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Biologically active quercetin from onion and its effect on health

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ABSTRACT

Quercetin is a phenolic compound that exhibits strong antioxidant activity and they are widely found in onion (*Allium cepa*). The concentration of quercetin in the onion of different species or different date of purchase varies within the range of 185-634mg/ kg of fresh onion. The naturally occurring quercetins in the onion are appeared in glucose conjugate forms such as quercetin-4′-O-β-glycopyranoside, quercetin-3,4′-O-β-diglycopyranoside, and quercetin -3,7,4′-O-β-triglycopyranoside. The attachment of glucose on the molecule of quercetin has brought to two adverse effects: i) Decrease of the antioxidant activity. ii) Limit the human intestinal absorption upon oral consumption. Previous studies have suggested several methods to remove the glucose from the molecule of quercetin including the strong acid hydrolysis, organic acid hydrolysis, enzymatic hydrolysis and lactic acid fermentation.

Introduction

Onion (*Allium cepa*) is a common food ingredient, also used for food decorative purpose and is widely consumed worldwide. Onion is usually consumed as fresh, in powder or as essential oil and approximately 5.5kg onion is consumed annually per person [1,2]. Since ancient time, onion has been used as medicinal plant in China to treat fever, headache, cholera and dysentery [3]. Corzo-Martinez*aet al*. have reviewed the biological properties of onion, evidences have shown that onion exhibits various bioactive functions such as antimicrobial activity, antiparasitic activity, antifungal activity, antibacterial activity, antiviral activity and antioxidant activity [1]. The onion extract was reported to have inhibited eosinophil peroxidase activity as well as controlling the protein content in bronchoalveolar lavage fluid of ovalbumin affected mice. These researchers also have reported that a herbal fraction of onion extracts helps in substantial reduction of lipid peroxidation in bronchoalveolar lavage fluid/ lung tissue [4]. Onion contains a great amount of phenolic compounds (flavonoids) that play an important role in exhibiting the antioxidant activity. Quercetin is one of the polyphenolic compounds that is abundant in everyday diet. The intake of flavonoids is essential to maintain our health as flavonoid help endogenous antioxidant in neutralizing the overload radical stress that cause the chronic diseases [5]. It is also important in curing of illness such as, flavonoids are found to have inhibited the proliferation of human lung cancer cell with the association of the capacity of flavonoids to trigger growth inhibition at G2/M phase of cancer [6]. However, quercetagetin a flavonoid showed ability to induce the apoptosis of cell growth responsible for colon cancer [7].

Basically, flavonoidshave a generic formula of C_6-C_3-C_6, in which each C_6 is a benzene ring and the variation of the connecting C_3 moiety determining the properties and the class of the compound. They can be categorized into flavone, flavanone, flavanonol, isoflavone, chalcone, dihydrochalcone, aurone, anthocyanidin, catechin, flava-3, 4-diol and flavonol. Flavonoids are widely spread among the plant material including the fruit, pollen, roots and heartwood but it does not occur naturally in animals. The natural flavonoids occur in glycoside form, in which one or more of the phenolic hydroxyl groups are bond with sugar residues such as glucose, rutinose and rhamnose[8].

Quercetin classified as flavonol and is widely found in fruits and vegetables. Hertog*et al*. have examined the total quercetin content in 28 vegetables and 9 fruits, the result indicated that the highest concentration of quercetin is found in onion follow by kale, French bean, broccoli, lettuce and tomatoes [9]. Among the 9 fruits examined, the highest concentration of quercetin is detected in apples. As the literature suggests, the high concentration of quercetin in onion suggested that onion is a potential source of dietary flavonoids. This review has compiled an up-to-date information on quercetin from different sources and articles. The aim of this review is to illuminate on the antioxidant capability of this flavonoid which can effectively inhibit cancer growth in human body and possible antidote for DNA damage. The ability of quercetin to a healthier testosterone is already proven, so it is a potential field for the researchers to investigate on the female fertility rate related to onion thus quercetin uptake.

Occurrences and distribution quercetin in onion

Major flavonoids found in onion is quercetin which appeared in sugar conjugates form such as, quercetin-4′-O-β-glycopyranoside, quercetin-3,4′-O-β-diglycopyranoside, and quercetin -3,7,4′-O-β-triglycopyranoside [10]. Besides, quercetin-4’-O-β-D-glucoside and quercetin-3,4’-O-β-D-diglucoside, isoquercitrin, kaempferol, luteolin, apigenin and rutin are found in onion [11]. The concentration of naturally occurring quercetinaglycone (sugar free quercetin) is relatively low in onion. According to Price and Rohdes , quercetin-4-O-monoglucoside and quercetin-3,4 O-diglucoside represent 85%

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of total flavonoids in onion [11]. The concentration of quercetinaglycone detected is less than 2 %. The remaining fraction of flavonoids comprises of 17 different components of which quercetin-3-O-glucoside and isorhamnetinglucoside are prominent. According to Lombard *et al*., quercetin-4-O-monoglucoside and quercetin-3,4 O-diglucoside represent 89-90% of the total flavonoid content in the onion and the ratios of quercetin-3,4 O-diglucoside to quercetin-4-O-monoglucosidein tissues ranged from 1: 1.3 and 1: 1.7 [12]. Similar result obtained by Roldan-Marin *et al*., quercetin-3,4-O-diglucoside and quercetin-4-O-monoglucoside represent the major flavonoids in onion [13]. The molecular structures of quercetin and its derivatives are shown in Figure 1.

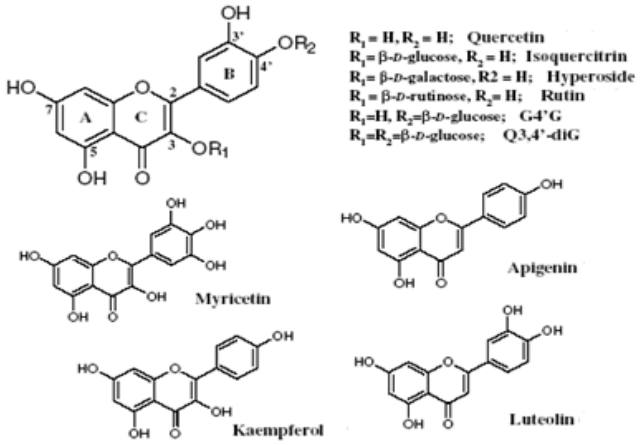

Figure 1. Molecular Structures of quercetin and its related compounds derived from Murakami *et al.* [59]

Total quercetin in the onion can be varied significantly according to the species as well as the origin of it. Prakash*et al*. have studied the total phenolic compound in four (red, violet, white and green) varieties of onion [14]. They found that red onion consists of the highest amount of phenolic compounds followed by violet, green and white onion. Crozier *et al*. have shown that the total quercetin content in white onion with different purchased dates varied from 185mg to 634mg per kg of fresh onion [15]. Meanwhile, 201mg/kg of fresh weighs is found in the red onion. In contrast, the approximate total quercetin concentration has been reported by the researchers in red onion of different sources as 360.25 mg/kg by Son *et al*., 348 mg/kg by Nuutila*et al*.and 367 mg/kg by Lombard *et al*. [15-17]. Therefore, it is suggested that variation of total quercetin content in red onion is less significant since approximately 200-370mg/kg of total quercetin is found in red onion. This amount of quercetin is higher than that in many other berry type fruits such as 6.51 to 9.44 mg per 100g of blueberry [18], 31.54 to 40.88 mg per 100 g of chokeberry fruits [19].Even lower values of it were reported (2.2-4.7 mg per 100 g fruits) by Häkkinen and Törrönen for the same fruits [20]. However, Zheng and Wang found blueberry to contain a comparatively higher amount of quercetin[21].

The quercetin is distributed in all parts of onion and the concentration varies accordingly. Bilyk*et al*.have examined quercetin content in the hydrolysed samples of dry skin, outer ring and inner ring of onion using high-performance liquid chromatography [22]. The result indicated that quercetin content in onion tissue decreases from dry skin towards the inner rings (pulps). Similar result was obtained by Prakash*et al*. in which the highest amount of phenolic compounds was found to present in the outer layers (74.1mg/g GAE) follow by middle layers (15.9 mg/g GAE) and inner layers (5.6 mg/g GAE) [14]. A similar phenomenon was also found in other medicinal plants, like Aloe Vera produces more minerals in its leaf gel with the plant's maturity from 1 to 4 years [23]. Meanwhile, a small quantity of quercetin is present in scallion leaves and none is detected in the bulb tissue. In addition, higher amount of naturally occurring quercetinaglycone is found in dry onion skin as compared to inner parts of onion [22].

Health effects and the absorption of quercetin

Quercetin is widely distributed in the plant kingdom and it is found abundantly in onion. Dietary suggestion for quercetin is receiving an increasing attention since many studies have evidenced the protective effects of quercetin against a variety of human diseases. The health effects of quercetin and its derivatives have been reported by several researchers. Yamamoto *et al.* found that quercetin has a significant inhibitory effect on copper ion-induced lipid peroxidation in human low density lipoprotein [24].Silva *et al*.have reported that quercetin effectively inhibit metal ion-induced lipid peroxidation in rat plasma [25]. Coskun*et al*.in a study on rat showed that quercetin significantly reduced the malondialdehyde and nitric oxide in its liver cells as well as increased the antioxidant enzyme activity, besides, quercetin treated rats apparently showed increased staining of insulin and prevention of islet cells [26]. Moreover, quercetin has been reported as to protect mouse liver from ethanol induced oxidative stress [27]. However, in a recent study Marieee*t al*. revealed that quercetin treated hipercholesteromic rats showed a reduced liver tiglycerides (24%), Lever total cholesterol (22%), Lever serum (20%) and thus the animals exhibited a reasonable improvement of hepatic antioxidant enzymes [28]. On an extensive study Khoo*et al*. have reported many aspects and mechanisms of quercetin in the body, to be specific, in the artery [29]. These researchers have reported that i) quercetin increases vasorelaxation by reducing vasoconstrictor sensitivity as well as increase cGMP levels in the rat aortic vessel. However, these researchers have claimed that quercetin induced vesorelaxation can be regulated in terms of antioxidants production. ii) thequercetin stimulated endothelial nitric oxide synthase (eNOS) phosphylation sites have been identified at serine and threonine protein kinases. Where it is found that quercetin treatment rapidly increased Ca^{2+}, stimulated eNOS phosphorylation at Ser^{1179} which results in NO generation in bovine aortic endothelial cells (BAECs). Moreover, this flavanoid increases the $NO^{\bullet}_2$ accumulation in BAECs. Iii) In aeortic ring segments quercetin increases cGMP which acts as a second messenger much like cyclic AMP. The similar result also has been reported by Kuhlmann*et al*. where it was found that quercetin induced stimulation in cellular cGMP level was 5 fold higher than that of controlled one due to the increased NO production [30]. While these researchers found that 50µmol/L is an optimum dosage for hyperpolarization capacitative Ca^{2+} influx in umbilical cord veins. The intracellular Ca^{2+} homeostasis is a major factor that influences the endothelial cell function. K^+ channel regulates the driving forces for Ca^{2+} in cells and thus are responsible for the capacitative hyperpolarization induced Ca^{2+} influx and the intracellular Ca^{2+} is also controlled by the activity of this K^+ channel. The Ca^{2+} activated K^+ channel (K_{ca}) belongs to the endothelial K^+channel which causes a strong hyperpolarization of the cell membrane with an increased activity [31].

Kuhlmann*et al*. have shown that, quercetin induced K_{ca}-dependent endothelial hyperpolarizationin human umbilical cord which leads to a capacitative influx of extracellular Ca^{2+}, as a result an increased production of NO was observed which helps reduce the endothelial proliferation [30].

Besides, quercetin increases antioxidant capacity *invivo* and demonstrates anti-inflammatory effect in vitro [32]. Again, quercetin in the body may induce the c-Jun expression in prostatic cell lines which is useful in prostate cancer prevention [33].Quercetin protects human hepatocytes from ethanol-induced oxidative stress and prevents lung cancer [34]. Moreover, quercetin also inhibits the growth of colon cancer cell by decreasing the expression of ErbB2 and ErbB3 proteins in HT-29 colon cancer cells [35]. Besides, this flavonoid also acts as anti-toxic for human body against some herbacides or pesticides. Atrazine is one of them. Exposure to ATZ interferes with spermatogenesis in rats and decreases spermatozoa motility [36,37]. However the serious finding has been reported that men living in agricultural areas and exposed to Atrazin suffer from reduced semen quality and fertility exposed to Atrazine [38].Abarikwu*et al.* have shown that quercetin can prevent the Atrazine induced testicular cell toxicity and also can recovered the normal level of cell viability [36]. Moreover, these researchers have stressed that quercetin alone increase the levels of glutathione, glutathione peroxidase (GSH-Px), and GR as well as the mRNA levels of GSH-Px, GR, GST, and can SOD-1.

Unfortunately, the intake of quercetin though oral ingestion of onion is not efficient as the absorption of quercetin by human body is relatively low as compare to other dietary antioxidant, consequently its ability as antioxidant is limited in plasma in *vivo* [39].According to Walle*et al*.approximately 36-53 % of the quercetin can be absorbed through the oral ingestion [40]. In contrast, some Individuals have the ability to absorb quercetin in high level which is possibly due to the polymorphisms of intestinal enzyme [1]. Therefore, this section reviews the mechanism and absorption pathway of quercetin through oral ingestion of onion.

Among the naturally occurring quercetin, a major part of it appeares as quercetinglucosides in the onion in which one or more glucoses attached on the molecular structure of quercetin[11]. Bokkenheuser*et al.* reported that the quercetin glucosides from the diet will pass through the small intestine and enter the large intestinal tract [41]. In the large intestine, the quercetinglucosides are hydrolyzed into quercetinaglycone by enterobacteria, in which the glucose moieties are removed from the molecular structure. Finally, quercetin is absorbed in the large intestine in the aglycone form. Quercetin glycosides are hardly absorbed in the intestinal tract due to their high hydrophilicity contributed by the sugar moieties attached. Murota*et al*.have investigated the intestinal absorption efficiency of quercetinaglycone and quercetinglucosides using human intestinal cell line caco-2 [42]. As compare to the quercetinaglycone, they found that none of the quercetinglucosides were absorbed efficiently from the apical side. The absorption efficiency is enhanced with the increasing lipophilicity of quercetin structures in the order of Quercetin-3,4'-O-β-D-diglucoside< Quercetin-3-O-β-D-glucoside< Quercetin-4-O-β-D-glucoside<Quercetinaglycone. It is suggested that quercetinaglycone can be absorbed easily in the large intestine as its higher lipophilicity facilitates its passage across the phospholipid bilayer of the epithelial cell membrane *via* passive diffusion of transcellular pathway.

In contrast, Hollman*et al*.reported that the absorption of quercetinglucosides (52± 5%) is better than quercetinaglycone (24±9%) when the healthy ileostomy are supplemented with quercetinglucosides (100mg), quercetinrutinoside and quercetinaglycone (100mg) in random order [43]. Gee *et al.* reported that quercetinglucosides isolated from onion including quercetin-3-O-monoglucoside, quercetin-4-O-monoglucoside and quercetin-3, 4'-diglucoside could be absorbed into small intestine [44]. The study demonstrated that the quercetinglucosides are capable to interact with the sodium dependent glucose transport-1 (SGLT1) receptors in the mucosal epithelium for the absorption in the small intestine in *vivo*. On the other hand, Day *et al*.reported that quercetinglucosides are absorbed in the small intestine in the form of aglycone as they demonstrated that the human small intestine and liver were capable of hydrolyzing of quercetinglucosides into quercetinaglycone by the enzyme β-glucosidases[45]. There were three human β-glucosidases have been reported including glucocerebrosidase, lactacephlorizin hydrolase (LPH) and cytosolic broad-specificity β-glucosidase. Cytosolic broad-specificity β-glucosidase is found in the small intestine, liver and kidney. While, lactacephlorizin hydrolase is membrane-bond enzyme found in the brush border of small intestine [46].However, Day *et al*.showed evidence that cytosolic broad-specificity β-glucosidase was responsible for hydrolyzing of quercetinglucosides in the human small intestine, but not the membrane-bond enzyme lactacephlorizin hydrolase [47]. However, later study reported that the hydrolysis activity of quercetinglucosides in the small intestine may depend on the position of the glucose attached to. In a consecutive study, Day *et al*. indicated that hydrolysis of quercetin-4'-glucoside involve both Cytosolic broad-specificity β-glucosidase and lactacephlorizin hydrolase [47]. Nevertheless, only lactacephlorizin hydrolase is responsible for the hydrolysis of quercetin-3-glucoside in the small intestine. Quercetin from the onion peel was found to the rapid rise of plasma quercetin, in just 30 min the level of quercetin was found higher in subjects plasma. The fast rise in quercetin level in plasma was found as an evidence that quercetin 3,4′- O-diglucoside and quercetin 4′-O-diglucoside (quercetin glycoside from onion peel) were absorbed in the stomach and/or upper part of intestine, further the total absorption of quercetin from onion was also higher from that of apple peel [48]. Meanwhile, recent study demonstrated that varieties of flavonoid glucosides including quercetin-4-glucosides and quercetin-3-glucoside can be hydrolyzed in the oral cavity. The hydrolysis are contributed by both bacteria and cytosol on the epithelial cell to deliver bioactive quercetinaglycone for better absorption across the phospholipid bilayer of the cellular membrane [49].

It is suggested that the quercetinglucosides scarcely absorbed through transcellular absorption as the hydrophilicity of sugar moieties limits the permeation across the phospholipid bilayer of epithelial cell [42].In addition to that, the hydrophilic compounds are difficult to pass through the intestinal barriers during the absorption in the intestinal tract. The intestinal barriers are represented by the mucous gel layer and the tight junction. The mucous gel layer is the first intestinal barrier as compounds need to pass through the mucous gel layer to reach on the surface of epithelial cells for absorption. In contrast to the transcellular pathway, a paracellular pathway required compounds to pass across the gaps between the epithelial cells (tight junction) which are well recognized as the second intestinal barriers. The mucous gel layer comprises of three dimensional network of glycoprotein and the tight junction comprises of complex protein tight junction that are responsible to control the permeation across the tight junction if paracelluar absorption takes place. In both cases, the hydrophilic nature of the compounds favors the interaction with the proteins present in the mucous gel layer and tight junction consequently limits the permeability to enter the circulation system. Therefore, studies have been carried out to incorporate permeation enhancers into

the hydrophilic compounds for better intestinal absorption [50,51].

Recent studies have demonstrated that the intestinal absorption of quercetin could be enhanced by modifying the physical condition of the oral intake. A new formulation of microemulsion system has been developed by Gao*et al.*which significantly enhances the absorption of quercetin especially in the colon [52].Besides, Li *et al.*used solid lipid nanoparticles (SLNs) as an oral delivery carrier to enhance the sorption of quercetin[53]. The result indicated that the absortion is significant increased and the relative bioavailability of quercetin –loaded solid lipid nanoparticles (QT-SLNs) to quercetin suspension was 571.4%. On the other hand, Silberberg *et al.*found that the Co-administration of quercetin and cathechin may reduce the intestinal absorption of quercetin[54]. It is likely that the oral intake of quercetin alone may provide better protective effects due to the higher bioavailability.

Antioxidant activity of quercetin

Much attention has been focused on the antioxidant potential of polyphenolic compounds or flavonoids, such as, cardiovascular protection, antimutagenic, antiviral and anticancer activities. However, the biological and pharmacological properties of antioxidant ingredients in modern scientific studies is relatively new [55,56]. Flavonoids and flavanols have been identified as strong antioxidants for quite long. Du *et al*. have shown that green tea polyphenols, including phenolic acids and flavonoids while flavan-3-ols or catechins are the most predominant compounds in green tea whereas, epigallocatechingallate is the major catechinwhis is responsible for much of the biological activity mediated by the green tea including cancer chemoprevention with adjuvant effect of other anticancer compounds [57].

Table 1: Radical scavenging activity of quercetin and its related compounds

Antioxidant	Radical scavenging activity (mol DPPH trapped/ mol antioxidant)	References
Cysteine	1	[59]
α-tocopherol	2.04	
Quercetin	6.56	
Isohamnetin	1.79	
Rhamnetin	6.48	
Q3G	4.71	
Q4'G	0.77	
Q7G	6.03	
Kaempferol	1.9	[24]
Apigenin	0	
Luteolin	3.9	
Myricetin	7.1	

Quercetin has been reported to have a very high antioxidant activity which is comparable to that of α-tocopherol[58]. The presence of the catechol group (ring B as shown in Figure 1) in quercetin contributes to its radical scavenging activity. Besides, the position and number of hydroxyl group attached to the flavonoid's structure dramatically affect the antioxidant activity.

In particular, the hydroxyl group at 3' and 4'-position in catechol group is the main determinant of antioxidant activity. Increase in hydroxyl group will increase the radical scavenging activity. The effectiveness of free radical scavenging activity by quercetin and its derivatives are shown in Table 1. Myricetin and quercetin with hydroxyl group at its 3' and 4'-position showing much higher radical scavenging activity as compare with those derivatives with attached sugar group such as quercetin-4'-*O*- β- D-glucoside (Q4'G). Myricetin has slightly higher antioxidant activity than quercetin as it has more hydroxyl group at its catechol group [59]. Again, quercetinaglycone and its glycosides have stronger antioxidant capabilities compared to corresponding aglycone and glycosides of kempherol, a flavonoid [60].

Ranawa*et al*.have concluded that quercetin possess both antioxidant and prooxidant characteristics [61]. As antioxidant quercetin shows a positive effect on male reproductive system. In a test with human semen, quercetin was identified to exert an irreversible and according to dose (5-200 µM) effect to reduce in sperm mortality, though sperm viability was decreased with higher concentration (50-100µM) of quercetin[62]. Quercetin helps scavenging free radical from the testicles thus prevents from DNA damage, Chromosome aberrations, sperm head abnormalities and germ cell depletion. However, quercetin may produce some metabolites which might be more susceptible to damage to sperm cell having a low glutathione level [61]. Reactive oxygen species such as superoxide ($O_2^{\bullet-}$) can cause production in the vasculature which results endothelial dysfunction. $O_2^{\bullet-}$ production is stimulated by the Endotheline -1 through nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation which leads to the endothelial dysfunction by a protein kinase pathway. Since quercetin is a broad protein kinases inhibitor, it was found that quercetin with isorhamnetin inhibited aortic $O_2^{\bullet-}$ production as well as reduced endotheline-1 induced overexpression of $p47^{phox}$ and endothelial dysfunction which is simulated by phorbol 12-myristate 13-acetate, a protein kinase activator [63].

The cytotoxicity of quercetin in heptoma HepG2 cells over a range of 1-100 µM concentration did not show any significant effect on cell viability, however, at 10 µM concentration quercetin showed a better result than many in the same groups of flavonoids against H_2O_2 induced oxidative stress. Whereas the cellular antioxidant capacities against oxidative stress induced by AAPH, Cu^{2+} or H_2O_2 was reported different from that of peroxyl radical scavenging capacity and reducing capacity where, isoquercitin found to be the strongest among the tested samples [77].

Deconjugation of quercetin glycoside

The intestinal absorption of quercetinglucosides can be occurred either through sodium dependent glucose transport-1 pathway or absorbed as aglycone after deconjugation by the intestinal enzymes) and enterobacteria[41, 44, 45].Recent study indicated that consumption of higher amount of quercetinaglycone onion extract provides greater protective effects against oxidative stress *in vivo* [16]. It is suggested that quercetinaglycone is superior for human consumption due to the better absorption efficiency and higher antioxidant activity [43, 59].Quercitin glycosides and isorhamnetin, a methylated metabolite of quercetin are involved in the antihypertensive response of oral quercetin. Conjugate metabolism inactivates quercetin initially during absorption and safely delivered to the inflamed arterial wall while the recruited metabolites become converted to aglycone in vascular smooth muscle cells thus exert the inhibitory activity on vascular tone. Altogether from these facts, it was suggested that deconjugation is required for the effect of quercetin metabolites [64].

Hence, proper method for hydrolysis of glycoside is essential in preparation of quercetinaglycone rich onion extract. Therefore, this section reviews several methods (strong acid hydrolysis, organic acid hydrolysis and bacterial hydrolysis) that are commonly used in hydrolysis of flavonoid glycoside.

Strong acid hydrolysis is the most common method used in food materials such as the bagasse hemicelluloses [65, 66], apple

pomace[67] and onion [17]. Generally, acid hydrolysis involves strong acid such as hydrochloric acid and sulfuric acid associated with high temperature (80-200^{o}C). The optimum condition for acid hydrolysis of quercetin glycoside in onion has been studied by Nuutila*et al*.[17]. Complete hydrolysis of rutin and isoquercetin in standard mixture can be achieved by refluxing at 80^{o}C with1.2 M HCL in 50% aqueous methanol for 2 hours. Although this protocol gives efficient hydrolysis of quercetin glycoside in onion, it is mainly used for quantification of total flavonoid in plant material. Quantification of individual flavonoid glycosides is difficult as flavonoids are occurred naturally in many varieties of glycoside. In terms of safety aspect, this method is not suitable for food processing due to the difficulties with the consideration of the production cost of downstream processing to remove the hydrochloric acid and methanol residues. On the other hand, the heat labile myricetin which is a flavonol present in onion will be significantly degraded in the high temperature (80^{o}C) during the hydrolysis [17].

Food grade organic acids in food materials are more susceptible in acid hydrolysis for human consumption. According to Cheetham, (0.1-25 %) organic acid can be used instead of a strong acid to hydrolysis flavonoid glycoside [68]. In addition, suitable food grade organic acids such as citric acid, lactic acid, mallic acid, acetic acid, propionic acid, tartaric acid and fumaric acid can be used. For efficient hydrolysis, it is desired that reactions take place at temperature between 80^{o}C to 120 ^{o}C for 1 to 10 hours. The results have shown that the suggested method gave nearly 100 % of hydrolysis of rutin and citrus waste.

Alternatively, enzymatic hydrolysis can be used in deconjugation of the quercetinglucosides in the onion and various enzymes glycosidase were studied comprehensively in the regard of their mechanism in breaking the glycosidic bond between sugar and flavonoids. Although enzymatic hydrolysis can work efficiently but the cost of enzyme for large scale production does not seem to be favorable. Wallace, suggested using endogenous enzyme within plant material to convert the flavonoid glycoside into flavonoid aglycone [69]. This method involves the release of endogenous enzyme by efficiently disrupt the plant cell and hence, the flavonoid glycoside will be hydrolyzed by the endogenous enzyme. It is likely that this method would not work efficiently in the plant material that has insufficient or no endogenous glycosidase enzyme such as onion.

Another potential alternative is through exogenous enzymes produced by the microorganisms during the fermentation of the plant material. According to Wallace, various types of microorganisms equipped with enzyme glycosidase such as fungi (*Aspergillusspp*), yeast (*Saccharomyces cerevisae*) and bacteria (*lactobacillus*) [69]. Lactic acid bacteria are most widely used in deconjugation of flavonoid glycosides in food material such as soymilk[70, 71]. In recent study, Bisakowski*et al*. have investigated the deconjugation of quercetinglucosides in the onion *via* lactic acid fermentation using *lactobacillus plantarum*[72]. The result indicated that the concentration of quercetindiglucoside in the onion decreased from the initial 58.3% to 18% but the amount of quercetinmonoglucoside and quercetinaglycone was accumulated after 72h of fermentation at 19^{o}C. The result demonstrated inefficient deconjugation of quercetinglucosides in onion by lactic acid fermentation as compared to the previous methods.

In contrast, many studies indicated that lactic acid bacteria give efficient deconjugation of flavonoid glycoside in soymilk within a shorter period (24h) [70, 73,74]. It was concluded that the fermentation condition is not the optimum in the study of Bisakowski*et al*. During the fermentation, the lactic acid bacteria will produce enzyme β-glucosidase that is responsible to break the glycoside bond between glucose and quercetin. However, the fermentation temperature (19 ^{o}C) used by Bisakowski*et al*. did not favour the enzyme activity of β-glucosidase[72]. According to Marazza*et al*., the enzyme activity of β-glucosidase from *lactobacillus* at 20^{o}C is approximately 20% of the maximum value at 42^{o}Cfurthermore, these researchers have investigated the β-glucosidase activity of 13 strains of *lactobacillus plantarum* and they found that no β-glucosidase activity was detected in all strains of *lactobacillus plantarum*[70]. It is likely that *lactobacillus plantarum* is not a potential species for the study of deconjugation of quercetinglucosides in the onion.

Conclusion:

The quercetin has been proved to be an active antioxidant and antiflamatory ingredient which is abundant and easily available through common food preparation with ordinary spices. Since it is easily accessible to food, its impact on human health is of relevance. However, the mechanism and many health effects, in vivo, are mostly unknown for quercetin. From many researches and tests done, it has been proved to be a multi-targeted agent. Besides, quercetin as an integration of various flavonoids, has the opportunity to be a nontoxic or low toxic. This phenomenon could be helpful for the future researches since the potentiality of inhibitors nontoxic behavior needs to be identified for clinical use in the multidrug resistance reversal [75]. To consider its pharmaceutical use, correct and safe dosage should have to be established. Quercetin effects on body cells and protein follows its concentration, so even the toxicity of quercetin may arise due to its dose and redox state of the body cell. Clinical trials are needed on cancer patients by administering this ingredient as a drug for the conformation of its effects against cancer, as till date this compound has only been implemented on healthy volunteers in order to establish its availability and metabolism [76]. However, to understand its functionality and reaction mechanism inside the living body more studies are needed to be conducted.

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