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An *in vitro* investigation on the physicochemical properties of different quercetin formulations

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Abstract

Objectives: Quercetin is a naturally occurring plant flavonoid commonly used as a nutritional supplement due to its antioxidant and anti-inflammatory properties. Its well-known low bioavailability has led to the design of different quercetin formulations by various commercial entities seeking to market a highly bioavailable quercetin product. This study investigates four different commercially available quercetin formulations (LMQ, QUX, QUO, and QUV) for their physicochemical properties that influence bioavailability. LMQ and QUX are liquid-based formulations while QUO and QUV are solid powder-based formulations.

Methods: Studies were conducted on particle size using a particle size analyzer; solubility (in water, simulated gastric and intestinal fluid) using Ultra High Performance Liquid Chromatography (UHPLC) to quantify the quercetin content; intestinal permeability and toxicity using Caco-2 cells and HepG2 liver cells.

Results: LMQ and QUX had the narrowest particle size distribution as well as the highest solubility while QUO and QUV had the widest particle size distribution but the poorest solubility. One formulation (QUO) exhibited a significant reduction in cell viability with HepG2 and Caco-2 cells including a significant decrease in TEER value change (−39.0 %; $p<0.01$); its higher Caco-2 cell permeability (P_{app} $2.85\times10^{-4}\pm4.22\times10^{-5}$; $p<0.05$) likely resulted from reduced membrane integrity. The other formulations significantly increased the TEER value within the first 4 h (≥22.7 %; $p<0.05$).

Conclusions: The particle size distribution of each of the individual formulations reflected their solubilities in water and gastrointestinal fluids. Despite QUO having the highest permeability, its negative change in TEER value over time revealed its evident cytotoxic effects. QUV performed poorly in terms of solubility, and permeability. LMQ and QUX were the most consistent across each study with LMQ performing better than QUX overall. Findings of this study present one formulation (LMQ) with superior intestinal absorption while maintaining high cell viability, thus making it one of the safer and more effective quercetin formulations.

Keywords: bioavailability; caco-2 cells; HepG2 liver cells; permeability; toxicity; quercetin

Introduction

Quercetin is a polyphenolic antioxidant classified in the flavonol subclass of flavonoids [1]. It occurs naturally across a variety of diverse fruits and vegetables such as apples, berries, red grapes, onions, cilantro and citrus fruits [2]. Quercetin has gained popularity as a phytonutrient for its antioxidant and anti-inflammatory properties [3] and is also believed to have a wide range of other health effects, including potential anti-cancer, anti-allergy, and anti-viral properties [4]. Quercetin is considered safe to consume with some studies into its toxicity reporting it is safe to take daily at concentrations upwards of 1,000 mg/day for up to three months [5]. However, toxicity studies have yielded somewhat contradictory results: several *in vitro* studies have detected mutagenicity with quercetin while many *in vivo* studies have not detected any carcinogenicity. That being said, the majority of the available evidence support its safety as a dietary supplement and as an addition to foods [6, 7]. The form of quercetin commonly present in plants is typically conjugated to other moieties such as ethers, phenolic acids, and, most commonly, to sugars or glycosylates. While the glycosylation of quercetin makes it more suitable for transportation within plant tissues, this form of quercetin is less readily available for absorption and utilization in humans than quercetin aglycone (free quercetin) [8, 9]. Since only the aglycone is absorbed into the enterocyte, conjugated forms

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require specific border enzymes to release the aglycone from bound moieties prior to absorption so that it can subsequently enter the bloodstream to exert potential health benefits [10]. As a hydrophobic compound, quercetin has low water solubility, which affects its dissolution and dispersion in the gastrointestinal tract [11, 12]. Furthermore, the compound has limited membrane permeability due to its poor solubility and instability and as such, requires specific transporters to facilitate its uptake into cells [13–15].

Orally administered quercetin is prone to degradation at multiple stages during the digestion process, which therefore contributes to its limited bioavailability. This means a significant amount of quercetin is degraded or converted to metabolites such as quercetin 3-*O*-glucoronide or quercetin 3'-*O*-sulfate before it is absorbed [16, 17]. Its low solubility, stability, brief biological half-life and low bioavailability consequently contribute to its reduced efficacy in most commercially formulated products [8]. As a result, researchers have explored various strategies to design a formulation with increased stability and bioavailability. Previous studies have shown that enhanced delivery systems which encapsulate quercetin with a water barrier are useful to provide sufficient blood concentrations of quercetin, likely due to higher gastrointestinal stability and solubility of the compound throughout the absorption process [18, 19]; some of the most common formulations include nanoparticles, micelles, or liposomes [20] which often utilize absorption enhancers such as certain types of phospholipids that are sometimes further co-administered with other bioflavonoids [21].

The objective of this paper was to examine different commercially available formulations regarding their physicochemical properties such as particle size, solubility, and cell permeability, and determine how much of these factors may affect the bioavailability of each quercetin formulation. Furthermore, another objective of this study was to investigate the cytotoxicity of the individual formulations toward intestinal and liver cells using Caco-2 and HepG2 cells. Prior to the *in vitro* experiments, extensive quality control testing was performed on all products used in this study.

Methods

Treatments

Four different quercetin formulations obtained from commercial sources were investigated in this study. The quercetin products and their ingredients are described in Table 1. All capsules were stored at room temperature, protected from heat, moisture, and direct light. QUV serves as control as it contains only standard quercetin and no other co-ingredients.

All commercial products used in this study were subjected to quality control testing for lead, mercury, cadmium, arsenic, and residual solvents. Determination of heavy metal contaminants (lead, mercury, cadmium, arsenic) was performed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) while residual solvent contaminants were analyzed with a Headspace Gas Chromatography-Mass Spectrometer (GC-MS) (see Supplementary files.). The products were further tested for potency, solubility, and particle

Table 1: Commercial quercetin products.

Product code	Brand	Product	Formulation	Ingredients per capsule
LMQ	Natural factors	LipoMicel® quercetin	Softgel capsule (liquid micelle matrix)	250 mg quercetin LipoMicel® (from *Sophora japonica* bud), softgel (gelatin, glycerin, purified water, carob powder), medium chain triglycerides (coconut), *Stevia rebaudiana* leaf, phosphatidylcholine lecithin (sunflower), peppermint essence.
QUX	Platinum naturals	Quercetin 15×	Hard capsule (liquid)	250 mg quercetin, 30 mg vitamin C, hard capsule (hypromellose, chlorophyll), grape seed oil, medium chain triglycerides, sunflower lecithin, d-alpha-tocopheryl acetate.
QUO	Life extension	Optimized quercetin	Hard capsule (powder)	250 mg quercetin, 30 mg vitamin C, 210 mg optimized food blend (bitter orange bioflavonoids, apple, and onion), and 60 mg camu-camu extract (wildcrafted berry), hard capsule (microcrystalline cellulose, vegetable cellulose, maltodextrin, silica, and vegetable stearate).
QUV[a]	Vital nutrients	Quercetin	Hard capsule (powder)	250 mg quercetin, hard capsule (cellulose, vegetable cellulose capsule, calcium carbonate, magnesium silicate, ascorbyl palmitate, and silica).

[a]QUV, is functionally the positive control.

size. In this work, quercetin denotes the anhydrous aglycone form of quercetin.

Potency determination

About 25 mg of each quercetin product was accurately weighed into a 50 mL volumetric flask and 40 mL of methanol was added. Samples were sonicated for 15 min using a Symphony sonicating water bath (VWR International, ON, Canada) to dissolve completely and then filled to volume with methanol. Each solution was filtered with a 0.45 µm Teflon syringe filter into labelled High-Performance Liquid Chromatography (HPLC) vials (Chromatographic Specialties, ON, Canada) and analyzed in an Ultimate 3,000 RS Ultra High-Performance Liquid Chromatography system (UHPLC; Thermo Fisher Scientific Inc., MA, USA) with a quaternary pump delivering a binary gradient of 0.2 % phosphoric acid (HPLC grade, VWR International, ON, Canada) in HPLC grade water (Thermo Fisher Scientific Inc., MA, USA) and HPLC grade acetonitrile (Thermo Fisher Scientific Inc., MA, USA) through a Poroshell EC-18 100 × 2.1 mm, 2.7 µm column (Agilent Technologies, CA, USA) at 0.400 mL/min. Gradient was linearly increased from 10 % acetonitrile to 90 % acetonitrile over a period of 6 min. The column was equilibrated with the starting conditions for 3.3 min before the next injection. Column oven was set to 40 °C and the detector set to collect UV absorption data at 375 nm. The results were calculated using Chromeleon 7.2 software (Thermo Fisher Scientific Inc., MA, USA). The content of quercetin in each sample was determined based on concentrations calculated from peak areas using external standard calibration.

Particle-size analysis

Particle size profiles of the different quercetin formulations were measured by a Mastersizer 3,000 Laser Diffraction Particle-size Analyzer (Malvern Panalytical, QC, Canada). One capsule of each quercetin sample was dispersed in water with continuous agitation, and particles were analyzed in a suspended and homogenized phase at room temperature.

Solubility study

Quercetin formulations used in this study were analyzed in terms of their solubilities in water, simulated gastric solution and simulated intestinal solution. One capsule of each quercetin samples was added to 10 mL of liquid test solution in a 15 mL centrifuge tube and allowed to reach saturation. For the determination of solubility in water, samples were vortexed briefly to suspend visible particles, and then sonicated for 15 min at 37 °C before filtering through 0.45 µm polytetrafluoroethylene (PTFE) filters (Chromatographic Specialties, ON, Canada) into glass HPLC vials for quercetin quantification. Filtered samples contained only particles smaller than 0.45 µm. The gastrointestinal (GI) media were prepared according to the method published by the United States Pharmacopoeia (USP) [22, 23]. For the determination of solubility in GI solutions, samples were vortexed and sonicated at 37 °C for 1 h before they were transferred to 1.5 mL glass vials. All filtered samples were analyzed using an Ultimate 3,000 RS UHPLC system as described in the *Potency* Section.

Caco-2 cell permeability

A human colon carcinoma cell line, Caco-2, was obtained from Cedarlane Laboratories (ON, Canada), suspended with culture media in a T-25 flask (Thermo Fisher Scientific Inc., MA, USA), and cultured in an HERACELL VIOS 160i CO_2 incubator (Thermo Fisher Scientific Inc., MA, USA) at 37 °C and 5.0 % CO_2. The composition of the cell culture media was as follows: Dulbecco's modified Eagle's medium (DMEM) (Sigma-Aldrich, MO, USA), 10 % heat-inactivated fetal bovine serum (FBS) (Thermo Fisher Scientific Inc., MA, USA), penicillin (100 units/mL) and streptomycin (100 units/mL) (Sigma-Aldrich, MO, USA). For permeability studies, cells were resuspended with 5 % trypsin (Thermo Fisher Scientific Inc., MA, USA) and seeded on a culturing plate with polycarbonate semipermeable membrane inserts formatted to a 24-well plate (6.5 mm diameter, 0.4 µm pore size; VWR International, ON, Canada). The seeding density was 1×10^{-4} cells/cm^2. Seeded cells were incubated with the culture media for a total of 21 days before the permeability assay. The culture media was refreshed every 48 h during the first 14 days, and then every 24 h prior to the test. EVOM2, a non-destructive epithelial monolayer voltmeter (World Precision Instruments, FL, USA), was used to measure the transepithelial electrical resistance (TEER) values of the cultured monolayers. Only Caco-2 monolayers with TEER values between 250 and 500 Ω cm^2 were selected for use in permeability experiments. The experiment was conducted according to a method previously published by Du et al. [24].

The apparent permeability coefficient (P_{app}) can be calculated from the permeation rate as determined by the concentrations of the test substance on both sides of the monolayer at t=4 h (see formula below). In this formula, dQ/dt is the amount of product present in the basal compartment as a function of time (nmol/s), A is the area of

each well (cm^2), and C_0 is the initial concentration of product applied in the apical compartment (μM).

$$P_{app} = \frac{dQ}{dt} \cdot \frac{1}{A \cdot C_0}$$

Trypan blue assay for cytotoxicity

Caco-2 and HepG2 liver cells (ATCC–American Type Culture Collection, Virginia, USA) were seeded in a 24-well plate for 5–7 days until they reach 80–90 % confluency. The complete culture medium was refreshed every 24 h. On the day of the cytotoxicity assays, DMEM medium was replaced by 600 μL of culture media containing saturated concentrations of the quercetin test products, and then the cells were placed in a CO_2 incubator at 37 °C. For cytotoxicity and TEER studies, the complete DMEM culture media were used as the control. After 4 h of incubation, cells were rinsed with 500 μL Hank's balanced salt solution (HBSS) and detached with 5 % trypsin, followed by centrifuging at 800×*g* for 3 min, and then the trypsin supernatant was removed. The cells were next incubated in trypan blue (Thermo Fisher Scientific Inc., MA, USA) diluted with HBSS (1:1 v/v) at room temperature for 5 min. The number of dead cells were counted using a hemacytometer under a microscope. The percentage of cell death was calculated using the following equation:

$$\text{Percentage of cell death} = \frac{\text{Trypan blue labeled cell}}{\text{All cells in the designated area}} \cdot 100\,\%$$

Tight junction integrity testing

TEER values of the Caco-2 monolayers were determined using an EVOM2 Epithelial Volt/ohm Meter (World Precision Instruments, Sarasota, FL, USA) before and after 4 h of incubation with culture media containing saturated concentrations of the quercetin test products. It was calculated by the following equation:

$$\text{TEER} = \text{Resistance } (\Omega) \cdot \text{insert membrane area } (cm^2)$$

Data analysis

Results were reported as means ± standard deviation (SD). As for statistical comparison, two-way ANOVA followed by post hoc Dunnett's multiple comparisons test was performed for solubility and permeability measurements and Kruskal–Wallis test with post hoc Dunn's correction for permeability experiments. Differences between sample groups were considered statistically significant at $p<0.05$.

Results

Potency

The products were analyzed using UHPLC to determine their actual quercetin content in comparison to their label claim (250 mg quercetin/capsule). The resulting chromatograms for each formulation showed quercetin appearing at a retention time of ~3.90 (Figure 1). The chromatograms show that more than 99 % of the flavonoids present in each product was quercetin with no appreciable amounts of other flavonoids or their glycosides detected. A small peak for isoquercetrin–a quercetin glucoside–was seen at retention time 3.37 in both QUO and QUV but not in the other formulations. LMQ, QUX and QUO were found to contain 102 , 110, and 114 % of their label claims of quercetin, respectively. QUV on the other hand, contained only 115 mg of quercetin, which was only 46 % of the amount stated in its label claim (Table 2).

UHPLC tests on other commercial formulas which contain quercetin such as Natural Factors' Bioactive Quercetin EMIQ and Webber Naturals' Extra Strength Ginkgo Biloba were performed to confirm the ability of the UHPLC method to differentiate quercetin from its glucosides and other flavonoid compounds (Figure 2). EMIQ not only produced a quercetin peak but also showed a peak for isoquercetrin and a prominent sharp peak for the flavonoid glycoside, rutin, along with a series of peaks representing other quercetin glycosides. *Ginkgo biloba*, on the other hand, showed clearly resolved peaks for quercetin, and other commonly occurring flavonoids isorhamnetin and kaempferol (Figure 2).

Particle size

All products contained particles between 1 μm and about 400 μm in size. QUO covered the widest particle size distribution ranging from about 1 to 400 μm while LMQ covered the narrowest particle-size distribution ranging from a few micrometers to 100 μm (Figure 3).

Solubility

The solubility of the quercetin formulations as determined by HPLC was studied in water, simulated gastric, and intestinal fluid. QUV showed the lowest solubility across all three media. LMQ had the highest solubility of all quercetin formulations in both water and simulated intestinal fluid; in

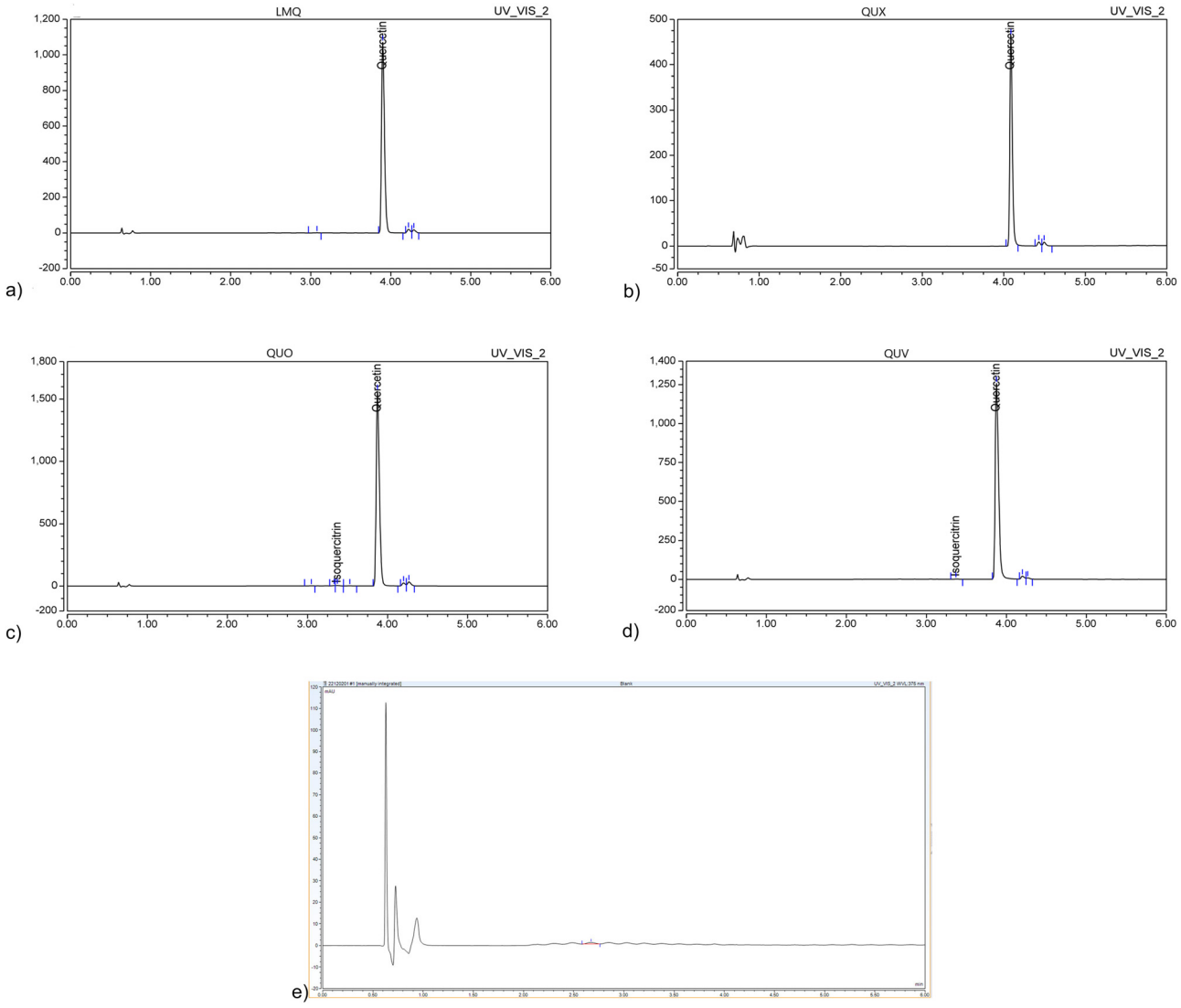


Figure 1: UHPLC chromatograms of (a) LMQ, (b) QUX, (c) QUO, and (d) QUV quercetin formulations and (e) a blank methanol injection.

Table 2: Potency testing results of the different commercial quercetin products.

Products	LMQ	QUX	QUO	QUV
Quercetin content (mg/capsule)	254	275	286	115
Potency %	102	110	114	46

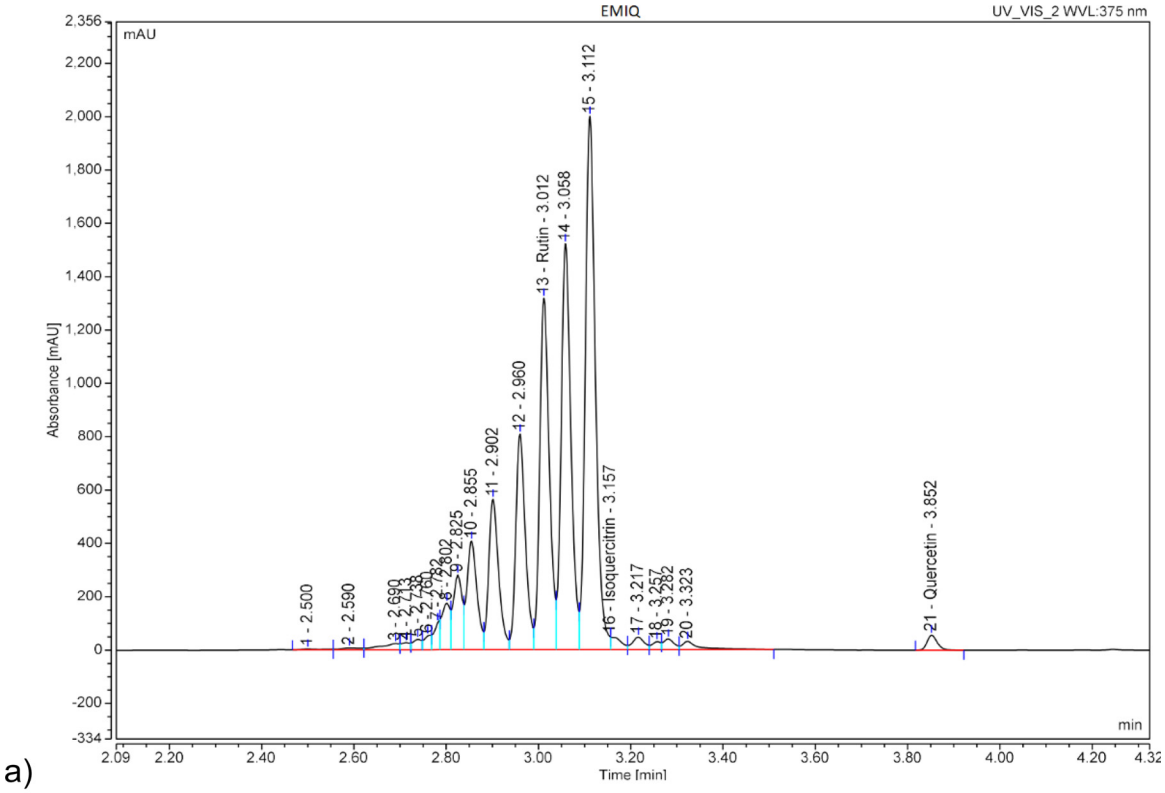
gastric media all four formulations exhibited similar solubility (Figure 4).

Permeability

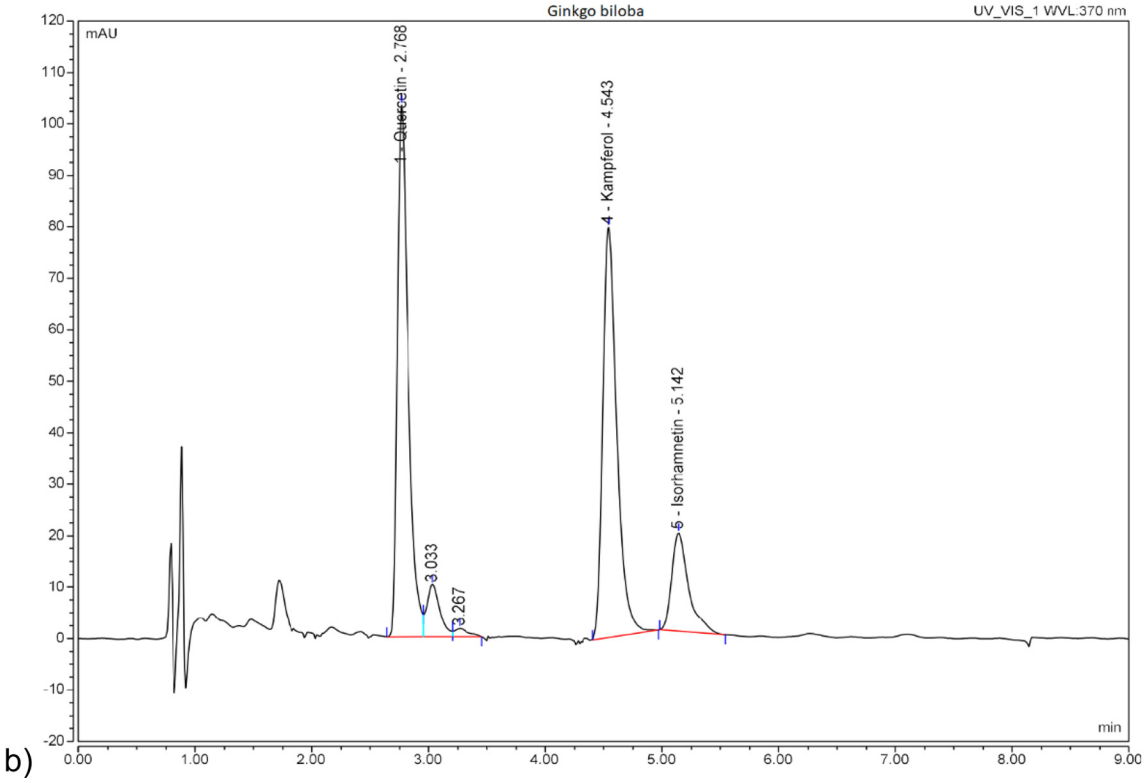
The intestinal permeability of the quercetin products (20 mM) was tested on a Caco-2 cell monolayer and reported as the P_{app} values as shown in Figure 5. QUO presented the highest permeability among the four products, whereas QUX showed the lowest permeability.

In-vitro toxicity

The toxicity of the quercetin products as determined by cell viability was tested by incubating the products (20 mM) for 4 h with HepG2 liver cells (Figure 6a) and Caco-2 cells (Figure 6b) and then calculating the percentage of remaining live cells. No significant changes in either Caco-2 cell or HepG2 cell viability were observed when treated with the different quercetin formulations–except for one product, QUO, which exhibited significant cytotoxic effects on both the HepG2 liver cells and the Caco-2 cells (10 and 20 % cell viability after 4 h, respectively, Table 3).



a)



b)

Figure 2: UHPLC chromatograms of commercial products containing quercetin and other flavonoids. (a) Shows natural factors' EMIQ, (b) shows natural factors ginkgo biloba extra strength.

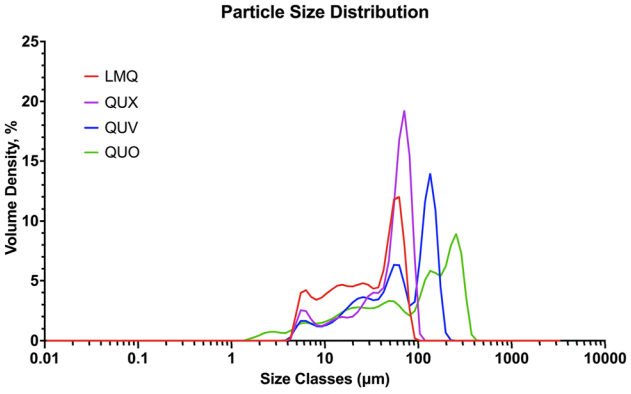


Figure 3: Particle size distribution in water for the different commercial quercetin products. Powdered products such as QUV and QUO shared similar characteristics in terms of water dispersion and particle size distribution; liquid-based products like LMQ and QUX showed lower particle size distribution.

Effect of quercetin products on TEER

Measurement of TEER value was performed to evaluate tight junction integrity of the cell monolayer. The TEER values were determined by measuring the potential difference between the two sides of the cell monolayer after 24 h of incubation with the test solution. Results (Figure 7, Table 4) showed that three out of the four tested quercetin products (at 20 mM) achieved a positive increase in TEER value within the first 4 h (from 22.7 ± 5.7 % up to 37.5 ± 8.3 % increase) indicating an improvement in the barrier function, and then slowly decreased back closer to baseline 100 % at 24 h. One product, QUO, caused a significant reduction in TEER value during incubation (–39.0 ± 5.3 %), which suggested that it compromised the integrity of the cell monolayer (Table 4).

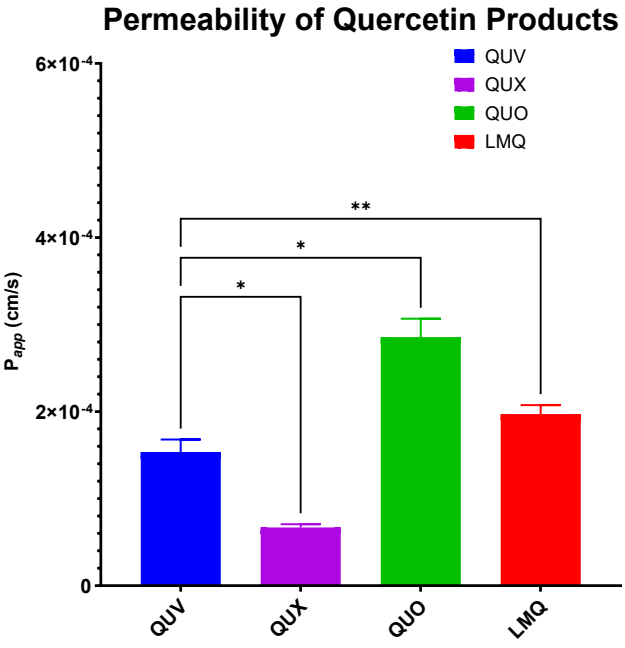


Figure 5: Permeability of the different quercetin products measured in CaCo-2 cell monolayer. Data are expressed as the mean ± SEM from n=4, *p<0.05, **p<0.01, as analyzed by repeated measure one-way ANOVA, with Dunnett's multiple comparison test.

Discussion

A notable drawback of quercetin supplementation is its low bioavailability. Using their physicochemical characteristics – such as solubility in water and simulated GI media, particle size distribution, and intestinal cell permeability – this work aims to predict the *in vivo* bioavailability of several quercetin formulations. UHPLC was used to determine the quercetin

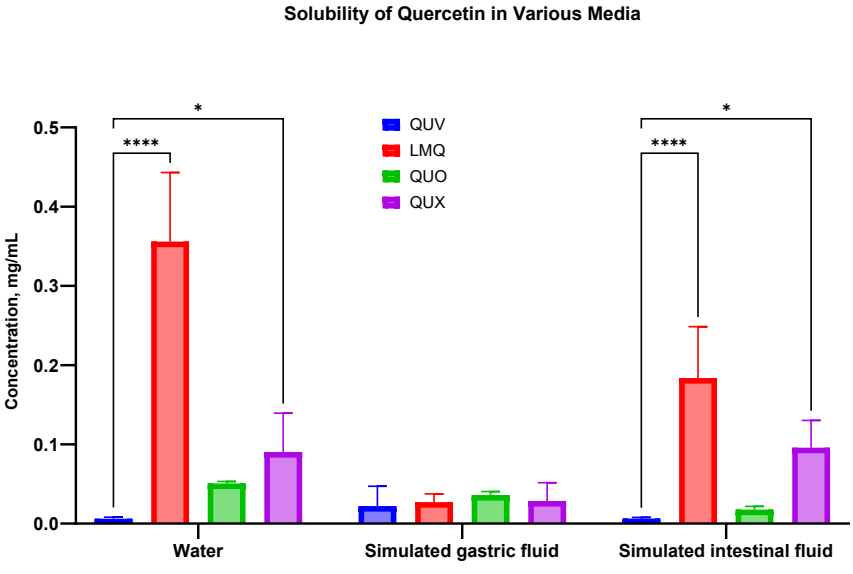


Figure 4: Solubility of the different quercetin formulations in water, simulated gastric fluid, and intestinal fluid. Data are expressed as the mean ± SD from n=3; *p<0.05, ****p<0.0001 (ANOVA and Dunnett's multiple comparisons test).

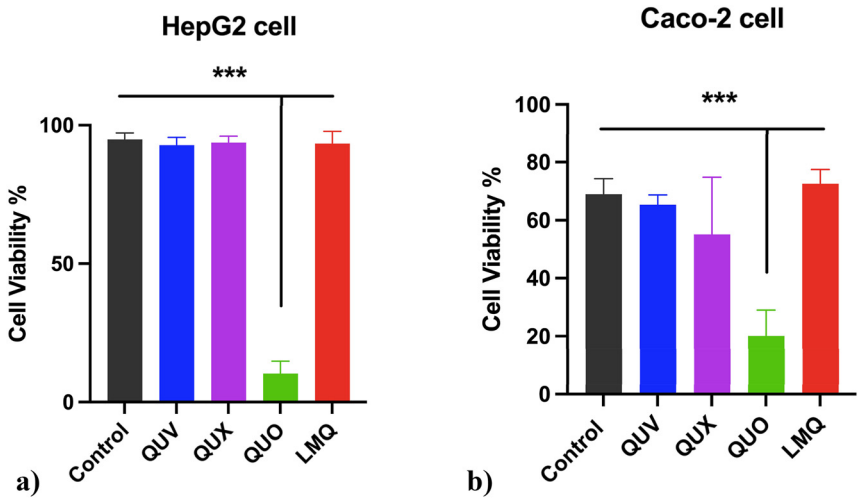


Figure 6: Cytotoxic effects of quercetin products on HepG2 liver cells (a) and Caco-2 cells (b) using trypan blue assay n=3, ***p<0.001. Data was analyzed by Kruskal–Wallis test followed by Dunn's correction for multiple comparisons.

Table 3: Cell viability in HEPG2 liver cells and CaCo-2 cells.

Viability, %	Control	QUV	QUX	QUO	LMQ
HepG2 cell	94.9 ± 2.36[a]	92.8 ± 2.82[a]	93.7 ± 2.37[a]	10.3 ± 4.46[b]	93.4 ± 4.46[a]
Caco-2 cell	69.0 ± 5.29[a]	65.4 ± 3.42[a]	55.1 ± 19.7[a]	20.1 ± 8.86[b]	72.6 ± 4.86[a]

[a,b]Means in each column without a common superscript letter differ by p<0.05, as analyzed by Kruskal–Wallis test followed by Dunn's correction for multiple comparisons; n=3 per treatment.

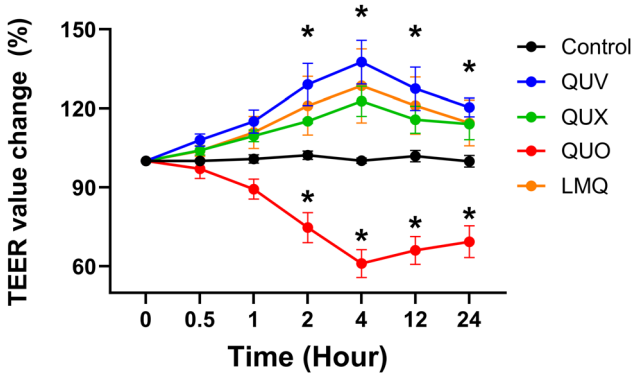


Figure 7: Effect of the different quercetin products on the TEER of Caco-2 cell monolayers over a 24 h period. Data are expressed as the means ± SD from n=3. *p<0.05 compared to control at each time point.

Table 4: Percentage of TEER value changes at 4 h and 24 h.

	Maximum TEER change, %	TEER recovery (% at 24 h)
Control	2.20 ± 1.57[a]	−0.10 ± 2.20[a]
QUV	37.5 ± 8.30[b]	20.33 ± 3.50[b]
QUX	22.7 ± 5.70[b]	14.0 ± 5.90[ab]
QUO	−39.0 ± 5.30[c]	−30.7 ± 6.00[c]
LMQ	28.5 ± 14.1[b]	14.5 ± 8.70[ab]

[a–c]Means in each column without a common superscript letter differ by p<0.05, as analyzed by 2-way ANOVA, followed by Tukey's correction for multiple comparisons; n=3 per treatment.

content within each of these four commercial quercetin formulations. The method was effective in isolating the quercetin compound from its glucosides and other flavonoids as evidenced by the tests of other flavonoid containing commercial formulations, EMIQ and Ginkgo Biloba, which showed a clear separation of quercetin from other glucosides and flavonoids. Of the four, one formulation, QUO, had the highest quercetin content per capsule. In fact, its potency well exceeded its label claim while being within the Canadian Good Manufacturing Practices (GMP) limits of 80–120 % [25]. However, given the presence of bioflavonoids from additional botanical ingredients such as onions, apples, and berries, which are known for their high quercetin content, it can be reasonably concluded that these contribute to the overall quercetin content of this formulation [26]. Nevertheless, by considering the physicochemical characteristics of the products under consideration, this study demonstrated that high quercetin content does not necessarily indicate better absorption.

Of the four products that were examined, two encapsulated quercetin in a liquid matrix (LMQ and QUX) while the other two contained quercetin in a powdered form (QUO and QUV). LMQ and QUX showed a lower particle size distribution spanning from a few micrometers to 100 µm, in contrast to the solid-based powdered products which had a wider particle size distribution. Additionally, when all formulations were evaluated in simulated intestinal fluid and

water, the smaller particle size correlated with increased solubility. Gupta et al. noted that enhanced dissolution velocity, which is closely related to solubility, results from increasing surface area, e.g., decreasing the particle size through micronization or nanonization. The solubility increases with decreasing particle size, and the dissolution velocity increases accordingly [27]. Sahoo et al. also supported this notion as the authors showed that quercetin nanocrystals had an augmented dissolution rate in comparison to standard quercetin particles, and the increase was attributed to an increased surface area resulting from a smaller particle size [28]. In this work, quercetin products which had the widest particle size distribution (>100 µm), such as QUO and QUV, exhibited the lowest solubility in water and simulated intestinal media, which may be related to the increased volume density of their large particles.

Particle size may not be the only factor to consider for the improved solubility of LMQ and QUX over QUO and QUV. Delivery systems that microencapsulate quercetin together with a liquid matrix can create a natural emulsion that reduce hydrodynamic volume, improve solubility, and facilitate the transport of the compound across the cell membrane. For example, Tran et al. found that a quercetin-containing self-nanoemulsifying drug delivery system (Q-SNEDDS) achieved better intestinal uptake and enhanced bioavailability compared to free quercetin by delivering approximately two times higher concentrations of quercetin (159.7 ng/mL for Q-SNEDDS vs. 85.1 ng/mL for free quercetin) [29].

Caco-2 monolayer permeability is an important model for measuring human drug absorption [30]. P_{app} values $<1 \times 10^{-6}$ cm/s indicate poor permeability (0–20 %) and low intestinal absorption; P_{app} values between 1×10^{-6} cm/s and 10×10^{-6} cm/s indicate moderate permeability (20–70 %) and absorption, and P_{app} values $>10 \times 10^{-6}$ cm/s represent high permeability (70–100 %) including high intestinal absorption [31]. Of the four products, QUO (a solid-based formulation) and LMQ (a liquid-based formulation) had the highest intestinal permeability when tested on a Caco-2 cell monolayer, reporting P_{app} values of $2.85 \times 10^{-4} \pm 4.22 \times 10^{-5}$ cm/s and $1.97 \times 10^{-4} \pm 1.00 \times 10^{-6}$ cm/s respectively. For comparison, another study investigating the permeability of 30 different flavonoids reported a much higher P_{app} value of $2.55 \pm 1.45 \times 10^{-6}$ cm/s for quercetin, though it is unclear which form of quercetin was being investigated [32]. Inada et al. found that a quercetin-casein hydrolysate complex with a reported P_{app} value of $0.0974 \pm 0.0045 \times 10^{-6}$ cm/s had a higher intestinal absorption (approximately 2.6 times) compared to free quercetin ($0.0373 \pm 0.00180 \times 10^{-6}$ cm/s) [33].

While both LMQ and QUO showed high intestinal permeability, LMQ increased transepithelial electrical resistance over time whereas QUO significantly decreased transepithelial electrical resistance (Figure 7). This appeared to be related to QUO's significant cytotoxic effects towards Caco-2 cells, resulting in disrupted membrane tight junctions (Figure 6 and Table 3). Damage to membrane tight junctions was indicated by the decrease in TEER value after 4 h (-39.0 ± 5.30 %; $p<0.01$) and after 24 h (-30.7 ± 6.0 %; $p<0.01$) of incubation. The additional co-active ingredients bitter orange bioflavonoids and camu-camu extract in QUO, may be a likely source to explain the cytotoxic effects on both HepG2 and Caco-2 cells. Studies have found that extracts from sweet orange peel containing high concentrations of polymethoxylated flavones (PMFs), which are naturally present in all forms of *citrus*, can inhibit human liver cancer HepG2 growth in *in vivo* models, with significant cytotoxic effects on HepG2 cells [34]. While the exact composition of the camu-camu extract in QUO is not known, the observed cytotoxic effects could be due to its cell growth inhibition properties on HepG2 and Caco-2 at concentrations of 500–2000 µg/mL as reported by Fidelis et al. [35].

Although two of the quercetin products, QUX and QUV, showed a significant increase in TEER, comparable to that of LMQ, they had correspondingly poor Caco-2 monolayer permeability. And even though QUV had a considerably higher permeability than QUX, its low potency of 46 % shows that it would still be providing less quercetin in comparison to the other formulations. In Caco-2 cells, LMQ treatment led to the highest cell viability (72.6 ± 4.86 %), even when compared to the control i.e., standard quercetin (69.0 ± 5.29 %). These results are supported by a previous study in which the standard quercetin product showed approximately 60 % viability on Caco-2 cells at a concentration of 0.1 mM, and the quercetin nanoemulsion had a higher Caco-2 cell permeability ($4.93 \pm 0.01 \times 10^{6}$ cm/s) than the standard quercetin ($\sim 3 \times 10^{6}$ cm/s) [36].

On the other hand, a new finding from this study was that most of the tested quercetin formulations increased trans-epithelial resistance (TEER) in Caco-2 monolayers over time (Figure 7). Earlier cell culture studies found increased expression of critical tight junction proteins such as occudens, occludins and claudin-1 from quercetin treatment of animal epithelial cell lines [37, 38]. *In-vivo* studies supplementing diets of piglets and hens with quercetin have further shown that quercetin protects the intestinal membrane from induced damage [39, 40]. Since intestinal barrier disruption is associated with several human conditions e.g., inflammatory bowel disease, irritable bowel syndrome (IBS), celiac disease, obesity, and formation of kidney stones [41, 42], TEER measurements in Caco-2 cells could serve as a preliminary screening tool for the effectiveness of formulations of quercetin and other flavonoids in treating these conditions.

Results of this study indicate that liquid-based quercetin formulations, such as LMQ and QUX, which have a smaller particle size distribution than the solid-based formulations are associated with higher solubility in water and GI media. Higher intestinal permeability was associated with a positive change in TEER value (22.7 ± 5.7 %, 28.5 ± 14.1 %, respectively) which is indicative of improved epithelial barrier function and tight junction integrity. However, one product, namely QUO, displayed the highest intestinal permeability possibly due to its cytotoxic effects on intestinal Caco-2 cells, disrupting the intestinal cell membrane as indicated by its negative change in TEER value over time (−39.0 ± 5.3 %).

Conclusions

Liquid-based, micellar, delivery systems such as LMQ (LipoMicel® Quercetin) that microencapsulate quercetin with fatty acid compounds in a soft-gel capsule are more soluble and better absorbed than powder-based ones. Compared to the other formulations, LMQ uses a micellar drug delivery system which shows superior physicochemical properties and intestinal absorption with the highest cell viability. Previous studies on LMQ have shown that it is a highly effective delivery system that can enhance the absorption of various natural compounds in the body [24, 43–45]. This study provides supporting evidence on the *in vitro* physicochemical properties of different commercial formulations used to enhance the bioavailability of quercetin in the body. In addition, significant differences in the physicochemical characteristics of liquid-based vs. solid-based quercetin formulations were highlighted in this study.

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