amount of quercetin consumed was excreted as quercetin or its conjugates. But the quercetin in form glycosides was reported to absorb in the rat stomach. Walgren[7], *et al*, established from his study with *invitro* studies of Caco-2 cells, that lack of absorption of the quercetin glucosides, happens primarily due to the effective efflux by the multi-drug resistance protein 2 transporters. In succeeding studies conducted with human subjects, it was observed that the quercetin glucosides were hydrolysed by bacterial enzymes in the small intestine.

In another study, it was reported that the absorption of quercetin and quercetin aglycone ranged from 36 per cent to 53 per cent and 65-81 per cent respectively. It was found that absorption of quercetin in ileostomists, as quercetin glucosides, quercetin rutinoside and quercetin aglycone was 52±5%, 17 ±15% and 24±9%, respectively[8-13].

3. EFFECT OF FOOD PROCESSING OF QUERCETIN

3.1 Thermal Processes on Quercetin

Thermal processes have a great influence on the availability of flavonoid from foods, based on their magnitude and duration of exposure. Various thermal processes like drying, microwaving, heating by an autoclave, roasting, pasteurisation, blanching have been used and their respective impact on the flavanoid was analysed. The Table 2 listed a few studies where the effect of heat treatment on the degradation of quercetin in foods was reported. The authors have reported that

Table 2. Effect of heat treatment on quercetin content

	Heat treatment	
Food product/flavonoids	**Processing conditions**	**Impact on flavonoids content**
Grapefruit juices	Pasteurisation (95 °C, 80 s)	Decrease of quercetin
Bean (quercetin)	Atmospheric (100 °C) and pressure boiling (121 °C) with and without soaking and draining	Increases of 1-90% of quercetin
Strawberry juices (quercetin)	High intensity pulsed electric fields Pasteurisation (90 °C, 60 s)	Stability of quercetin

that quercetin was labile to heat degradation.

Ranilla[14], *et al*, reported that boiling and soaking of Brazilian beans at 100 °C along with or without draining, induced a loss percentage of 1-90 per cent of quercetin. Thermal pasteurisation of strawberry juices when performed at 90 °C for 60 s was reported to have no effect on detrimental effect on quercetin contents[15], whereas it was proved to reduce quercetin content in grapefruit juices[16,17]. The flavonoids in aqueous solutions have showed to posses different degrees of sensitivity to heat treatment based on their structures. The degradation of flavonoids may also be influenced by other parameters such as pH, phytochemicals and their structure and also by the presence or absence of oxygen[18].

3.2 Mechanical Processes on Quercetin

The commonly processes like peeling, trimming, and cutting has been studied for its effect on bioactive compounds in flavonoid-rich foods; which is expected to influence the content, activity and availability of those bioactive compounds[19]. High proportions of flavonoids are lost during the pre-processing step when undesired parts of the product was removed or cut off. For instance, during peeling and trimming of onions it said to result in 39 per cent of flavonoids to lose[20] and great losses were noticed while peeling and dicing of tomatoes[21]. However, in another study, it was reported that cutting increased flavonol content in fresh-cut potatoes[22] and onions[23].

3.3 Domestic Processes on Quercetin

Several studies were investigated to find out the effects on flavonoid degradation under simulated home food preparation conditions as shown in Table 3. Common domestic processes methods like boiling, frying, baking, sautéing were performed. Boiling was reported to result in flavonoids losses of 43.9 per cent for asparagus spears and 20.5 per cent for onions, due to leaching of flavanoids into the cooking water[24]. Similarly, flavanoid losses in onions were reported[25-27]. Lombard[28], *et al.* reported that sautéing resulted in a 25 per cent increase in the flavonoid content of onion. But the frying process was reported to decrease about 25-33 per cent of flavonoid content in onion[25,27]. Conversely, baking was found to increase the amount of quercetin conjugate and total flavonol content (7 per cent) in onions, due to the loss of water and other volatiles during cooking and thereby making these compounds concentrated in the tissues[25,26].

Table 3. Effects of domestic treatment on quercetin content

Domestic processes		
Food product	**Processing condition**	**Impact on flavonoid content**
Onions	Sautéing (5 min), baking (15 min, 176 °C)	Increase of quercetin conjugates
Brown skinned onions	Boiling (20 min)	A 14.3% loss of quercetin conjugates
	Frying (5 min, 15 min)	23-29% Losses of quercetin conjugates
Red skinned onions	Boiling (20 min)	A 21.9% loss of quercetin conjugates
	Frying (5 min, 15 min)	23-29% Losses of quercetin conjugates

4. EFFECT OF STORAGE ON QUERCETIN

The effect of storage conditions on the nutritional quality of food can be a limiting step[28]. The degradation of flavanoid is influenced by the storage duration, temperature and the presence and intensity of light. The influence of storage temperature and time need to be controlled to reduce the effect on the flavanoid degradation. The quality degradation has been found to vary with state of food (fresh or processed) when evaluated for the effect of storage temperature (0 °C and 20 °C) under dark or light exposure conditions as shown in Table 4.

The lower temperature storage of fresh has been reported to result in minimal effect on the flavanoid degradation. Price[27], *et al.* reported that nil effect was acquired in onion quercetin conjugate content when stored under dark condition at 4°C temperature for 6 months. Lopez-Rubira[29], *et al.* reported the effect on the antioxidant activity was insignificant in the pomegranates stored at 1 °C for 13 days. In contrary to this, Vina and Chaves[30] reported 47 per cent loss in total flavanoid content of pre-cut celery, when stored at 0 °C for 21 days. An increase in the flavanoid content was reported[22,31]. The potato strips when stored at 4 °C and exposed to light, was reported to have a higher flavanoid accumulation rate[22]. Wang[31], *et al.* reported that storage of raspberries 16 °C/24 °C for 4 days induced an increase in it's the phenolic content.

The quercetin content of strawberry juices was found to decrease progressively on storage at 4 °C in darkness for 56 days[15]. In another study on raspberry jams, 40 per cent loss of quercetin 3-glycoside was reported when it was stored in dark at 20 °C for a period of 6 months[32]. Though the studies have proved the effect of storage conditions such as time,

Table 4. Effect of storage conditions on quercetin

Effect of storage conditions		
Food product/ flavonoids	**Storage conditions**	**Impact on flavonoids content**
Fresh foods pomegranate	Storage (13 days, 1°C)	No effect on antioxidant activity
Fresh-cut potatoes (flavonols) onions (quercetin conjugates)	Storage at 4 °C under light Long-term storage in darkness (6 months,4 °C)	A 100% increase of flavonol content and no effect on quercetin conjugates
Enriched tea drink Strawberry juice (quercetin)	Storage (6 months, 4 °C) Refrigeration in darkness (56 days, 4 °C)	Decrease of quercetin
Raspberry jams (quercetin 3-glycoside)	Storage (6 months, 20 °C)	40% loss of quercetin3-glycoside

temperature and lighting, on the degradation of the flavanoids, the effect cannot generalised, as the degree or type of influence varies with the type of product and its nature, and with storage condition.

5. EFFECT OF LIGHT ON QUERCETIN

The photodegradation of flavanoid has been investigated earlier[33-35]. The photodegradative effect on the flavanoids was reported to either increase or decrease, based on the state of the food (fresh or processed foods). The stress signal caused by the light exposure enhances the flavanols in fresh foods[56] such as fresh-cut potatoes and onions[22,25]. In a study on the effect of illumination on fresh cut onons, 8 per cent increase in the quercetin was reported. The exposure of the blueberries to UV-C increased the flavonoid content and the antioxidant activity as well. Wang[31], *et al.* reported an increase in the phenolic content of the raspberries when exposed light.

The effect of light, primarily on the photodegradation of phenols was reported to be dependent on various variables like wavelength of light, pH, concentration and its structure, based which positive or negative effects were obtained.

According to Tommasini[36], *et al.* structural rearrangement of 3-hydroxyflavone, occurred rapidly when irradiated at 254 nm than when it was exposed at 350 nm. It was also reported to be highly influenced by the physicochemical properties of the solvent, which played a vital in determination of the occurrence of a photo-oxidation or photo-induced molecular rearrangement.

6. HEALTH BENEFITS OF QUERCETIN

The versatile nature of quercetin contributes too many beneficial biological properties such as antioxidant action, canker sores, neurological effect, antiviral activity, anti-inflammatory, asthma, cardiovascular properties and as a anticancer agent.

6.1 Antioxidant Action

The antioxidant property of the flavanoids principally neutralises the free radicals by donating hydrogen atoms to it. Pietta[37], *et al.* observed that difficulty aroused on correlating the structure of flavonoids and their accountability for radical-scavenging.

The formation of reactive oxygen species (ROS) has been reported to contribute to the diabetes, atherosclerosis, hypertension, ischemic heart disease and heart failure. Quercetin acts as an antioxidant by preventing oxidative stress, the major cause for generation of ROS. The flavonoids comprising 3-OH and 3`, 4`-catechol were known to be ten times more effective towards peroxynitrite than ebselen, a RNS scavenger[38].

Quercetin is widely known for its antioxidant property that is for its ability to scavenge free radicals and bind transition metal ions. The ultimate effects on the humans by quercetin for its antioxidant property need to streamline to obtain significant impact on the biomarkers/ indices to be measured. In a study, the consumption of a test meal of fried onions was reported to have significantly increased the plasma quercetin levels. Though an increase in the total antioxidant activity of the plasma was noted, not much difference occurred in the oxidation of the plasma or isolated low density lipoprotein (LDL) over the 48-h period following the consumption of the fried onions. The two weeks supplementation of quercetin in healthy subjects (150 mg/day) was reported to have minimal influence on the plasma, its antioxidant capacity, oxidised LDL, or alpha- or gamma-tocopherols.

6.2 Neurological Effects

Quercetin has been proved to be neuroprotective as well as neurotoxic. Joseph[34], *et al.* reported to be neuroprotector in rat brain when used in combination to fish oil, where it beneficial effects against neurodegenerative diseases (e.g. Alzheimer's disease). Choi[40], *et al.* showed inhibitory effects against acetylcholinesterase. Quercetin was reported to have decreased the 6-hydroxydopamine induced oxidative stress in the neurons of brain striatum of rats[56]. In another study, it was reported that the quercetin treatment affected the working of the nervous system by depleting the intracellular glutathione contents[41]. On the other hand, whether the prolonged usage of antioxidant supplements can be considered safe for human health is still a big question.

6.3 Antiviral Activity

Quercetin has been reported to be effective against viruses i.e. it posses antiviral activity against enveloped viruses such as herpes simplex type I, parainfluenza type 3, respiratory syncytial, pseudorabies, and Sindbis. It was also proved to be protecting from the cardio virus[42]. The antiviral activity of quercetin was due to its ability to bind to viral coat protein and polymerases and also to damage DNA. The mutagenic, carcinogenic, and anti carcinogenic activity of quercetin was found to be related to its ability to impose or prevent damage to DNA. It was reported that on stabilisation of Quercetin with ascorbate it enhances its antiviral activity, which was similar to the effect induced by ascorbate enhanced antiproliferative effect on squamous cell carcinoma[43]. Quercetin was also proved to have enhance the antiviral activity of agents like interferon and 5°ethyl-2'-deoxyuridine.

6.4 Anticancer Agent

Quercetin and other flavonoids, derived from fruits and vegetables have been marked important compounds as it was considered to positively help in preventing cancer. Various studies has been performed to evaluate the anti-carcinogenic effect of quercetin on cell cultures, where it was found that slow the growth of cancer cells was effected and it also helped to foster apoptosis. The induction of apoptosis in cancer cells has been proved to be an vital step in the development of novel anticancer drug[44]. Some animal studies conducted have shown that quercetin helps in the protecting form certain type of cancers, especially colon cancer[45].

6.5 Canker Sores

Small and shallow lesions known as the canker sores (aphthous ulcers), occur on the soft tissues in mouth or at the base of gums. Sharma[45], *et al.* proved that quercetin reduced the occurrence of mouth sores and also helps to induce mild symptomatic relief.

6.6 Cardiovascular Properties

Heart diseases have been identified to be the primary and leading cause of mortality in the developed countries. Though the exact reason for the cause and the mechanism involved in the occurrence of heart disease still remains a mystery, oxidative stress and inflammation has been identified to play a vital role. Quercetin has been investigated for its possible utilisation as a safe alternative to the antioxidant and anti-inflammatory drugs used for conditions like cardiovascular disease. The studies have revealed that, both preclinical and clinical study, quercetin positively reduced several of the risk factors related with heart disease, including blood pressure and cytokine-induced C-reactive protein (CRP) expression. It has also been identified as a potent vasodilatory agent.

6.7 Anti-inflammatory

The normal biological process in response to injuries, microbial infection or intoxication and chemical irritation was known as inflammation. Inflammation was generally considered to be initiated by the migration of immune cells from blood vessels to the infected/ injured area and discharge of mediators to combat the infection/injury.

Quercetin has been widely known for its anti-inflammatory activity. During a in vivo study conducted by Lin[46], *et al.*, when the rats were treated with quercetin mixed with polysorbate 80, it resulted in the inhibition of edema in the paw of the rats. The applications of quercetin glycoside through the skin surface were found to be ineffective against inflammation due to low absorption value. The quercetin pentamethylether formulation was highly absorbed through the skin route in rat, and it was found to be effective against inflammation, therby proving it to be a potent anti-inflammatory agent.

6.8 Asthma

Asthma is a chronic lung-disease that swells and narrows the airways, thereby causing difficulty in breathing. Quercetin was found to ease the symptoms of asthma. It was found to induce reduction in the inflammatory immune cells number and activation, cuts off the histamine level and also eases the airway smooth muscle. Rigolin[47], *et al.* reported that even at the minimum concentration, quercetin was effective against asthma, in comparison to the standard asthma maintenance medications and steroid inhalers that reduces the resistance to air flow.

Quercetin was also reported to reduce pathologies of asthma, such as eosinophil and neutrophil enrollment, bronchial epithelial cell activation, mucus and collagen production and airway hyperactivity. The dietary intake levels of quercetin were reported to influence the asthma symptoms. The clinical studies performed revealed the possible application of quercetin to prevent or treat asthma in human patients.

7. NEGATIVE EFFECTS OF QUERCETIN

Quercetin which was generally considered to be safe was reported to result in few side effects like headache and discomfort of stomach. A preliminary study conducted, suggested that the byproduct of quercetin leads to the loss of protein function. It has also been reported that very high doses of quercetin may harm the kidneys and thereby it was suggested to take intermittent breaks during the consumption of quercetin. It was advised that pregnant and breastfeeding women and people with kidney disorder should avoid quercetin. Consumption of doses greater than 1 g per day, have been reported to have caused damage to the kidneys.

In a four-week rat study performed by Azuma[48], *et al.* increase in the ratio of weight of liver and kidney to the body weight ratios was observed when fed with more than 314 mg and 157 mg quercetin/kg body weight/day, respectively. The consumption of doses above 157 mg quercetin/kg body weight/ day resulted in a pro-oxidant effect.

In human studies, it was seen that the quercetin content has commonly been well tolerated. It was proved that consumption of doses up to 1,000 mg/day for several months have not induced any adverse effects on blood parameters of liver and kidney function, hematology, or serum electrolytes. At present, the principal concern for toxicity was the co-administration of high quercetin doses with digoxin. Though it has been proved to be toxic, until more studies pertaining to the safe dosage level determination, it suggested to avoid consumption of quercetin along with digoxin[31].

8. SAFETY AND DOSAGE OF QUERCETIN

Several studies, have shown that the higher doses of quercetin more than 200 μm reduced the cell viability[49]; but low doses of quercetin (<200 μm) was reported to increase the cell viability and to be fixed as therapeutic dose. It was reported that a low dose of quercetin also resulted in the inhibition of the proliferation of breast cancer cells, mild cytotoxic effect, and also induce mild DNA damage. The fruit and vegetable consumption was reported to contribute to an average of 15 mg to 40 mg of quercetin per day from the diet. It was suggested that an increase in quercetin intake could be accomplished by increasing the consumption of more fruit and vegetables. Therapeutic dosages of quercetin intake were denoted to range from 250 mg to 500 mg three times per day. Quercetin was generally available, in the form of capsules or tablets ranging in doses from 50 mg to 500 mg, as dietary supplements. The dosage of quercetin has been recommended based on the health condition to be treated and no standard dose for quercetin has been suggested. According to Werbach[50], *et al,* for allergic conditions and for chronic hives, a dose of 250 mg - 600 mg per day and 200 mg - 400 mg has been recommended. It was reported that a low dose of quercetin was sufficient to inhibit the proliferation of breast cancer cells, mild cytotoxic effect, and to induce mild DNA damage[51].

9. DIETARY SOURCE OF QUERCETIN

Scientists have involved in the process of identification and quantification of quercetin from various food sources. It was reported that on comparing the quercetin content in onion peel with that in its flesh, the highest concentration was found in the peels of onion[52]. It has been normally found in a variety of foods like onions, apples, berries, tea, tomatoes, grapes, shallots, brassica vegetables, many seeds, nuts, flowers, barks, leaves and also in some medical plants (ginkgo biloba, cranberries and St. John's wort). The aglycone form

of quercetin was found in much lesser amounts in the diet generally consumed. Hollman[8], *et al.* analysed the glycoside and aglycone form of quercetin extracted from plants, and quantified using High-performance Liquid Chromatography (HPLC). The Table 5 represents the list of items and their quercetin content as reported by the United States Department of Agriculture[53].

Table 5. Quercetin content in foods

Food source	Quercetin content (mg/100g)
Raw onions	13.27
Black tea	1.99
Apple, with skin	4.42
Green tea	2.69
Broccoli, raw	3.21
Red wine	0.84
Spinach, raw	4.86
Cocoa powder, unsweetened	20.10
Cranberries, raw	14.00

10. QUANTIFICATION METHODS

Extraction has been the critical and important step in the employment and development of analytical methods for analysis of plant extracts. Table represents the summary of optimised experimental conditions for various extraction protocols. In general, the basic unit operations of extraction involve drying and milling of source to acquire a homogenous powder and also to improve the extraction kinetic of the molecules. Methods such as ultrasonication, heating under reflux, extraction with Soxhlet apparatus were the most used techniques[54]. However, these methods were time consuming and require large volumes of organic solvents like methanol, ethanol with low extraction rates. Molecules of interest can be of polar, non-polar or heat sensitive in nature; thus the selection of extraction method need to be done by considering all of these parameters. The various extraction and experimental methods for the extraction of quercetin has been represented in Table 6.

The Raman spectrum analysis of quercetin in the ethanol solution at a concentration of 1.0 x 10^{-2} mol/L was observed. The bands obtained were at 600 cm^{-1} and 1616 cm^{-1}, appeared without interference from the solvent peak. The quantitative measurement and confirmation of quercetin was done using with HPLC and UV-Vis absorption spectrometry. The HPLC analysis of quercetin was performed with a UV detector at 254 nm. The UV-Vis absorption spectra were determined by measuring the absorbance at 374 nm for quercetin molecule[55].

11. CONCLUSIONS

Quercetin, a flavonoid has been proved to be a powerful antioxidant which can be derived from raw foods such as fruit and vegetables, cocoa, tea, coffee, etc. Quercetin was also known to provide various beneficial properties for human health as a anti-oxidant, anti-inflammatory agent, antiviral activity, cardiovascular properties and anticancer properties. The health benefits of quercetin can be preserved in the processed foods by adapting less intense and non-aggressive processes. However, providing the consumers, antioxidants enriched food products may not be an easy task, despite the several studies conducted on the effect of food processes on the degradation of quercetin and their functional activities, it has been difficult to generalize the results and adapt. Many factors such as:

(i) The type of raw food
(ii) Standardisation of processing and analytical methods
(iii) The influence of the food matrix, affect the quercetin content extraction, analysis and it properties as well.

The usage of quercetin for its anti-oxidative and anti-inflammatory property has been well established. Interestingly, these were the two effects of quercetin which has been widely dealt to combat the oxidative stress and inflammation proving it to be a major source of supplementation for those who have been suffering from this problem.

The major toxicological work related to the quercetin and its by product has been widely studied with the in vitro studies. The formation of toxic compounds from quercetin upon oxidation, during its ROS scavenging activities were likely to occur. The primary oxidation product of quercetin formed was found to be orthoquinone, which has been proved to be toxic. As a result, the supplementation of quercetin during the in vivo studies has to be done with utmost care, considering the possibility of toxic compounds/ metabolite formation, especially in the treatment of chronic ailments.

REFERENCE

1. Abirami, G. & Vetrichelv T. Development and validation of RP-HPLC method for the determination of cefpodoxime proxetil and ambroxol hydrochloride in pharmaceutical formulation. *IJPT*, 2013, **4**(4), 5028-5037.
2. Aherne, S.A. & O'Brien, N.M. Dietary flavonoids: chemistry, food content, and metabolism. *Nutrition*, 2002, **18**(1), 75-81.
doi: 10.1016/S0899-9007(01)00695-5
3. Aherne, S.A.; O'Brien, N.M. Dietary flavonols: chemistry, food content, and metabolism. *Nutrition,* 2002, **18**(1), 75-81.
doi: 10.1016/S0899-9007(01)00695-5

Table 6. Experimental conditions for the extraction of quercetin

Extraction method	Solvents	Temperature (°C)	Pressure	Time
Ultrasonication	CH_3OH, C_2H_5OH	Above room temperature	120 Watt	1 h
Microwave assisted extraction	CH_3OH, C_2H_5OH	60	15 Watt	2 min
Shaking-water bath	60% aqueous CH_3OH	30		60 min
Ultrasonication using a ultrasonic liquid processor	60% aqueous CH_3OH	Room temperature	10 Watt	30 s
Accelerated solvent extraction	60% aqueous CH_3OH	40	1500 psi	2 min

4. Aramwit, P.; Bang, N. & Srichana, T. The properties and stability of anthocyanins in mulberry fruits. *Food Res. Int.*, 2010, **43**, 1093-1097. doi: 10.1016/j.foodres.2010.01.022
5. Azuma, K.; Ippoushi, K & Terao, J. Evaluation of tolerable levels of dietary quercetin for exerting its antioxidative effect in high cholesterol-fed rats. *Food Chem. Toxicol.*, 2010, **48**, 1117-1122. doi: 10.1016/j.fct.2010.02.005
6. Beecher, G.R. Overview of dietary flavonoids: nomenclature, occurrence and intake. *Journal Nutrition*, 2003, **10**, 3248S-3254S.
7. Bordignon-Luiz, M.T.; Gauche, C.; Gris, E.F. & Falcoao, L.D. Colour stability of anthocyanins from Isabel grapes (*Vitis labrusca* L.) in model systems. *LWT- Food Sci. Technol.*, 2007, **40**, 594–599.
8. Buchner, N.; Krumbein, A.; Rhon, S. & Kroh, L.W. Effect of thermal processing on the flavonols rutin and quercetin. *Rapid Commun. Mass Spectro.*, 2006, **20**, 3229-3235. doi: 10.1002/rcm.2720
9. Cao, X.G.; Li, X.X. & Bao, Y.Z. Responses of human lens epithelial cells to quercetin and DMSO. *Invest Ophthalmol Vis Sci.*, 2007, **48**, 3714-3718. doi: 10.1167/iovs.06-1304
10. Carlsen, C. & Stapelfeldt, H. Light sensitivity of elderberry extract. Quantum yields for photodegradation in aqueous solution. *Food Chemistry*, 1997, **60**, 383-387. doi: 10.1016/S0308-8146(96)00356-1
11. Chandrasekara, N. & Shahidi, F. Effect of roasting on phenolic content and antioxidant activities of whole cashew nuts, kernels and testa. *J. Agr. Food Chem.*, 2011, **59**, 5006-5014. doi: 10.1021/jf2000772
12. Choi, G. N.; Kim, J. H.; Kwak, J. H.; Jeong, C.H.; Jeong, H. R.; Lee, U. & Heo, H. J. Effect of quercetin on learning and memory performance in ICR mice under neurotoxic trimethyltin exposure. *Food Chemistry*, 2012, **132**, 1019-1024. doi: 10.1016/j.foodchem.2011.11.089
13. Cisneros-Zevallos, L. The use of controlled postharvest abiotic stresses as a tool for enhancing the nutraceutical content and adding-value of fresh fruits and vegetables. *J. Food Sci.*, 2003, **68** (5), 1560–1564. doi: 10.1111/j.1365-2621.2003.tb12291.x
14. Cornard, J.P.; Dangleterre, L. & Lapouge, C. Computational and Spectroscopic Characterization of the Molecular and Electronic Structure of the Pb(II)−Quercetin Complex. *J. Phys. Chem. A.*, 2005, **109**, 10044-10051. doi: 10.1021/jp053506i
15. Crespy, V.; Morand, C.; Besson, C.; Manach, C.; Demigne, C.; & Remesy, C. Quercetin, but not its glycosides, is absorbed from the rat stomach. *J. Agr. Food Chem.*, 2002, **50**, 618-621. doi: 10.1021/jf010919h
16. Dyrby, M.; Westergaard, N. & Stapelfeldt. H. Light and heat sensitivity of redcabbage extract in soft drink model systems. *Food Chemistry*, 2001, **72**, 431-437. doi: 10.1016/S0308-8146(00)00251-X
17. Elliott Middleton, J.R.; Kandaswami ,C. & Theoharides, C.T. Effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacological Reviews*, 2010, **52**(4), 47/867401.
18. Ewald, C.; Fjelkner-Moding, S.; Johansson, K.; Sjoholm, I. & Akesson, B. Effect of processing on major flavonoids processed onions, green beans, and peas. *Food Chemistry*, 1999, **64**, 231-235. doi: 10.1016/S0308-8146(98)00136-8
19. Friedman, M. Chemistry, biochemistry and dietary role of potato polyphenols- review. *J. Agr. Food Chem.*, 1997, **45**(5), 1523-1540. doi: 10.1021/jf960900s
20. Fuleki, T. & Ricardo-Da-Silva, J.M. Effects of cultivar and processing method on the contents of catechins and procyanidins in grape Juice. *J Agr. Food Chem*., 2003, **51**, 640-646. doi: 10.1021/jf020689m
21. Graefe, E.U.; Wittig, J. & Mueller, S. Pharmacokinetics and bioavailability of quercetin glycosides in humans. *J. Clin. Pharmacol.*, 2001, **1**, 492-499. doi: 10.1177/00912700122010366
22. Haenen, G.R.; Paquay, J.B.; Korthouwer, R.E. & Bast, A. Peroxynitrite scavenging by flavonoids. *Biochem. Biophy. Res. Comm.*, 1997, **236**(3), 591-593. doi: 10.1006/bbrc.1997.7016
23. Haleagrahara, N.; Siew, C.J. & Ponnusamy, K. Effect of quercetin and desferrioxamine on 6-hydroxydopamine (6-OHDA) induced neurotoxicity in striatum of rats. *J. Toxicol. Sci.*, 2013, **38,** 25-33. doi: 10.2131/jts.38.25
24. Hartmann, A.; Patz, C.D.; Andlauer, W.; Dietrich, H. & Ludwig, M. Influence of processing on quality parameters of strawberries. *J. Agr. Food Chem.*, 2008, **56**(20), 9484-9489. doi: 10.1021/jf801555q
25. Hertog, M.G. & Hollman, P.C. Potential health effects of the dietary flavonol quercetin. *Eur. J .Clin. Nutr.*, 1996, **50**, 63-71.
26. Hertog, M.G.L.; Hollman, P.C.H. & Katan, M.B. Content of potentially anticarcinogenic avonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *J. Agr. Food Chem.*, 1992, **40**, 2379-2383. doi: 10.1021/jf00024a011
27. Higashio, H.; Hirokane, H.; Sato, F.; Tokuda, S. & Uragami, A. Enhancement of functional compounds in allium vegetables with UV radiation. *Acta Horticulturae*, 2007, **744**, 357-362. doi: 10.17660/ActaHortic.2007.744.39
28. Hollman, P.C.H.; Gaag, M.V.D.; Mengelers, M.J.B.; Van Trijp, J.M.P.; De Vries, J.H.M. & Kata, M.B. Absorption and disposition kinetics of the dietary antioxidant quercetin in man. *Free Radic Biol Med.*,1996, **21**,703–707. doi: 10.1016/0891-5849(96)00129-3
29. Igual, M.; García-Martínez, E.; Camacho, M.M. and Martínez-Navarrete, N. Changes in flavonoid content of grapefruit juice caused by thermal treatment and storage. *Innovative Food Sci. Emerging Technol.,* 2011, **12**, 153-

162.
doi: 10.1016/j.ifset.2010.12.010

30. Indap, M.A.; Radhika, S.; Motiwale, L. & Rao, K. Quercetin: Antitumor activity and pharmacological manipulations for increased therapeutic gains. *Ind. J. Pharm. Sci.*, 2006, **68**, 465-469. doi: 10.4103/0250-474X.27819
31. Jae-Hoon J.; Jee Young.; An, Yong Tae Kwon.; Juong, G. Rhee & Yong, J. Lee. Effects of low dose quercetin: Cancer cell-specific inhibition of cell cycle progression. *J. Cell Biochem.*, 2009, **106**(1), 73-82. doi: 10.1002/jcb.21977
32. Jazvinscak, J.M.; Cipak, G.A.; Vukovic, L.; Vlainic. J.; Zarkovic. N. & Orsolic N. Quercetin supplementation: insight into the potentially harmful outcomes of neurodegenerative prevention. *N-S Arch Pharmacol.*, 2012, **385**, 1185-1197. doi: 10.1007/s00210-012-0799-y
33. Joseph, D.; Muralidhara, K.M. Enhanced neuroprotective effect of fish oil in combination with quercetin against 3-nitropropionic acid induced oxidative stress in rat brain. *Prog. Neuro-Psychopharmacol. Biol Psychiatry.*, 2013, **40**, 83-92. doi: 10.1016/j.pnpbp.2012.08.018
34. Jurasekova, Z.; Domingo, C.; Garcia-Ramosa, J.V. & Sanchez-Cortesa, S. Adsorption and catalysis of flavonoid quercetin on different plasmonic metal nanoparticles monitored by SERS. *J. Raman Spectr*., 2012, **43**, 1913-1919. doi: 10.1002/jrs.4114
35. Kaul, T.N.; Middleton, E. & Ogra, P.L. Antiviral effect of flavonoids on human viruses. *J. Med. Virology*, 1985, **15**, 71-79. doi: 10.1002/jmv.1890150110
36. Lee, S.U.; Lee, J.H.; Choi, S.H.; Lee, J.S.; Ohnisi-Kameyama, M.; Kozukue, N.; Levin, C.E. & Friedman, M. Flavonoid content in fresh, home-processed and light-exposed onions and in dehydrated commercial onion products. *J. Agr. Food Chem.*, 2008, **56**, 8541-8548. doi: 10.1021/jf801009p
37. Lin, C.F.; Leu, Y.L.; Al-Suwayeh, S.A.; Ku, M.C.; Hwang, T.L & Fang, J.Y. Anti-inflammatory activity and percutaneous absorption of quercetin and its polymethoxylated compound and glycosides: the relationships to chemical structures. *Eur. J. Pharm Sci.*, 2012, **47**, 857-864. doi: 10.1016/j.ejps.2012.04.024
38. Lombard, K.; Peffley, E.; Geoffriau, E.; Thompson, L. & Herring, A. Quercetin in onion (Allium cepa L.) after heat-treatment simulating home preparation. *J. Food Composition Anal.*, 2005, **18**, 571-581. doi: 10.1016/j.jfca.2004.03.027
39. Lopez-Rubira, V.; Conesa, A.; Allende, A. & Artes, F. Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C. *Postharvest Bio. Technol.*, 2005, **37**,174-185. doi: 10.1016/j.postharvbio.2005.04.003
40. Makris, D.P. & Rossiter, J.T. Heat-induced, metal-catalyzed oxidative degradation of quercetin and rutin (Quercetin 3-O-Rhamnosylglucoside) in aqueous model. *J. Agr. Food Chem.*, 2000, **48**, 3830-3838. doi: 10.1021/jf0001280
41. Małgorzata, Materska. Quercetin and its derivatives: chemical structure and bioactivity-A review. *Pol. J. Food Nutr. Sci.*, 2008, **58**(4), 407-413.
42. Manach, C.; Morand, C. & Crespy, V. Quercetin is recovered in human plasma as conjugated derivatives which retain antioxidant properties. *FEBS Letter*, 1998, **426**,331-336. doi: 10.1016/S0014-5793(98)00367-6
43. Marco, P.H.; Poppi, R.J.; Scarmino, I.S. & Tauler, R. Investigation of the pH effect and UV radiation on kinetic degradation of anthocyanin mixtures extractedfrom Hibiscus acetosella. *Food Chemistry*, 2011, **125**, 1020-1027. doi: 10.1016/j.foodchem.2010.10.005
44. Morand, C.; Crespy, V.; Manach, C.; Besson, C.; Demigne, C.; & Remesy, C. Plasma metabolites of quercetin and their antioxidant properties. *Am. J. Physiol.*, 1998, **275**, R212-219.
45. Murakami, M.; Yamaguchi. T.; Takamura, H. & Matoba, T. Effects of thermal treatment on radical-scavenging activity of single and mixed polyphenolics compounds. *Food Chem. Toxicol.*, 2004, **69**, FCT 7-10.
46. Nicoli, M.C.; Anese, M. & Parpinel, M. Influence of processing on the antioxidant properties of fruit and vegetables. *Trends Food Sci. Technol*., 1999, **10**(3), 94-100. doi: 10.1016/S0924-2244(99)00023-0
47. Odriozola-Serrano, I.; Soliva-Fortuny, R. & Martín-Belloso, O. Phenolic acids, flavonoids, vitamin C and antioxidant capacity of strawberry juices processed by high-intensity pulsed electric fields or heat treatments. *Euro. Food Res. Technol.*, 2008, **228**, 239-248. doi: 10.1007/s00217-008-0928-5
48. Ong, C.S.; Tran, E.; Nguyen, T.T.T.; Ong, C.K.; Lee, S .K.; Lee, J.J.; Ng,C.P.; Leong, C. & Huynh H. Quercetin-induced growth inhibition and cell death in nasopharyngeal carcinoma cells are associated with increase in bad and hypo phosphorylated retinoblastoma expressions. *Oncol Rep.,* 2004, **11**(3), 727-733.
49. Pandey, K.B. & Rizvi, S. Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine Cellular Longevity*, 2009, **2**(5), 270-278. doi: 10.4161/oxim.2.5.9498
50. Pérez-Gregorio, M.R.; Garcia-Falcon, M.S. & Simal-Gandara, J. Flavonoids changes in fresh-cut onions during storage in different packaging systems. *Food Chemistry*, 2011, **124**, 652-658. doi: 10.1016/j.foodchem.2010.06.090
51. Pietta, P.G. Flavonoids as antioxidants. *J. Natural Products*, 2000, **63**(7), 1035-1042. doi: 10.1021/np9904509
52. Price, K.R.; Bacon, J.R. & Rhodes, M.J.C. Effect of storage and domestic processing on the content and

composition of flavonol glucosides in onion (Allium cepa). *J. Agr. Food Chem.*, 1997, **45**, 938-942. doi: 10.1021/jf9605916
53. Pubchem, http://pubchem.ncbi.nlm.nih.gov/compound/quercetin (Accessed on 22 April 2017).
54. Ranilla, L.G.; Genovese, M.I. & Lajolo, F.M. Effect of different cooking conditions on phenolic compounds and antioxidant capacity of some selected brazilian bean (*Phaselous vulgaris* L.) cultivars. *J. Agr. Food Chem.*, 2009, **57**(13), 5734-5742. doi: 10.1021/jf900527v
55. Ratty, A. & Das, N. Effects of flavonoids on nonenzymatic lipid peroxidation: Structure-activity relationship. *Biochem. Med. Metabolic Bio.*, 1988, **39**(1), 69-79. doi: 10.1016/0885-4505(88)90060-6
56. Rietveld, A. & Wiseman, S. Antioxidant effect of tea: evidence from human clinical trials. *Journal Nutrition*, 2003, **133**(10), 3285S-3292S.
57. Rigolin, L.; Fortunato Freitas, D.C.; Alves Martins, M.; Teixeira P. & Rogerio, A. Quercetin: A flavonoid with the potential to treat asthma. *Braz. J. Pharm. Sci.*, 2012, **48**(4), 589-599. doi: 10.1590/S1984-82502012000400002
58. Sharma, A. & Gupta, H. Quercetin-A flavonoid. *Chron Young Sci.*, 2010, **1**, 10-15.
59. Sharma, P. & Gujral, H.S. Effect of sand roasting and microwave cooking on antioxidant activity of barley. *Food Res. Int*., 2011, **44**, 235-240. doi: 10.1016/j.foodres.2010.10.030
60. Sirinet, P. & Wanlaya, U.T. Anticancer and apoptosis-inducing activities of microbial metabolites. *Electronic J. Biotechnol.*, 2010, **13**, 1-12.
61. Slimestad, F. & Vagen. Onions: a source of unique dietary flavonoids. *J. Agr. Food Chem.*, 2007, **55**(25), 10067-10080. doi: 10.1021/jf0712503
62. Takahama, U. Spectrophotometric study on the oxidation of rutin by horse radish peroxidase and characteristics of the oxidized products. *BBA - General Subjects*, 1986, **882**(3), 445-451.
63. Tommasini. S.; Calabro, M.L.; Donato, P.; Raneri, D.; Guglielmo, G.; Ficarra, P. & Ficarra, R. Comparative photodegradation studies on 3-hydroxyflavone: influence of different media, pH and light sources. *J. Pharmaceutical Biomedical Anal.*, 2004, **35**, 389–397. doi: 10.1016/S0731-7085(03)00586-7
64. Tudela, J.A.; Cantos, E.; Espin, J.C.; Tomás-Barberán. F.A. & Gil, M.I. Induction of antioxidant flavonol biosynthesis in fresh-cut potatoes-effect of domestic cooking. *J. Agr. Food Chem.*, 2002, **50**, 5925-5931. doi: 10.1021/jf020330y
65. Turner, C.; Turner, P.; Jacobson, G.; Almgren, K.; Waldeback, M.; Sjoberg, P.; Karlsson, E.N. & Markides, K.E. Subcritical water extraction and [small beta]-glucosidase-catalyzed hydrolysis of quercetin glycosides in onion waste. *Green Chemistry*, 2006, **8**, 949-959. doi: 10.1039/B608011A
66. Valverdú-Queralt, A.; Medina-Remón, A.; Andres-Lacueva, C. & Lamuela-Raventos, R.M. Changes in phenolic profile and antioxidant activity during production of diced tomatoes. *Food Chem.*, 2011, **126**, 1700-1707. doi: 10.1016/j.foodchem.2010.12.061
67. Viña, S.Z. & Chaves, A.R. Effect of heat treatment and refrigerated storage on antioxidant properties of pre-cut celery (Apium graveolens L). *Int. J. Food Sci. Tech.*, 2008, **43**, 44-51. doi: 10.1111/j.1365-2621.2006.01380.x
68. Walgren, R.A.; Lin, J.T. & Kinne, R.K. Cellular uptake of dietary flavonoids quercetin-4-beta-glucoside by sodium-dependant glucoside transporter SGLTI. *J. Pharmacol. Exp. Ther.*, 2000, **294** 837-843.
69. Walle, T.; Walle, U.K.; & Halushka, P.V. Carbon dioxide is the major metabolite of quercetin in humans. *Journal Nutrition*, 2001, **131**, 2648-2652.
70. Wang YH, Chao PD and Hsiu SL. Lethal quercetin-digoxin interaction in pigs. *Life Sci.*, 2004, **74**, 1191-1197. doi: 10.1016/j.lfs.2003.06.044
71. Wang, S.; Meckling, K. A.; Marcone, M.F.; Kakuda, Y. & Tsao, R. Synergistic, Additive and antagonistic effects of food mixtures on total antioxidant capacities. *J. Agr. Food Chem.,* 2011, **59**, 960-968. doi: 10.1021/jf1040977
72. Werbach, M.R. Nutritional influences on illness. Ed. 2nd, Third Line Press, Tarzana, California, 1993, pp 207-220.
73. Wiczkowski, W.; Nemeth, K.; Bucinski, A. & Piskua, K.M. Bioavailability of quercetin from flesh scales and dry skin of onion in rats. *Pol. J. Food Nutr. Sci.*, 2003, **12,** (53, SI), 95-99.
74. Wu, X.; Cao, G. & Prior, R.L. Absorption and metabolism of anthocyanins in human subjects following consumption of elderberry or blueberry. *Journal Nutrition*, 2002, **132**, 1865-1871.
75. Zafrilla, P.; Ferreres, F.; & Tomas-Barberan F.A. Effect of processing and storage on the antioxidant ellagic acid derivatives and flavonoids of red raspberry (Rubus idaeus L) jams. *J. Agr. Food Chem.*, 2001, **49**, 3651–3655. doi: 10.1021/jf010192x
76. Zheng, H. & Lu, H. Use of kinetic, Weibull and PLSR models to predict the retention of ascorbic acid, total phenols and antioxidant activity during storage of pasteurized pineapple juice. *LWT- Food Sci. Technol.*, 2011, **44** (5), 1273-1281. doi: 10.1016/j.lwt.2010.12.023

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