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(54) Title: FLAVONOID-ENRICHED SPRAY-DRIED POWDER

(57) Abstract: The invention relates to a spray-dried powder that comprises a hydrophobic flavonoid and an edible phosphate salt such as a sodium phosphate or potassium phosphate salt. A hydrophobic flavonoid in the form of the spray-dried powder has much higher dispersibility and solubility compared to the raw flavonoid.

FLAVONOID-ENRICHED SPRAY-DRIED POWDER

1. FIELD OF THE INVENTION

The invention relates generally to a spray-dried powder that comprises a hydrophobic flavonoid and an edible phosphate salt such as a sodium phosphate or potassium phosphate salt. The spray-dried powder has properties that make it especially suitable for the incorporation into foods and beverages to increase their flavonoid content.

2. BACKGROUND OF THE INVENTION

Flavonoids are polyphenolic compounds produced as secondary metabolites by many plants. They are defined by the presence of a structure consisting of two benzene rings interconnected by a C3 connector (a heterocylic pyrane ring). The most common flavonoids include the followings: rutin, naringenin and hesperetin (flavanones); apigenin (flavones); isorhamnetin, kaempferol and quercetin (flavonols); genistein and daidzein (isoflavones); epigallocatechin, epicatechin, and gallocatechin (flavan-3-ols/catechins) and cyanidin, delphinidin, pelargonidin and malvidin (anthocyanins).

Many flavonoids have therapeutic and pharmacological properties related to their antioxidant, anti-bacterial, and/or anti-inflammatory qualities. Unfortunately, few people have access to the type of food supply that would allow them to receive the full benefits of these compounds.

For example, rutin (quercetin-3-rhamnosylglucoside) is a well-known flavonoid glycoside, plentifully found in natural sources such as buckwheat seed and fruits (especially, citrus and their rinds). The molecule comprises the flavonol quercetin and the disaccharide rutinose. Rutin possesses potent antioxidant properties on a molecular level. Due to its substantial radical-scavenging properties, rutin demonstrates therapeutic and pharmacological effects such as anti-inflammatory, antidiabetic, hypolipidaemic, and anticarcinogenic properties. However, a high dosage of this flavonoid compound is required in the daily diet to achieve such benefits. Current supplements (nutraceuticals) in the market recommend an oral dosage of 500 mg per day. The daily intake of flavonoids such as rutin in a typical Western diet is much lower - the median intake is only about 10 mg/day.

While nutraceutical supplements in the form of capsules, tablets, and sachets provide benefits, they can lose efficacy due to flavonoid instability and may taste and/or smell unpalatable. Therefore, many people do not like to consume them or simply forget to take them regularly enough to provide the benefits. Hence, the addition of flavonoids to

food products would allow a wider range of people to benefit from their therapeutic properties.

Like many other beneficial flavonoids, rutin is hydrophobic in nature. Other hydrophobic flavonoids include curcumin, hesperidin, naringenin, and catechin. Unfortunately, it is difficult to fortify foods with hydrophobic flavonoids, which are poorly soluble in both oil and water. Their low solubility means that the added flavonoids will sediment in liquid food products (beverages) and produce gritty textures in semi-solid or solid food.

Many flavonoids can also interact with food components such as proteins and fats, changing the physicochemical and sensorial properties of the food. Flavonoids can also undergo chemical and enzymatic degradation themselves. Furthermore, poorly-soluble flavonoids have a very low dissolution rate, as well as a limited release profile in the gastro-intestinal tract, that result in their low bioavailability in the human body.

Therefore, there is increasing interest in methods of encapsulating/entrapping hydrophobic flavonoids, so that they can be successfully added to food systems. A wide range of delivery systems has already been developed; e.g., emulsions, liposomes, coacervates, and gels, which are composed of different natural polymers such as polysaccharides, proteins, and phospholipids. However, options are somewhat limited because of the need to use GRAS (generally regarded as safe) materials, and a strong consumer preference for natural ingredients only.

In addition, the preparation of many flavonoid delivery vehicles involves chemical cross-linking and/or organic solvents such as ethanol and methanol. These agents are undesirable in products for human consumption and the removal of solvents from food products is not cost-effective. Currently available encapsulation/delivery methods also often have low encapsulation efficiency and/or loading capacity. Other processes incorporate manufacturing steps that are expensive or technically difficult to scale up.

Food proteins such as caseins, whey protein, soy proteins and the like have been used extensively as components of delivery vehicles for nutraceuticals. The caseins, in particular, form part of many nutraceutical delivery systems that take advantage of their micellar structure. Caseins are known to self-assemble to form casein micelles of about 40 to 300 nm diameter, which can encapsulate some chemical compounds, if dissociated then re-assembled in the presence of the compound to be encapsulated. Dissociation of casein micelles can be achieved physically, for example, using hydrostatic pressure, or chemically, such as by heating in aqueous ethanol. Casein micelles can also be dissociated under alkaline conditions.

For example, Pan *et al*. (Pan, 2014), describes the production of casein nanoparticles of about 100 nm by alkaline dissociation of sodium caseinate (NaCas), followed by the addition of acid to reach neutral pH. The addition of curcumin to an alkaline solution of NaCas, followed by neutralisation gives a product in which curcumin is encapsulated in the reassembled casein particles. Unfortunately, this does not provide a product that is useful for food fortification.

Firstly, the micellar structure will only reassemble at neutral pH in dilute solutions. So, the process incorporates relatively low amounts of curcumin (1 mg/ml) and NaCas (2.0%), leaving an uneconomically large volume of water to be removed before the encapsulated product can be recovered. Increasing the concentration of curcumin only decreases the encapsulation efficiency (EE) of the process, which is not high to begin with; (1 mg/ml curcumin gives an EE of only about 70%, at the longest incubation time).

Also, the encapsulated product has a low loading capacity (LC) of the flavonoid, so the proportion of the flavonoid in the product is low. This means that to provide a therapeutic benefit, such a large amount of product would need to be incorporated into a food, that the properties of the food would be compromised.

A new flavonoid delivery system described in WO2020/095238 overcomes many of the difficulties described above. This system comprises a co-precipitate of a hydrophobic flavonoid and a protein entrapped in a protein matrix. The co-precipitate is prepared by alkaline solubilisation of the flavonoid followed by co-precipitation with a protein. The dried co-precipitated product has a high loading capacity of flavonoid yet is highly soluble and dispersible, making it suitable for use as a food additive.

Unfortunately, due to its relatively large particle size in aqueous solution, this co-precipitated flavonoid/protein product produces opaque dispersions, and therefore is not suitable for incorporation into transparent or semi-transparent liquid food products.

Accordingly, there is still a need for a product that goes at least partway to overcoming the challenges associated with the delivery of hydrophobic flavonoids, or at least provides the public with a useful choice. Other objects of the invention may become apparent from the following description which is given by way of example only.

In this specification, where reference has been made to external sources of information, including patent specifications and other documents, this is generally to provide a context for discussing the features of the present invention. Unless stated otherwise, reference to such sources of information is not to be construed, in any jurisdiction, as an admission that such sources of information are prior art or form part of the common general knowledge in the art.

3. SUMMARY OF THE INVENTION

In one aspect, the invention provides a spray-dried powder comprising, consisting essentially of, or consisting of a hydrophobic flavonoid and a soluble, edible phosphate salt.

In one embodiment, the spray-dried powder further comprises one or more macromolecular compounds selected from the group consisting of a protein, a polysaccharide, a lipid, and a non-ionic surfactant.

In one embodiment, the hydrophobic flavonoid has a hydrophobicity of about 2 to about 4 and/or is soluble in an aqueous solution at high pH, preferably above about 10.

In one embodiment, the hydrophobic flavonoid is selected from the group consisting of rutin, naringenin, quercetin, curcumin, hesperidin, alpha-naphthoflavone (ANF), beta-naphthoflavone (BNF), catechin and catechin derivatives, chrysin, luteolin, myricetin, and an anthocyanin.

In one embodiment, the hydrophobic flavonoid is selected from the group consisting of rutin, naringenin, catechin, curcumin and hesperidin.

In another aspect, the invention provides a process for producing a spray-dried powder comprising a hydrophobic flavonoid and a soluble, edible phosphate salt, the process comprising the steps of:

(a) adding a hydrophobic flavonoid to an aqueous solution of a soluble, edible phosphate salt, which has a starting pH of about 7.1 to about 10,

(b) stirring the mixture at a temperature of about 20 to about 85°C

until the hydrophobic flavonoid has dissolved, while maintaining the pH at about the starting pH;

(c) adjusting the pH to be about 7.0 to about 7.5; and

(d) spray-drying the solution to provide the powder product.

In one embodiment, one or more macromolecular compounds selected from the group consisting of a protein, a polysaccharide, a lipid and a non-ionic surfactant are added to the dissolved solution of hydrophobic flavonoid following step (b) prior to pH adjustment in step (c).

In one aspect, the invention provides a food product including a spray-dried powder of the invention.

In one embodiment, the food product also comprises one or more macromolecular compounds selected from the group consisting of a protein, a polysaccharide, a lipid and a non-ionic surfactant, preferably a protein.

4. BRIEF DESCRIPTION OF THE FIGURES

The invention will now be described by way of example only and with reference to the drawings in which:

Figure 1 shows particle size measurements of the dispersed particles of untreated/raw rutin and a series of rutin spray-dried powders, measured in phosphate buffer (pH 7.0) at room temperature.

Figure 2 shows particle size measurements of the dispersed particles of untreated/raw naringenin and a series of naringenin spray-dried powders, measured in phosphate buffer (pH 7.0) at room temperature.

Figure 3 shows particle size measurements of the dispersed particles of untreated/raw hesperidin and a series of hesperidin spray-dried powders, measured in phosphate buffer (pH 7.0) at room temperature.

Figure 4 shows particle size measurements of the dispersed particles of untreated/raw curcumin and a series of curcumin spray-dried powders, measured in phosphate buffer (pH 7.0) at room temperature.

Figure 5 shows particle size measurements of the dispersed particles of untreated/raw catechin and a series of catechin spray-dried powders, measured in phosphate buffer (pH 7.0) at room temperature.

Figure 6 shows the water solubility of the dispersed particles of untreated/raw rutin and a series of rutin spray-dried powders, measured after 24 hours in phosphate buffer (pH 7.0) at room temperature.

Figure 7 shows the water solubility of the dispersed particles of untreated/raw naringenin and a series of naringenin spray-dried powders, measured after 24 hours in phosphate buffer (pH 7.0) at room temperature.

Figure 8 shows the water solubility of the dispersed particles of untreated/raw hesperidin and a series of hesperidin spray-dried powders, measured after 24 hours in phosphate buffer (pH 7.0) at room temperature.

Figure 9 shows the water solubility of the dispersed particles of untreated/raw curcumin and a series of curcumin spray-dried powders, measured after 24 hours in phosphate buffer (pH 7.0) at room temperature.

Figure 10 shows water solubility of the dispersed particles of untreated/raw catechin and a series of catechin spray-dried powders, measured after 24 hours in phosphate buffer (pH 7.0) at room temperature.

Figure 11 shows the XRD analysis comparing the crystallinity of untreated/raw naringenin with a series of naringenin spray-dried powders. The edible phosphate salts used were K_2HPO_4 (potassium hydrogen phosphate) and TPP (trisodium phosphate).

Figure 12 is a series of scanning electron micrographs (SEMs) showing the morphology of the particles of untreated/raw catechin (**A**) and catechin spray-dried powders, where **B** is catechin + K_2HPO_4 (dipotassium phosphate), **C** is catechin + K_2HPO_4 + sodium caseinate, **D** is catechin + K_2HPO_4 + soy protein isolate, and **E** is catechin + K_2HPO_4 + pectin. Scale bars are found at the bottom of each micrograph.

Figure 13 is a graph showing the overall liking scores (**A**) and difference-from-control scores (**B**) obtained from banana-flavoured milk and instant flat white coffee fortified with rutin spray-dried powder of the invention, as described in Example 9 (n= 28). FP2 (designated as FP) 500mg: contains 500 mg rutin per serve, FP2 (designated as FP) 250mg: contains 250 mg rutin per serve.

Figure 14 is a graph showing changes in pH of the control and PF2 fortified banana-flavoured milk (designated FP250mg and FP500mg) during storage.

Figure 15 is a graph showing changes in the viscosity of control (no flavonoid) and PF2 fortified banana-flavoured milks (designated FP250mg and FP500mg) stored at 4 °C for 14 days.

Figure 16 is a photograph of rutin and rutin-containing powders mixed in phosphate buffer. Each vial comprises 100 mg rutin. From left to right, the vials comprise raw/ungeared rutin, FlavoPlus 1 (the subject of WO2020/095238) and FlavoPlus 2 (the product of the present invention).

Figure 17 is a graph showing the effect of digested banana milk fortified with FP2 (FlavoPlus2) vs RH (Rutin hydrate) on intracellular antioxidant activity over 60 minutes. Intracellular antioxidant activity was quantified with DCFH-DA assay using relative fluorescence values of the samples. Data represents the mean of three biological replicates with three replicates in each assay. Error bars correspond to the standard

error of the mean. Samples that do not share the same letters are significantly different ($p \leq 0.05$).

Figure 18 is a graph showing the total phenolic content (TPC) of FP2 vs RH expressed as rutin equivalent (µg/sample). Data represents the mean of three replicates with error bars corresponding to the standard error of the mean. Samples that do not share the same letters are significantly different ($p \leq 0.05$).

Figure 19 is a series of graphs showing the effect of FP2 vs RH on intracellular antioxidant activity over 60 minutes. Intracellular antioxidant activity was quantified with DCFH-DA (2′,7′-Dichlorofluorescin diacetate) assay using relative fluorescence values of the samples. Data represents the mean of three biological replicates with three replicates in each assay. Error bars correspond to the standard error of the mean. Samples that do not share the same letters are significantly different ($p \leq 0.05$).

Figure 20 is a series of graphs showing the effect of digested banana milk fortified with FP2 vs RH on intracellular antioxidant activity over 60 minutes. Intracellular antioxidant activity was quantified with DCFH-DA assay using relative fluorescence values of the samples. Data represents the mean of three biological replicates with three replicates in each assay. Error bars correspond to the standard error of the mean. Samples that do not share the same letters are significantly different ($p \leq 0.05$).

Figure 21 is a graph showing the *in vitro* bioaccessibility of rutin in FP2, which was incorporated into a banana milk product, obtained during the intestinal digestion phase. Data represents the mean of three replicates with error bars corresponding to the standard error of the mean. Samples that do not share the same letters are significantly different ($p \leq 0.05$).

5. DETAILED DESCRIPTION OF THE INVENTION

The invention comprises a spray-dried powder comprising a hydrophobic flavonoid and a soluble, edible phosphate salt that can be used to fortify foods and beverages with health-promoting flavonoids. The spray-dried powder is surprisingly simple to prepare. It demonstrates a high loading capacity as well as superior solubility and dispersibility, when compared to the comparable raw flavonoid compound.

5.1 The spray-dried powder of the invention

Flavonoids are a class of compounds having a 15-carbon skeleton consisting of two phenyl rings and a connecting heterocyclic ring. Different sub-classes are defined by

differences in the degree of unsaturation and oxidation state of the heterocyclic connector.

The term "flavonoid" as used herein includes flavanols, flavonols, anthoxanthins, flavanones, isoflavones, flavones, flavans, and anthocyanidines. "Flavonoid" also encompasses isoflavonoids and neofavonoids.

Many flavonoids are hydrophobic and hence cannot be easily incorporated into water-based food products.

The term "hydrophobic flavonoid" as used herein, means a flavonoid that has a hydrophobicity of greater than about 2. Hydrophobicity is measured as Log P, wherein P is the partition coefficient (the solubility of the compound in 1-octanol divided by its solubility in water). Such compounds have very low solubility in aqueous solutions at neutral pH.

Pure hydrophobic flavonoids generally exist in solid form. The solubility of a hydrophobic flavonoid depends on several factors including the ionic strength of the flavonoid, the pH, temperature, and chemical structure of the solvent in which it is to be dissolved.

Raising the pH and heating the solvent can increase the proportion of most hydrophobic flavonoids in an aqueous solution. However, the dried products of such high-pH flavonoid solutions cannot be used in food products without changing the sensory properties of the food. In contact with water, the hydroxy ions present in the dried flavonoid product will raise the pH of the food.

Restoring the solution to neutral pH prior to drying the flavonoid product is also not an option, because the hydrophobic flavonoids will precipitate out of the solution as the pH lowers.

Despite the challenges outlined above, the present inventors have developed a process that results in a powdered hydrophobic flavonoid product of neutral pH that is highly soluble and dispersible in the aqueous medium. The product is prepared according to a process of the invention, which utilises a soluble, edible phosphate salt, such as a sodium, potassium or ammonium salt. Such dried products have a long shelf life and can be incorporated into various food formulations (including clear or semi-clear beverages, or used as a ready-to-mix sachet product) at high concentrations.

In one aspect, the invention provides a spray-dried powder comprising, consisting essentially of, or consisting of a hydrophobic flavonoid and a soluble, edible phosphate salt.

In one embodiment, the hydrophobic flavonoid has a hydrophobicity of about 2 to about 4. In one embodiment, the hydrophobic flavonoid is soluble in an aqueous solution at high pH, preferably above about 10.

In one embodiment, the hydrophobic flavonoid is selected from the group consisting of rutin, naringenin, quercetin, curcumin, hesperidin, alpha-naphthoflavone (ANF), beta-naphthoflavone (BNF), catechin and catechin derivatives, chrysin, luteolin, myricetin, and anthocyanins.

In one embodiment, the hydrophobic flavonoid is selected from the group consisting of rutin, naringenin, catechin, curcumin, and hesperidin.

To prepare the spray-dried powder of the invention, a hydrophobic flavonoid is first dissolved in an aqueous solution of a soluble, edible phosphate salt. The term "soluble, edible phosphate salt" means a salt with a solubility in water of at least 50 wt% at 25°C, which is non-toxic to mammals when ingested in reasonable quantities (up to about 3 g per day).

In one embodiment, the soluble, edible phosphate salt is a sodium, potassium or ammonium phosphate salt, preferably a sodium or potassium phosphate salt.

In one embodiment, the soluble, edible phosphate salt is a monophosphate, diphosphate or polyphosphate salt.

In one embodiment, the soluble, edible phosphate salt is a monosodium or monopotassium phosphate salt. In one embodiment, the soluble, edible phosphate salt is a disodium or dipotassium phosphate salt. In one embodiment, the soluble, edible phosphate salt is a trisodium or tripotassium phosphate salt.

In one embodiment, the soluble, edible phosphate salt is a monophosphate. In one embodiment, the monophosphate salt is derived from orthophosphate, hydrogen phosphate or dihydrogen phosphate.

In one embodiment the soluble, edible phosphate salt is selected from the group consisting of monosodium phosphate, disodium phosphate, trisodium phosphate (TPP), disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium triphosphate, monopotassium phosphate, dipotassium phosphate, tripotassium phosphate, potassium hydrogen phosphate (K_2HPO_4), dipotassium hydrogen phosphate, potassium dihydrogen phosphate, tetra potassium diphosphate, sodium acid pyrophosphate, tetrasodium pyrophosphate, tetrapotassium pyrophosphate, sodium tripolyphosphate, potassium trypolyphosphate, sodium hexametaphosphate, monoammonium phosphate and diammonium phosphate.

In one embodiment, the soluble, edible phosphate salt is a sodium phosphate or potassium phosphate salt, which is selected from the group consisting of K_2HPO_4, tetra potassium diphosphate, and sodium triphosphate, preferably K_2HPO_4.

In one aspect, the invention provides a spray-dried powder comprising, consisting essentially of, or consisting of, a hydrophobic flavonoid and a sodium phosphate or a potassium phosphate salt.

In one embodiment, the spray-dried powder of the invention comprises about 1 to about 70 wt% edible phosphate salt, preferably about 3 to 50 wt%, more preferably about 5 wt% phosphate salt.

In one embodiment, the spray-dried powder of the invention has a flavonoid concentration of about 2 to about 70 wt%, preferably about 20 to about 50 wt%, more preferably about 33 wt%.

In one embodiment, the spray-dried powder of the invention has a flavonoid loading capacity (LC) of about 5 to about 90%, preferably about 10 to about 70%, more preferably about 25 to about 35%, and most preferably, about 30 to about 35%.

In one embodiment, the mass ratio of phosphate salt:flavonoid in the spray-dried powder is about 20:1 to about 1:10, preferably about 15:1 to about 1:7, more preferably about 10:1 to about 1:6.

In one embodiment the spray-dried powder has a pH of about 7 to about 7.5 when dissolved in an aqueous solution.

The spray-dried powder of the invention comprises a hydrophobic flavonoid in solid form that is dispersible and soluble in aqueous mediums. The spray-dried powder of the invention can be stored at room temperature for long periods before use. However, unlike many powdered products, it can be easily incorporated into food products without adversely affecting the properties of the food.

To be effective as a food ingredient, a powdered material must be able to rehydrate in aqueous media. Dispersibility (the ability of a substance to disperse into single particles throughout the medium) is an important step in rehydration. The hydrophobic flavonoid present in the spray-dried powder of the invention is much more dispersible in aqueous solution than an equivalent powdered hydrophobic flavonoid. Both dispersibility and solubility are improved further by incorporating a macromolecular compound into the spray-dried product.

In one embodiment, the spray-dried powder of the invention further comprises one or more macromolecular compounds selected from a protein, a polysaccharide, a lipid, and a non-ionic surfactant.

In one embodiment, the protein is selected from the group consisting of sodium caseinate (NaCas), whey protein isolate (WPI), milk protein concentrate (MPC), milk protein isolate (MPI), soy protein isolate (SPI), pea protein isolate, rice protein isolate (RPI), and gelatine, and/or a hydrolysate of any of these proteins, preferably NaCas.

In one embodiment, the polysaccharide is selected from the group consisting of pectin, carrageenan, dextrin, gum arabic, alginate, chitosan, starch, carboxymethyl cellulose (CMC), and agar-agar.

In one embodiment, the lipid is selected from the group consisting of lecithin, milk fat, coconut oil, and cacao butter.

In one embodiment, the non-ionic surfactant is a polysorbate-type non-ionic surfactant. In one embodiment, the polysorbate-type non-ionic surfactant is selected from the group consisting of polysorbate 20, polysorbate 40, polysorbate 60, and polysorbate 80 (Tween® 20, 40, 60, and 80, respectively).

In one embodiment, the mass ratio of phosphate salt:macromolecular compound:flavonoid in the spray-dried powder is about 5:5:1 to about 1:1:10, preferably about 2:2:1 to about 1:1:5, and more preferably about 1:1:1 to about 1:1:8.

In one embodiment, the macromolecular compound is a protein.

In one aspect, the invention provides a spray-dried powder comprising, consisting essentially of, or consisting of a hydrophobic flavonoid, a soluble, edible phosphate salt, and one or more macromolecular compounds selected from the group consisting of a protein, a polysaccharide, a lipid and a non-ionic surfactant.

As shown in **Figures 1-5**, the flavonoid-containing spray-dried powders of the invention are much more dispersible in aqueous solution than the comparative flavonoid that has not undergone the process of the invention (herein referred to as "raw" or "untreated" flavonoid).

In one embodiment, the hydrophobic flavonoid in the spray-dried powder of the invention is about 10x, 20x, 30x, 40x, 50x, 100x, 200x, 300x or 400x more dispersible in aqueous solution than the same raw, solid flavonoid.

In one embodiment, the spray-dried powder of the invention is completely dispersed in aqueous solution when present at a concentration of 1 to 12 wt%. In one embodiment,

the spray-dried powder of the invention is completely dispersed in aqueous solution when present at a concentration of at least about 8 wt%, preferably at about 12 wt%.

As shown in **Figures 6–10**, the flavonoid-containing spray-dried powders of the invention are much more soluble in aqueous solution than the comparative raw flavonoid.

In one embodiment, the hydrophobic flavonoid in the spray-dried powder of the invention is about 10x, 15x, 20x, 30x, 40x, 50x, 60x, 70x, 80s, 90x, or 100x more soluble in aqueous solution than the same raw flavonoid.

In one embodiment, the spray-dried powder comprises rutin, NaCas, and K_2HPO_4 and the rutin in the powder is at least 10x more soluble in aqueous solution than raw rutin.

In one embodiment, the spray-dried powder comprises rutin, NaCas, and K_2HPO_4 and the rutin in the powder is at least 20x more soluble in aqueous solution than raw rutin.

In one embodiment, the spray-dried powder comprises naringenin, NaCas, and K_2HPO_4 and the naringenin in the powder is at least 30x more soluble in aqueous solution than raw naringenin.

In one embodiment, the spray-dried powder comprises naringenin, NaCas, and K_2HPO_4 and the naringenin in the powder is at least 60x more soluble in aqueous solution than raw naringenin.

In one embodiment, the spray-dried powder comprises hesperidin, NaCas, and K_2HPO_4 and the hesperidin in the powder is at least 70x more soluble in aqueous solution than raw hesperidin.

In one embodiment, the spray-dried powder comprises hesperidin, NaCas, and K_2HPO_4 and the hesperidin in the powder is at least 140x more soluble in aqueous solution than raw hesperidin.

In one embodiment, the spray-dried powder comprises curcumin, NaCas, and K_2HPO_4 and the curcumin in the powder is at least 15x more soluble in aqueous solution than raw curcumin.

In one embodiment, the spray-dried powder comprises curcumin, NaCas, and K_2HPO_4 and the curcumin in the powder is at least 30x more soluble in aqueous solution than raw curcumin.

In one embodiment, the spray-dried powder comprises catechin, NaCas, and K_2HPO_4 and the catechin in the powder is at least 45x more soluble in aqueous solution than raw catechin.

In one embodiment, the spray-dried powder comprises catechin, NaCas, and K_2HPO_4 and the catechin in the powder is at least 90x more soluble in aqueous solution than raw catechin.

In one embodiment, the flavonoid spray-dried powder of the invention has an *in vitro* flavonoid bioaccessiblity of 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 X greater than the raw flavonoid.

Preferably, the flavonoid spray-dried powder of the invention has an *in vitro* flavonoid bioaccessiblity of at least 20 X greater than the raw flavonoid, preferably at least 50 X greater than the raw flavonoid.

The above embodiments also apply to the other aspects of the invention *mutatis mutandi*s.

A relatively large amount of the spray-dried powder of the invention can be added to food products, because the powder remains completely soluble and dispersed even when present in high concentrations.

The flavonoid spray-dried powder of the invention has a pH of about 7.0 to 7.5 in aqueous solution and so will not change the pH of food products to which it is added.

Figure 11 is an X-ray diffractogram comparing untreated/raw naringenin with a series of naringenin-containing spray-dried powders of the invention. The data shows that the crystallinity of naringenin has decreased substantially due to the process of the invention.

This effect is seen in all the spray-dried powders of the invention. Following the process of the invention, the previously crystalline structure of naringenin becomes almost amorphous. This change in structure is the reason for the higher dispersibility/solubility of the spray-dried powders of the invention when compared to the raw flavonoid.

The scanning electron micrographs in **Figure 12** show that the highly crystalline structure of untreated catechin changes dramatically on processing, to form small, spherical spray-dried particles.

5.2 Preparation of the spray-dried powder of the invention

The spray-dried powder of the invention can be easily prepared at a large scale, using only consumable ingredients.

In one aspect, the invention provides a process for producing a spray-dried powder comprising a hydrophobic flavonoid and a soluble, edible phosphate salt, the process comprising the steps of:

(a) adding a hydrophobic flavonoid to an aqueous solution of a soluble, edible phosphate salt which has a starting pH of about 7.1 to about 10,

(b) stirring the mixture at a temperature of about 20 to about 85°C,

until the hydrophobic flavonoid has dissolved, while maintaining the pH at about the starting pH;

(c) adjusting the pH to be about 7.0 to about 7.5; and

(d) spray-drying the mixture to provide the powder product.

Generally, the soluble, edible phosphate salt for use in the process of the invention will be food grade, so that the spray-dried product may be incorporated into food products.

In one embodiment, the concentration of phosphate salt in the aqueous solution of step (a) is about 0.5 to about 10% (w/v), preferably about 3 to about 7% (w/v), more preferably about 5% (w/v).

In one embodiment, the amount of hydrophobic flavonoid added in step (a) is an amount that results in a concentration of about 0.1 to about 10% (w/v) hydrophobic flavonoid in the aqueous solution, preferably about 3 to about 6% (w/v).

The starting pH in step (a) should be about 7.1 to 10.0. If the concentration of phosphate salt in step (a) is relatively low, the pH may need to be adjusted by the addition of a suitable base such as NaOH or KOH to reach the starting pH range. Suitable bases are food grade bases.

In one embodiment, the starting pH is about 7.6 to 8.5, preferably about 8.0.

In step (b), the hydrophobic flavonoid is dissolved in the phosphate salt solution. While some hydrophobic flavonoids may dissolve quite readily, others will be less soluble and may be difficult to dissolve, particularly if added to the phosphate salt solution in large amounts.

The solubility of the hydrophobic flavonoid may be increased by raising the pH and/or temperature of the solution and/or increasing the concentration of phosphate salt in the solution. A person skilled in the art will understand how to vary these factors to achieve complete dissolution of the flavonoid in the solution.

For example, the pK_a of the hydrophobic flavonoid provides an indication of the pH at which the compound will be most soluble. Although hydrophobic flavonoids are generally more soluble at the higher pH used in the process, heating may be required to completely dissolve the solid flavonoid. The mixture may be heated up to about 85°C.

For hydrophobic flavonoids that may be unstable at high temperatures, the minimum amount of heat should be applied to achieve dissolution. In these circumstances, the hydrophobic flavonoid may be dissolved in a phosphate salt solution of higher pH – up to about 10.

In one embodiment, the mixture in step (b) is heated to a temperature of about 30 to about 85°C, preferably about 30 to about 60°C, more preferably to about 30 to 45°C.

In one embodiment, the mixture in step (b) is not heated.

During the dissolution process in step (b), the pH of the solution may start to drop. If this occurs, a base should be added to maintain the pH in the "starting pH range" so that the flavonoid will continue to dissolve.

In one embodiment, one or more macromolecular compounds selected from the group consisting of a protein, a polysaccharide, a lipid, and a non-ionic surfactant are added to the solution of dissolved hydrophobic flavonoid produced in step (b).

In one embodiment, the amount of macromolecular compound added to the solution in step (b) is an amount that results in a concentration of about 0.1 to about 7% (w/v), preferably about 0.5 to about 2.5% (w/v), more preferably about 0.3 to about 1.5% (w/v).

In one embodiment, the macromolecular compound is a protein, preferably NaCas.

In step (c) the pH of the phosphate salt solution of hydrophobic flavonoid is lowered to about neutral. If the mixture required heating to dissolve the flavonoid, it may be cooled to be less than about 60°C before the pH is adjusted.

Surprisingly, the hydrophobic flavonoid remains in solution under these conditions. Without being bound by theory, it is proposed that the phosphate salt interacts with the flavonoid to sterically hinder the aggregation of the flavonoid when the temperature and pH of the solution return to neutral conditions.

The solution containing the hydrophobic flavonoid dissolved in phosphate salt solution is then spray-dried to provide the powdered product of the invention.

In one embodiment, the solution is spray-dried with an inlet temperature of about 150 to 180°C. In one embodiment, the solution is spray-dried with an outlet temperature of

about 75 to 90°C. In one embodiment, the solution is spray-dried with a flow rate of about 10 to 30 mL/min, preferably 20 mL/min.

The invention also provides a product produced by the above processes.

5.3 Food products comprising the spray-dried powder of the invention

The spray-dried powder of the invention can be used in many applications. It is especially useful for the incorporation into food and nutraceutical products.

The spray-dried powder can be incorporated into a range of food products (including liquid, solid, and semi-solid food products) as a fortifying agent to increase the content of health-enhancing flavonoids in the food.

The spray-dried powder may also be used in the form of a ready-to-mix sachet that can be dissolved in water (or the liquid/beverage of choice) immediately before consumption.

In one aspect, the invention provides a food product comprising a spray-dried powder of the invention.

In one embodiment, the food product comprises about 0.3 to about 12 wt% of the spray-dried powder of the invention, preferably about 1 to 9 wt%, more preferably about 4 wt%.

The high LC achieved in the preparation of the flavonoid-containing spray-dried powders of the invention makes them economical to use as fortification agents, as only a small amount needs to be added to greatly increase the flavonoid content of the food product. The smaller amounts needed also make it less likely that the spray-dried powders will affect the sensory properties of the food.

The spray-dried powders of the invention are surprisingly transparent when in solution. This makes them particularly suited for use in transparent foods and beverages, where the addition of an opaque ingredient would be unacceptable. They can also be used in a sachet form where the consumer dissolves the product in water (or a liquid/beverage of their choice) immediately before consumption.

In one embodiment, the food product of the invention is a transparent or semi-transparent food or beverage. Examples of transparent or semi-transparent beverages include, but are not limited to, flavoured water, protein-fortified drink formulations, filtered fruit juices, iced tea, energy drinks, alkaline water, tonic water, mineral water, soft drinks, and energy drinks.

In one embodiment, the transparent or semi-transparent food or beverage comprises about 0.3 to about 9 wt% of the spray-dried powder of the invention, preferably about 0.5 to 5 wt%, more preferably about 3 wt%.

The spray-dried powder of the invention is also particularly suited for the incorporation into dairy products including but not limited to milk and milk-based beverages, yogurt, dairy food, cheese, ice-cream, sorbet, jellies, single-served shot products, honey and honey-based products, and the like; protein bars; powdered beverages, beverages, in particular, semi-solid protein beverages such as smoothies and shakes: cereals; and spreads, for example, peanut butter.

In one embodiment, the food product is a dairy product including but not limited to a yoghurt, dairy food including dairy powders, cheese, ice-cream or sorbet, preferably yogurt.

In one embodiment, the dairy product comprises about 0.2 to about 8 wt% of the spray-dried powder of the invention, preferably about 0.6 to about 6 wt%, more preferably about 1.5 to about 4 wt%. In one embodiment the dairy product is a yoghurt.

In one embodiment, the food product is a protein beverage. In one embodiment, the protein beverage comprises about 0.2 to about 8 (w/v) spray-dried powder of the invention, preferably about 0.15 to about 6, more preferably about 4 (w/v).

In one embodiment, the food product is a protein bar. In one embodiment, the protein bar comprises about 0.5 to about 17 wt% spray-dried powder of the invention, preferably about 1 to about 10 wt%, more preferably about 3 to about 8 wt%.

In one aspect, the invention provides a food product comprising greater than about 0.3 wt% hydrophobic flavonoid, preferably greater than 0.5 wt% hydrophobic flavonoid. In one embodiment, the food product is a dairy product, preferably a yogurt.

While the spray-dried powder of the invention is particularly suited for food fortification, it may also be used as a dietary supplement. A dietary supplement is generally in the form of a pill, capsule, tablet, sachet, gels, or liquid, taken separately or with food to supplement the diet.

In one aspect, the invention provides a dietary supplement comprising a spray-dried powder of the invention.

As used herein the term "comprising" means "consisting at least in part of". When interpreting each statement in this specification that includes the term "comprising", features other than that or those prefaced by the term may also be present. Related terms such as "comprise" and "comprises" are to be interpreted in the same manner.

The term "consisting essentially of" as used herein means the specified materials or steps and those that do not materially affect the basic and novel characteristic(s) of the claimed invention.

In this specification, where reference has been made to patent specifications, other external documents, or other sources of information, this is generally to provide a context for discussing the features of the invention. Unless specifically stated otherwise, reference to such external documents is not to be construed as an admission that such documents, or such sources of information, in any jurisdiction, are prior art, or form part of the common general knowledge in the art.

It is intended that reference to a range of numbers disclosed herein (for example, 1 to 10) also incorporates reference to all rational numbers within that range (for example, 1, 1.1, 2, 3, 3.9, 4, 5, 6, 6.5, 7, 8, 9, and 10) and also any range of rational numbers within that range (for example, 2 to 8, 1.5 to 5.5, and 3.1 to 4.7) and, therefore, all sub-ranges of all ranges expressly disclosed herein are hereby expressly disclosed. These are only examples of what is specifically intended and all possible combinations of numerical values between the lowest value and the highest value enumerated are to be considered to be expressly stated in this application in a similar manner.

Whenever a range is given in the specification, for example, a temperature range, a time range, or a composition range, all intermediate ranges, and subranges, as well as all individual values included in the ranges given are intended to be included in the disclosure. In the disclosure and the claims, "and/or" means additionally or alternatively. Moreover, any use of a term in the singular also encompasses plural forms.

The term "about" as used herein means a reasonable amount of deviation of the modified term such that the end result is not significantly changed. For example, when applied to a value, the term should be construed as including a deviation of+/- 5% of the value.

6. EXAMPLES

Chemicals

Rutin, naringenin, hesperidin, curcumin, catechin, low methoxy pectin, and carrageenan were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). Sodium caseinate and whey protein isolate (WPI) were purchased from Fonterra Co-operative Ltd. (Auckland, New Zealand). Soy protein isolate (SPI) (Ajipron SU®) was purchased from Ajinomoto Co., Inc. Potassium dihydrogen phosphate and trisodium phosphate were purchased from Merck (Darmstadt, FRG). All other chemicals or reagents used were of analytical

reagent grade, obtained from either Sigma-Aldrich (Auckland, New Zealand) or Thermo Fisher Scientific (Auckland, New Zealand).

Statistical analysis

Samples were prepared in triplicate and all measurements were repeated three times (despite X-ray). Mean values of data and standard deviations were calculated using Excel 2016 (Microsoft Redmond, VA, USA) and significant differences between treatments were evaluated using SPSS 20 Advanced Statistics (IBM, Armonk, NY, USA) at $p<0.05$.

Example 1: Preparation of rutin:NaCas:K_2HPO_4 spray-dried powder

One litre of a 5% (w/v) aqueous solution of K_2HPO_4 was prepared and stirred for 30 min to fully dissolve the K_2HPO_4. Rutin (1.5 wt%) was added to the K_2HPO_4 solution and the mixture was heated to 75°C and stirred until the complete dissolution of rutin (about 20 min). The pH was recorded (about 8.0 to 8.5).

To this solution, 2.5 wt% of NaCas was added and the mixture was stirred until the complete dissolution of the protein (about 15 min), while the pH was continually monitored. The temperature was decreased to 45°C and the pH adjusted to 7.3 by the addition of HCl.

The warm solution was spray-dried (inlet and outlet temperatures of 150 and 75°C, respectively) and the resulting spray-dried powder was packaged in aluminium pouches and stored at 4°C until further use.

The LC of the product was found to be 16.7%.

Example 2: Preparation of rutin:K_2HPO_4 spray-dried powder

One litre of a 5% (w/v) aqueous solution of K_2HPO_4 was prepared and stirred for 30 min to fully dissolve the K_2HPO_4. Rutin (1.5 wt%) was added to the K_2HPO_4 solution and the mixture was heated to 75°C and stirred until the complete dissolution of rutin (about 20 min). The pH was recorded (about 8.0 to 8.5).

The temperature was decreased to about 60°C and the pH adjusted to 7.3 by the addition of HCl.

The warm solution was spray-dried (inlet and outlet temperatures of 150 and 75°C, respectively) and the resulting spray-dried powder was packaged in aluminium pouches and stored at 4°C until further use.

The LC of the product was found to be 23.1%.

Example 3: Preparation of further flavonoid: phosphate salt spray-dried powders

The processes set out in Examples 1 and 2 were used to produce a range of rutin-containing spray-dried powders of the invention as exemplified in Table 1 below. NaCas was replaced with alternative macromolecular compounds such as WPI, gelatine, pectin, soy lecithin and carrageenan. K_2HPO_4 was replaced with TPP in some instances. Analogous spray-dried powders were also made replacing rutin with other flavonoids including naringenin, hesperidin, curcumin, and catechin.

Table 1: **Examples of the spray-dried powders of rutin, manufactured using different combinations of phosphate salts and macromolecular compounds**

Flavonoid	Macromolecular compound	Phosphate salt
Rutin	NaCas	K_2HPO_4
Rutin	NaCas	TPP
Rutin	WPI	K_2HPO_4
Rutin	WPI	TPP
Rutin	SPI	K_2HPO_4
Rutin	SPI	TPP
Rutin	Gelatine	K_2HPO_4
Rutin	Gelatine	TPP
Rutin	Pectin	K_2HPO_4
Rutin	Pectin	TPP
Rutin	Soy lecithin	K_2HPO_4
Rutin	Soy lecithin	TPP
Rutin	Carrageenan	K_2HPO_4
Rutin	Carrageenan	TPP
Rutin	-	K_2HPO_4
Rutin	-	TPP
Rutin	-	K_2HPO_4 + TPP

NaCas: sodium caseinate, WPI: whey protein isolate, SPI: soy protein isolate, K2HPO4: potassium hydrogen phosphate, TPP: trisodium phosphate.

Example 4: Loading capacity (LC) determination

The flavonoid LCs of spray-dried powders made according to the process of the invention were calculated according to the method from Ahmad et al. (2016) using the following equation;

LC (%) = Total flavonoid / weight of spray-dried powder × 100

The "total flavonoid" is the amount of solid hydrophobic flavonoid added to the phosphate salt solution in step (a) of the process of the invention.

The spray-dried powders of the invention have LCs of about 5 to about 90%. The LC is generally higher where the flavonoid component has higher aqueous solubility.

For example, the LC values of the products of Examples 1 and 2 were 16.7 and 23.1, respectively. The LC in the case of the flavonoids with higher aqueous solubility was much higher. For instance, a catechin LC of 90% can be achieved in a formulation of catechin:K_2HPO_4 prepared by the process of the invention

Example 5: Dispersibility of the spray-dried powders of the invention

The spray-dried powders of each flavonoid were dispersed in phosphate buffer (pH 7.0) and left stirring at 2000 rpm for 120 min over which time the size properties (dispersibility) of the particles were studied. As suggested by Fang *et al*. (Fang, 2011), after the surface materials of particles are released in the aqueous medium, their particle size decreases. Such a decrease in size can be an indication of improved dispersibility. That means, measuring the size of the particles of a specific powder over a specific period of time (e.g., 120 minutes) in an aqueous medium, is an indication of the dispersion behaviour of that powder in food products with the same medium.

Thus, the change in the size of the particles during distribution in phosphate buffer (pH 7.0) and during agitation was used as an applicable technique to observe the dispersion behaviour of the spray-dried powder or raw flavonoid (control) over time, according to the method from Ji *et al*. (2016).

A Malvern Mastersizer 3000 (Malvern Instruments Ltd, Worcestershire, UK) equipped with a 4 mW He-Ne laser operating was used for spray-dried powders of the invention with a mean particle size above 600 nm. A Malvern Zetasizer Nano (Malvern Instruments Ltd, Worcestershire, UK) was used for powders with a mean particle size smaller than 600 nm, where the obscuration was so low (due to the high solubility of the particles) that they could not be measured in the Mastersizer.

About 30 mg of each powder was weighed (to achieve the ideal level of obscuration in the instrument), added to phosphate buffer (pH 7.0) in the dispersion unit. The wavelength of 632.8 nm was used to continuously measure the particle size properties at 2 min intervals. Size distributions, D 50 (µm) for each measurement were collected and analysed. The results are shown in **Figures 1-5**.

Example 6: Solubility of spray-dried powders of the invention

A known amount of each spray-dried powder to be tested was added to 10 mL of the aqueous medium used for the dispersibility experiment in Example 5 (phosphate buffer) and stirred for 24 h. The samples were then centrifuged (3000 x g, 20°C, 10 min) and the supernatant was collected and filtered (0.45 µm; Thermo Scientific, Waltham, MA, USA). The soluble flavonoid present in the supernatant was then extracted in ethanol and quantified using the high-pressure liquid chromatography (HPLC) method described below, following the method of Dammak *et al*. (Dammak, 2017).

The HPLC machine was equipped with UV/Visible and diodray detectors (Agilent Technologies, 1200 Series, Santa Clara, CA, USA). The column was a reverse-phase Prevail™ C18 with the dimensions of 4.6 cm × 150 mm, and 5 µm particle size (Grace Alltech, Columbia, MD, USA). The mobile phase consisted of acidic Milli-Q water (pH 3.50, 1% acetic acid, v/v) and methanol at the volume ratio of 50: 50 and a flow rate of 1 mL/min with the sample injection volume of 5 µL. Each flavonoid was detected at its specific wavelength when eluted at a specific retention time.

For the calibration of the HPLC column and quantification of flavonoid in the samples, standard solutions (0.01-1 mg/ml) of pure raw flavonoids (>97%) in the mobile phase were used and the standard curves were plotted accordingly. The chromatographic peaks of analytes were obtained by comparison of retention times with the standard and peak integration using the external standard method.

To release the total fraction of the remaining flavonoid, the supernatants were disrupted in heated ethanol (70°C) and filtered (0.45 µm; Thermo Scientific, Waltham, MA, USA) before injecting into the HPLC column.

The results are shown in **Figures 6-10**.

Example 7: *X-ray diffraction (XRD) of the spray-dried powders*

The XRD analysis was performed at 20.0°C on a Rigaku RAPID image-plate detector (Rigaku, The Woodlands, Texas, USA) set at 127.40 mm. Cu Kα radiation (λ = 1.540562 Å) generated by a Rigaku MicroMax007 Microfocus rotating anode generator (Rigaku, USA) and focused by an Osmic-Rigaku metal multi-layer optic device (Rigaku, USA), was

used. Spray-dried powders of the invention, as well as untreated flavonoids and control macromolecular compounds (as the references), were mounted in Hampton CryoLoops (Hampton Research, CA, USA) with a tiny amount of Fomblin oil. Data collection was under the control of RAPID II software (Version 2.4.2, Rigaku, USA), where the data were background-corrected and converted to a line profile with the 2DP programme (Version 1.0.3.4, Rigaku, USA), and compared using CrystalDiffract software (Version 6.5.5, CrystalMaker Software Ltd., Oxfordshire, UK). As sample sizes in the cryo-loops were variable, data were scaled to the same rise in the background caused by beam-stop shadow. All samples were analysed in the 2θ angle range of 5° to 100°. A narrow oscillation range of 5° was used in order to highlight the number of crystals in the X-ray beam.

The results for naringenin are shown in **Figure 11**. Spray-dried powders comprising other hydrophobic flavonoids (rutin, curcumin, catechin, and hesperidin) gave similar results.

Example 8: Morphology of the spray-dried powders of the invention using scanning electron microscopy (SEM)

An environmental scanning electron microscope (FEI Quanta 200, The Netherlands) was used to study the morphology of the spray-dried powders. Small amounts of the spray-dried flavonoids (as well as untreated flavonoids and control macromolecular compounds, as the references) were mounted onto aluminium stubs using double-sided tape (stuck to them). When the backing was peeled off, the sample was scooped onto the exposed tape and any excess sample was puffed off. Afterwards, the samples were sputter-coated with approximately 100 nm of gold (Baltec SCD 149 050 sputter coater), and then viewed under the microscope at an accelerating voltage of 20kV, using different magnifications.

The results are shown in **Figure 12**. Spray-dried powders comprising other hydrophobic flavonoids (rutin, curcumin, catechin, and hesperidin) gave similar results.

Example 9: Incorporation of spray-dried powders of the invention into food products

A rutin-containing spray-dried powder of the invention (FlavoPlus II) was prepared at the pilot plant scale, according to the following process:

1. Weigh 181.6 g of dipotassium phosphate and dissolve in 18.16 L of distilled water.
2. Add 273.4 g of food grade rutin slowly.

3. Increase the pH to 9.0 (using NaOH) and heat up the solution to 75℃ and stir until dissolved.
4. Add 545 g of sodium caseinate slowly and stir until dissolved.
5. Adjust the pH to 7.5 and leave stirring at 75℃.
6. Spray dry the above solution as it is hot (inlet and outlet temperature of 150 ℃ and 75℃, respectively).
7. Vacuum pack the dried powder.

Table 2: Specification of FlavoPlus II

Material	Percentage (wt%)	Quantity (g)	Standard Quality (g)
Rutin	1.5	286	273.4
K_2HPO_4	1	190	181.6
NaCas	3	570	545

The sensory attributes and consumers' behaviour towards the consumption of food products fortified with the spray-dried powder of the invention were assessed. The products tested were banana-flavoured milk and instant flat white coffee.

Products contained 250 mg or 500 mg of rutin per serve, added through FlavoPlus II ingredient. The serving size of banana-flavoured milk and instant flat white coffee was 250 and 200 mL, respectively. The concentration of FlavoPlus II in the banana milk was 3.66 g/L for the 250 mg rutin per serving of the product and 7.32 g/L for the 500 mg rutin per serving of the product. The concentration of FlavoPlus II in the instant flat white coffee was 4.56 g/L for the 250 mg rutin per serving of the product and 9.15 g/L for the 500 mg rutin per serving of the product.

To make the banana milk, the FlavoPlus II powder was stirred into the milk for 15 min. The mixture was then homogenised, pasteurised and bottled. To make the instant flat white coffee, the FlavoPlus II powder was blended with the dried coffee mixture (coffee, milk powder and sugar), which was added to hot water and stirred until dissolved.

The level of acceptability of banana-flavoured milk and instant flat white coffee was investigated using a 9-point hedonic scale, where 1 means 'dislike extremely' and 9 means 'like extremely'. Participants were also asked to identify differences between FlavoPlus II-fortified products and control products (without FlavoPlus II). The level of difference was recorded with a 7-point scale, where 1 means 'no difference' and 7 means 'very large difference'. Finally, participants were asked to choose which of the products

fortified with FlavoPlus II they would buy if that was available in the supermarket. The results are shown in **Figure 13**.

The products fortified with the spray-dried powders of the invention were relatively acceptable for the consumers, showing average liking scores between 6 and 7. Participants liked both products to a very similar level, meaning there was no particular preference for one of them (**Fig 13A**).

When participants were asked to identify differences between a control sample with no FlavoPlus II and two formulations tested (FP250mg and FP500mg), they found a 'light difference' in products fortified with the lowest content of FlavoPlus II and a 'moderate difference' in products with the highest content (**Fig 13B**). These differences were more pronounced in banana-flavoured milk than in coffee. These results suggest that the addition of the spray-dried powder of the invention to both products does not considerably affect the original sensory attributes of the food product.

The addition of the rutin-containing spray-dried powder of the invention increased the pH of the banana milk sample (6.60) to 6.90 and 7.0 when 250 and 500 mg/serve of powder were added, respectively. The higher the content in the spray-dried powder, the higher the pH. Nonetheless, during the storage, none of the samples showed significant changes in the pH over time. This is shown in **Figure 14**.

The apparent viscosity of the two beverages was calculated from rheological measurements, using a cone-plate geometry. The viscosity of control samples did not change as the storage time progressed. However, the addition of the spray-dried powder of the invention to milk caused a small increase in the viscosity regardless of the concentration used in the formulation. The results are shown in **Figure 15**. Changes in viscosity were not negatively perceived by the consumer, as observed in the results of the sensory test that show the fortified products were reasonably acceptable to the consumers.

Example 10: Stability of spray-dried powders of the invention in beverages

FlavoPlus II (as prepared in Example 9) was dissolved in water, alkaline water, milk, coconut water and vegetable juice (to deliver 500 mg rutin per serve) and the solubility and stability of the flavonoid component were assessed. Physical stability, pH, particle size, zeta potential and UV-visible spectra of the beverages were monitored and compared over 9 days storage at 4°C.

All beverages were found to be stable over that time period.

Example 11: Use of the spray-dried powder of the invention in transparent beverages

Three 50 ml vials of rutin in phosphate buffer (20.21 g dibasic sodium phosphate and 3.39 g monobasic sodium phosphate in 1 L MilliQ water) were prepared to comprise 100 mg of rutin per sample (2g/L). The vials are shown in **Figure 16**, with the pH of each vial recorded.

The vial on the left comprises 100 mg raw/untreated rutin dissolved in phosphate buffer. The vial on the right comprises 0.767g FlavoPlus II (as prepared in Example 9) corresponding to about 100 mg rutin.

The vial in the middle comprises FlavoPlus I, as described in WO2020/095238, at a concentration equivalent to 100 mg rutin. FlavoPlus I was prepared by the following process:

One litre of a 10% (w/v) aqueous solution of sodium caseinate (NaCas) was prepared and left to fully hydrate overnight. The solution was then brought to pH 11.0 using 4 M NaOH and left stirring (300 rpm) at room temperature for 30 min for the complete dissociation of NaCas. 100 g (10%, w/v) of food grade rutin was added to this solution and the pH was increased to 11.0 again, as rutin decreased the pH dramatically.

The mixture was stirred at room temperature until all of the added rutin was dissolved while the pH of the solution was constantly monitored and adjusted to 11.0, when required. From the time that all rutin was dissolved in the NaCas solution, the mixed solution was stirred for another 30 min while the pH was continually monitored.

The solution was acidified rapidly to pH 4.6 (the pI of casein) using 4M HCl, causing the rutin and NaCas to co-precipitate. The resulting mixture was centrifuged at 3000 g at room temperature for 10 min.

The co-precipitated product (10% dry wt/v) was then dispersed in a potassium phosphate solution and spray-dried under the following conditions: inlet temperature 180°C, outlet temperature 75°C, flow rate 20 mL/min.

As can be seen in **Figure 16**, FlavoPlus II dissolves in the phosphate buffer to provide a transparent solution whereas FlavoPlus I gives an opaque suspension. The former spray-dried powder is suitable for fortification of transparent and semi-transparent beverages, whereas the latter powder is not.

Example 12: Maximum solubility of FlavoPlus 2 in water

Rutin-containing spray-dried powders of the invention (FlavoPlus 2) prepared in accordance with Example 2 were dissolved in water at the concentrations shown in Table

2 by mixing for 30 min. The solutions were then stored at room temperature to check the solution stability (phase separation) as shown in Figure 16.

As can be seen in Table 2, 30 min was enough to dissolve up to 83.3 g of FlavoPlus 2 powder in 1 L of water. This is equal to 45 g of pure rutin, which is a dramatic increase in the solubility of this hydrophobic flavonoid compared to its untreated form. Figure 16 shows the appearance of the soluble FlavoPlus 2 powder.

Table 2: Solubility of FlavoPlus 2 in water

Sample	Rutin concentration (g/l)	Powder concentration (g/l)	Solubility	Physical stability
1	30	55.5	Dissolved	60
2	35	64.8	Dissolved	60
3	40	74	Dissolved	30
4	45	83.3	Dissolved	< 30
5	50	92.6	Not fully Dissolved	NA

Example 13: Antioxidant potential of FlavoPlus 2 (FP2)

The antioxidant potential of rutin-casein composite FlavoPlus 2 (FP2) was compared with rutin hydrate (RH) using an *in vitro* cell and digestion model. The experimental methodology was divided into 2 phases with each phase serving its categorical objectives. **Phase 1** demonstrates the potential antioxidant effect of the FP2 on human intestinal epithelium cells (Caco-2) through cell-based and biochemical assays.

In **phase 2**, the banana milk beverage fortified with FP2 (Example 9) and digested using an INFOGEST *in vitro* digestion model. Subsequently, its physiochemical characteristics were measured along with its intracellular antioxidant potential.

Phase 1: Cellular and biochemical estimation of antioxidant potential of FP2 (data collected and processed by Anubhavi Singh, Massey University, Palmerston North, New Zealand, and Dr Raise Ahmed, AgResearch Ltd., Palmerston North, New Zealand)

Human intestinal epithelium cell line, Caco-2 was used to test the antioxidant activity of FP2. Caco-2 cells were cultured in Minimum Essential Media (MEM) for 72 hours and always maintained at 37°C in 5% CO_2 humified incubator. Cells were treated with DMSO at doses varying from 0% to 5% prior to testing the cytotoxic effect of FP2 and RH.

Optimisation of DMSO concentration on Caco-2 cells was tested by investigating the cell viability through various experimental trials. Simultaneously, the cytotoxic effect of FP2 and RH on Caco-2 cells was determined by MTT assay, as demonstrated below.

Evaluation of *in vitro* antioxidant activity of FP2 in comparison to RH through a Caco-2 cellular model was determined by a fluorescent antioxidant assay. DCFH-DA, a fluorescent dye that helps in the detection of ROS in cells, was used as the probe for detecting intracellular activity. Cells were pre-treated with various concentrations of antioxidant medium containing FP2 and RH for 1 hour. DCFH-DA dye was added to these pre-treated cells and incubated for 1 hour, where it converts into an oxidisable form DCFH. To determine the effective intracellular antioxidant activity of FP2 and RH, a free-radical generator, called AAPH was added to these pre-treated cells and relative fluorescence over a time period of 1 hour was monitored to indicate any antioxidant activity within the cells.

Cell line, medium and reagents

Caco-2 cells (ATCC ®; American Tissue Culture Collection, # HTB-37) with passage number 19 were kindly provided by Dr Rachel Anderson (AgResearch Ltd., Palmerston North, New Zealand). Cells were maintained and cultured in complete minimum essential media (MEM) and seeded in 96 well plates for the experiment at 70% confluency.

Cell culture medium and reagents

Complete Minimum essential media

Complete MEM was prepared by supplementing Gibco™ MEM (L-Glutamine; catalogue number 11095080, ThermoFisher Scientific™, Waltham, MA, USA) with 10% Fetal Bovine Serum (FBS, Australian sourced, ɣ-radiated, catalogue number FBSF, Thermo Fisher Scientific, Waltham, MA, USA), 1% NEAA (MEM non-essential amino acids 100x solution; catalogue #11140-050) and 1% penicillin-streptomycin (Pen-Strep, 10,000units/mL penicillin G sodium salt and 10,000 µg/mL streptomycin sulfate in 0.85% saline) were procured from Gibco, Invitrogen, MA, USA. Cells were sub-cultured every third day using trypsin (TrypLETM, Gibco™ , Invitrogen Corporation, Carlsbad, CA, USA) and Gibco™ PBS, pH 7.4 (catalogue #10010023). The complete MEM was stored at 4°C.

3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide (MTT) stock solution

MTT stock solution was prepared by dissolving 5 mg of the dye (catalogue #M6494, Invitrogen by Thermo Fisher Scientific™, Waltham, MA, USA) in 1 ml of phenol-free Gibco™ MEM. The solution was syringe filtered (0.22 µm) and stored at -20^{0}C until further use. The working solution of MTT dye was prepared by diluting the stock solution

by 10-fold (1:10) in phenol-free MEM. The stock solution was covered in the foil due to MTT's light-sensitive nature.

Dimethyl sulfoxide (DMSO)

A stock solution of 0.3% DMSO (catalogue #D4540, Sigma-Aldrich®, St. Louis, MO, USA) was prepared which was further diluted in PBS, pH 7.4 (catalogue #10010023) and the solution was syringe filtered (0.22 µm) and stored at -20°C until used stored at -20°C.

DCFH-DA/Cellular antioxidant assay

All reagents and buffers for DCFH-DA assay were prepared according to Ma et al. (2018)

2',7'-Dichlorofluorescin diacetate (DCFH-DA) stock solution

To prepare a 12.5 mM stock solution, 12.182 mg of DCFH-DA (catalogue #4091-99-0, Sigma-Aldrich®, St. Louis, MO, USA) was dissolved in 2 ml of DMSO (catalogue #D4540, Sigma-Aldrich®, St. Louis, MO, USA) and stored in 100 µl single-use aliquots at -20°C to avoid repeated freeze-thaw cycles.

2,2'-Azobis(2-methylpropionamidine) dihydrochloride (AAPH) stock solution

To prepare a 60 mM stock solution, 32.542 mg of AAPH (catalogue #2997-92-4, Sigma-Aldrich®, St. Louis, MO, USA) was dissolved in 2 ml of Gibco™ HBSS (catalogue #14175095, Thermo Fisher Scientific™, Waltham, MA, USA) and stored in 100 µl single-use aliquots at -20°C to avoid repeated freeze-thaw cycles.

Preparation and resuspension of bioactive compounds

Rutin hydrate stock solution

A stock solution of rutin hydrate (≥94%; catalogue #207671-50-9; (Thermo Fisher Scientific™, Waltham, MA, USA) was prepared by dissolving 1 mg powder in 0.3% DMSO solution before further dilution in treatment medium. The solution was syringe-filtered (0.22 µm) and stored at -20°C until use.

FlavoPlus 2 (FP2) stock solution

FP2 powder tested in this example was composed of sodium caseinate (54.5%), rutin (27.3%) and dipotassium phosphate (18.2%), prepared following the method described in Example 1.

A stock solution was prepared by dissolving 2 mg powder in 1 ml Milli-Q (Millipore Corp., SAS – 67120, Bedford, MA, USA) water before further dilution in the treatment medium. The solution was syringe-filtered (0.22 µm) and stored at -20°C until use.

DPPH stock solution

The DPPH stock solution was prepared by dissolving 0.025 g DPPH reagent (procured from Sigma-Aldrich Co., Inc. Darmstadt, Germany) in 100 ml methanol (Analytical grade, catalogue #67561, Sigma-Aldrich®, St. Louis, MO, USA). The stock solution was covered by foil due to its light sensitivity and stored in a dark and cool place until used.

Total phenolic content (TPC) stock solution

Folin-Ciocalteu's reagent (procured from Merck Co., Inc. New Jersey, USA) was mixed with milli-Q water in a ratio of 1:1 to generate a stock solution. The stock solution was covered by foil due to its light sensitivity and stored in a dark and cool place until used.

Methods

Maintenance and culture of Caco-2 cells

The Caco-2 cells were grown and maintained in complete MEM at 37°C, 5% CO_2 in a humified incubator (Heracell™ VIOS 160i CO_2 Incubator, Thermo Fisher Scientific™, Waltham, MA, USA). The growth of cells was monitored daily under an inverted microscope (Nikon eclipse TS100, Japan) until it reached 70% confluency. Cells were sub-cultured every third day by washing with PBS, pH 7.4 (catalogue #10010023) and incubating with trypsin for 4 minutes to remove adherent cells from the surface of the flasks (TrypLETM, Gibco™, Invitrogen Corporation, Carlsbad, CA, USA). The cells were then centrifuged at 110 RCF for 4 minutes (MegaFuge™ 8 centrifuge, Thermo Fisher Scientific™, Waltham, MA, USA) and resuspended in MEM medium. Subsequently, they were seeded into 96 well plates for the corresponding assays (Natoli et al., 2012).

Differentiation of Caco-2 cells

Differentiation of Caco-2 cells was done to perform absorption experiments and barrier integrity assessment of the cell monolayer. The cells were tested for viability using trypan blue solution (0.4%, catalogue #T10282, Invitrogen™ Thermo Fisher Scientific™, Waltham, MA, USA) where the cell suspension was mixed with trypan blue dye in a 1:1 ratio and mixed in an Eppendorf tube. About 12 µl of the sample was loaded onto a disposable Countess™ chamber slide (ThermoFisher Scientific™, Waltham, MA, USA) and observed under a Countess™ automated cell counter (ThermoFisher Scientific™, Waltham, MA, USA). Live and viable cells were transparent and clear while dead cells were stained blue with trypan blue.

Caco-2 cells were seeded at a density of 8×10^4 cells/ Transwell (6.5 mm, polyester, 0.33 µm pore size; Corning® Inc., catalogue #CLS3470, ThermoFisher Scientific™, Waltham, MA, USA). The volume of fresh MEM medium in the apical compartment was 200 µl and the basolateral compartment was 810 µl. The plates were incubated in humified incubators at 37°C at 5% CO_2. The medium in the transwell was changed after

every second day. Differentiation of Caco-2 cells occurs after 15-17 days of incubation. The background transepithelial electrical resistance (TEER) across Caco-2 cell monolayer was measured after cells reached confluency using EndOhm TEER cup (World Precision Instruments, Sarasota, FL, USA) connected to the EVOM^2 Epithelial Voltohmmeter (World Precision Instruments, Sarasota, FL, USA), to determine the barrier integrity of the cells before absorption experiments.

Treatment of Caco-2 cells with FlavoPlus 2TM (FP2)

Caco-2 cells were treated with FP2 and RH at various concentrations ranging from 0.1 µg/ml to 50 µg/ml. These concentrations were achieved after dilutions from the stock solutions in a fresh MEM medium. In order to understand the effect of the samples, control vehicles such as DMSO were optimised at varying concentrations from 0.01% to 5%. The concentration of DMSO used in diluting RH corresponds to the concentration range of DMSO used for its optimisation. After seeding cells at a density of 4 $\times 10^5$ cells/ml in a 96-well plate (Corning® Co-star 3596 Inc., catalogue # 07-200-90, ThermoFisher Scientific™, Waltham, MA, USA), the incubation of 24 hours was needed for the cells to adhere to the surface of the well. After incubation, cells were treated with DMSO (0.01% to 5%). These cells were quickly assessed via MTT Assay to optimise the cytotoxic concentrations of DMSO.

MTT Assay

After the treatment with DMSO, FP2 and RH, the cells were washed with phenol-red free MEM medium. The MTT dye working solution (0.5mg/ml) was added to each well and incubated for 3-4 hours. The negative control was DMSO + cells, positive control was without sample, and blank wells were regarded as wells without cells. After incubation, the wells had a dark blue/purple formazan crystal precipitation. After carefully aspirating the MEM medium from the wells, 100 µl/well DMSO was added. Further incubation of 10 minutes with occasional shaking at 37°C was needed to dissolve the formazan crystal formation. Absorbance was measured at 570 nm using a micro-plate reader (FlexStation 3™ Multi-Mode Microplate Reader, Bio-Strategy, NZ). The cell viability was calculated using the following equation (Eq. 1). This method was adapted from Kuntz et al. (1999) with some modifications.

$$\%\text{Cell viability} = \frac{Absorbance\ of\ sample - Absorbance\ of\ blank}{Absorbance\ of\ control - Absorbacnce\ of\ blank} \times 100 \ldots\ldots\ldots \text{(Eq. 1)}$$

Intracellular antioxidant activity

Intracellular antioxidant activity was measured by using 2′,7′-Dichlorofluorescin diacetate DCFH-DA assay as mentioned by Wan et al. (2015) with slight modifications. The cells were seeded at a density of 6 $\times 10^5$ cells/mL in a 96-well plate (Corning® Co-

star 3596 Inc., catalogue #07-200-90, ThermoFisher Scientific™, Waltham, MA, USA), where the incubation of 24 hours was needed for the cells to adhere to the surface of the well. After the incubation, MEM medium was discarded and cells were washed with Gibco™ PBS, pH 7.4 (catalogue #10010023, ThermoFisher Scientific™, Waltham, MA, USA). The cells were treated with samples at a concentration and their response to FP2 and RH (i.e., cell viability) was quantified using Eq. 1. All the reagents were stored at 4°C and were equilibrated to room temperature before use. The assay was performed by applying the method developed by Ma et al. (2018) with some modifications. The cells were treated with 100 µl of samples in conjunction with 25 µM DCFH-DA (Catalogue #4091-99-0, Sigma-Aldrich®, St. Louis, MO, USA) dye solution, which was incubated at 37°C for 1 hour. After an incubation of 1 hour, the cells were washed with PBS 3 times. After washing the cells, 100 µl of (600 µM) AAPH (Catalogue #2997-92-4, Sigma-Aldrich®, St. Louis, MO, USA) was added into a 96 well-plate. The fluorescence intensity was immediately measured at 37°C with an excitation of 480 nm and emission wavelength of 530 nm by a fluorescence micro-plate reader (FlexStation 3™ Multi-Mode Microplate Reader, Bio-Strategy, NZ) for every 5 minutes for 1 hour. The control group was without any samples and blank group was without AAPH and samples, only PBS treatment was regarded as blank. The fluorescence intensity of the samples was calculated using Equation 2.

$$\text{Fluorescence intensity \%} = \frac{F_{sample}}{F_{control}} \times 100 \quad \text{(Eq. 2)}$$

where, F_{sample} is the fluorescence of sample and $F_{control}$ is the fluorescence of control.

UV-Spectrophotometric DPPH Assay

DPPH (2,2-diphenyl-1-picryl-hydrazyl) free radical scavenging method (Vogrincic et al., 2010) was used to test the antioxidant scavenging capacity of FP2 and RH via UV-spectrophotometric chemical-based assay.100 µl of the samples was mixed with 1:10 dilution of the DPPH stock solution in a cuvette and was then incubated in the dark for 30 minutes at room temperature (25°C) until recording the absorbance. For the control sample, 3.9 mL of the DPPH solution was added to the cuvette and its absorbance was recorded immediately at 517 nm using a spectrophotometer (Multiskan™ GO Microplate spectrophotometer, ThermoFisher Scientific™, Waltham, MA, USA). The blank sample consisted of water with a DPPH solution. With the increase in the concentration of the bioactive compound (i.e., rutin), stable DPPH radical was reduced to its non-radical form changing the colour of the solution from purple to yellow. The scavenging activity or inhibition % was calculated against control using the following equation (Eq. 3).

$$\text{Scavenging activity (\%)} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100 \quad \text{(Eq. 3)}$$

where the absorbance of the control is at t= 0 mins and the absorbance of the sample is at t= 30 mins. All measurements were carried out at room temperature.

Total phenolic content (TPC) estimation

To evaluate the TPC values in FP2 and RH, the method adapted from Gangwar et al. (2014) was applied using Folin-Ciocalteu's (FC) reagent (procured from Merck Co., Inc. New Jersey, USA). An aliquot of 0.1 ml was taken from sample stock and 2.5 ml of FC reagent was added and incubated for 10 minutes. After the incubation, 2 mL of sodium carbonate (75 g/L) was added, and samples were vortexed and incubated in dark at room temperature for 2 hours. The absorbance was measured by spectrophotometer at 765 nm (Multiskan™ GO Microplate spectrophotometer, ThermoFisher Scientific™, Waltham, MA, USA). The absorbance values were compared with the gallic acid standard curve to express the results as µg of the gallic acid equivalent of the sample.

Results of Phase 1 **(data collected and processed by Anubhavi Singh, Palmerston North, New Zealand, and Dr Raise Ahmed, AgResearch Ltd., Palmerston North, New Zealand)**

As shown in Figure 17, the antioxidant activity (expressed in form scavenging activity) of FP2 significantly exceeds that of RH, demonstrating that the rutin in the FP2 product is a much more effective antioxidant than that in RH. Samples that do not share the same letters are significantly different ($p \leq 0.05$). It must be noted that the concentration of rutin in the FP2 powder tested in this example is much lower (around three times lower) than that of pure rutin in RH powder; nonetheless, the antioxidant activity of this powder is much higher than pure rutin (i.e., RH). For clarity, we have also provided the data in the form of rutin equivalent for both samples (lines in Figure 17), where the differences are seen much clearer.

Figure 18 presents the results for the total phenolic content of FP2 powder vs untreated form of rutin (RH). Samples that do not share the same letters are significantly different ($p \leq 0.05$). We tested the same amount of both powders for this example, but the concentration of rutin in the FP2 powder is around three times that of RH powder. This has resulted in some variation in the results especially in the case of low concentration, but overall, even at these unequal concentrations, FP2 showed a stronger phenolic content. When the data was normalised for the rutin equivalent in this powder (lines in Figure 18), FP2 was dramatically stronger in terms of phenolic properties. This confirms the results shown in Figure 17 as generally speaking, a positive and strong correlation between total phenolic content and antioxidant activity in the case of such samples is expected.

The effect of FP2 vs untreated rutin (RH) on the intracellular antioxidant activity over 60 minutes is shown in Figure 18. Intracellular antioxidant activity was quantified with DCFH-DA (2′,7′-Dichlorofluorescin diacetate) assay using relative fluorescence values of the samples, and the data represents the mean of three biological replicates with three replicates in each assay. Samples that do not share the same letters are significantly different ($p \leq 0.05$). As can be seen in this figure (Figure 18), there is a significant difference ($p \leq 0.05$) between the antioxidant activity exhibited by FP2 and that of RH at almost every time point. These differences confirm that the treatment of RH using the process invented here can result in higher antioxidant activity, which is in line with the results obtained from solubility and crystallinity tests. It is notable that the concentration of rutin in FP2 is much lower than that of pure RH, yet the same concentrations of the powder (not rutin) are compared.

Figure 20 shows the effect of digested banana milk fortified with FP2 vs RH on intracellular antioxidant activity over 60 minutes. Intracellular antioxidant activity was quantified with DCFH-DA assay using relative fluorescence values of the samples, with data representing the mean of three biological replicates with three replicates in each assay. Samples that do not share the same letters are significantly different ($p \leq 0.05$). Based on these results (Figure 20), at every time point (A-D), the banana milk containing FP2 showed a much stronger intracellular antioxidant activity when compared to the same milk containing RH. This is in line with the results presented in Figure 19 (the powders alone and not in food). Notably, the concentration of rutin in FP2 is much lower than that of pure RH, yet the same concentrations of the powder (not rutin) are compared.

Phase 2: Preparation of banana milk beverage fortified with FP2 and characterisation of its physicochemical and antioxidant properties

This part of the study aimed at preparing a banana-flavoured milk beverage fortified with FP2 or RH. The milk beverages were developed with a concentration of 7.32 g/L powder for 500 mg rutin per serve of 250 mL of banana flavoured milk.

This banana-flavoured milk was digested through an *in vitro* static digestion model. *In vitro* digestion was performed to understand the changes in the structural and functional properties of bioaccessible rutin in banana flavoured milk beverage. High-performance liquid chromatography (HPLC) was used for the quantification of rutin in the samples at 0, 30, 60 and 120 mins after digestion; i.e., to study the release of rutin, which determines the bioaccessible amount of rutin available after digestion. Subsequently, a bioaccessible amount of the samples were added to a differentiated Caco-2 cell

monolayer to assess the impact on cytotoxicity, barrier integrity and intracellular antioxidant activity.

Materials, chemicals and reagents

Commercial banana milk used in the experimental trials was purchased from a local supermarket (Pak n Save, Palmerston North, New Zealand), the composition and nutritional information of which is presented in Table 3.

Table 3: Composition/nutritional information of the banana-flavoured milk

Composition	Average quantity per 250ml serving	Average value per 100 ml	% Daily intake per serving
Energy	650kJ	260kJ	7%
Protein	11g	3.3g	4.4%
Fat, total	3.8g	1.5g	5%
- saturated	2.3g	0.9g	10%
Carbohydrate	18.8g	7.5g	6%
- sugars	18.8g	7.5g	21%
Sodium	90mg	35mg	4%
Calcium	365mg	145mg	46% RDI
Vitamin D	1.3mg	0.5mg	13% RDI

The enzymes used in the *in vitro* digestion were of porcine origin; pepsin (Catalogue #P7012-5G, Sigma-Aldrich®, St. Louis, MO, USA) was stored at 0°C, pancreatin (Catalogue #P7545-100G, Sigma-Aldrich®, St. Louis, MO, USA) was stored at 0°C, and bile salts (Catalogue #B8631-100G) was stored at room temperature. Milli-Q water (Millipore Corp., SAS – 67120, Bedford, MA, USA) was used for the preparations of all the solutions. High purity (HPLC grade) rutin and quercetin standards were purchased from Sigma-Aldrich®, St. Louis, MO, USA. All chemicals and reagents used for this study were of analytical grade.

Methods

Preparation of banana flavoured milk fortified with FP2 and RH

A serving size of 500 mg rutin per serve of 250 mL of milk was chosen, and 400 ml of fortified banana milk was prepared. The concentration of the FP2 and RH powders in the banana milk product was 7.32 g/L and 2 g/L, respectively. The powders were stirred into

the milk for 15 mins and the mixture was heated at 70°C for 30 mins and cooled before storage. Fortified milk was bottled and stored at 4°C until further use.

In vitro digestion

In vitro digestion of the banana-flavoured milks fortified with FP2 and RH was performed according to Minekus et al. (2014) with some modifications. Freshly made fortified banana milk beverage was used for the *in vitro* digestion studies (in triplicates). The banana milk samples fortified with FP2 were compared to banana milk fortified with RH. Banana milk without fortification was used as the control along with Milli-Q water regarded as the blank. Three aliquots were taken for each time point to generate enough samples for further experiments, while two tubes were used for performing *in vitro* digestion for each replicate. A shaking water bath was maintained at 37 ± 1°C to maintain the human body temperature.

The 10 mL samples were mixed with simulated gastric fluid (SGF) to a final ratio of 1:1 containing 5 µl $CaCl_2$, 8 mL SGF and 0.5 mL pepsin (at a final pepsin activity of 2000 U/ml), and the pH of the tubes was adjusted to 3 ± 0.1. The first time point (at 0 mins) marked the initiation of the gastric phase in this experiment. During the gastric phase, samples were collected at 0, 30, 60 and 120 minutes. In the case of the intestinal phase, samples were obtained from the remaining tubes in the water bath, which had a final volume of 20 mL from the gastric phase. The digesta was mixed with simulated intestinal fluid (SIF) to a final ratio of 1:1 containing 40 µl $CaCl_2$, 2.5 mL bile salts (at 10 mM) and 5 ml pancreatin (at a final 100 U/ml trypsin activity), and the pH of the tubes was adjusted to 7 ± 0.1 with regular stirring. During the intestinal phase, samples were collected at 0, 30, 60 and 120 minutes.

To stop the enzymatic reactions, enzyme inhibitors were immediately added to the samples. 10 µl of Pepstatin A (catalogue #ab141416, Abcam, UK) in methanol (0.5 mg/ml) was added to every 1 mL of gastric digesta sample taken and 0.45 mL of the protease inhibitor cocktail (1 tablet in 50 ml Milli-Q water; Sigmafast®, Catalogue #S8820, Sigma-Aldrich®, St. Louis, MO, USA) solution was added to every 1 mL of the intestinal digesta sample. All digesta samples were stored at -20°C for further experimental analysis.

High performance liquid chromatography (HPLC)

Rutin standards were analysed based on the method reported by Naveen et al. (2017) and Acevedo-Fani et al. (2021) with slight modifications. An Agilent 1200 series, HPLC machine equipped with UV/visible diode array detector (Agilent technologies, 1200 Series, Santa Clara, CA, USA) and Kinetex XB-C18 column (100 A°, 100 mm × 4.6 mm, 2.6 µm pore size) was used to measure the amount of rutin bioaccessible in the banana

fortified milk product after extraction. The mobile phases were composed of two different solutions; acetic acid 0.5% acting as A and acetonitrile acting as B. The flow rate was 1 mL/min with a sample injection volume of 5 µl. The column temperature was maintained at 26°C and the UV detector was set at 356 nm. Rutin was detected at 356 nm and peak height and peak area were obtained by integration using EZ Chrome software (Agilent OpenLab Technologies, USA). The identification of rutin was dependent on the retention time of the peaks and their spectra compared with the calibration curve made from standards (105-421 ppm).

Particle size

The particle size of the sample was obtained after *in vitro* digestion using a Mastersizer (Malvern MasterSizer Hydro 2000MU, Malvern Instruments Ltd., Malvern, UK) with two laser sources. The refractive index used was 1.460 for milk and 1.33 for water at an obscuration level maintained at 9.5%. A small amount (2-3 mL) of digested milk sample was added to the measurement cell containing 800 mL of water to reach the obscuration level.

Zeta potential

The zeta potential of the samples was determined by Zetasizer Nano ZS (Malvern Panalytical Ltd, UK) at 25°C. The milk samples were diluted by deionised water before analysis to prevent multiple scattering events during the experiment. The samples were equilibrated for 2 mins before measurement.

MTT assay

The procedure of the MTT assay is as described above. The cells were treated with a bioaccessible amount of FP2 and RH after *in vitro* digestion and their cell viability was calculated by measuring absorbance at 570 nm using a microplate reader (FlexStation 3™ Multi-Mode Microplate Reader, Bio-Strategy, NZ). The cell viability was calculated using Equation 1.

DCFHDA assay

After quantification of cell viability, the cells were seeded on Transwell inserts (6.5 mm, polyester, 0.33 µm pore size; Corning® Inc., Catalogue #CLS3470, ThermoFisher Scientific™, Waltham, MA, USA) to become a differentiated cell monolayer after 15-17 days. The procedure of the DCFH-DA assay is described above. The DCFH-DA assay quantifies the intracellular antioxidant capacity of the bioaccessible samples after 24 hours of absorption on the Caco-2 monolayer with a change in its fluorescence values.

Transepithelial electrical resistance (TEER)

TEER measures the electrical resistance across a cellular monolayer. This is a reliable and sensitive method that confirms the integrity and permeability of the monolayer. In this example, Caco-2 cells were seeded at a density of 8 × 10^4 cells/Transwell (6.5 mm, polyester, 0.33 µm pore size; Corning® Inc., catalogue #CLS3470, ThermoFisher Scientific™, Waltham, MA, USA) in Gibco™ MEM medium in an apical compartment with 200 µl and a basolateral compartment with 810 µl. The plates were incubated in humified incubators at 37°C at 5% CO_2 (Heracell™ VIOS 160i CO_2 Incubator, ThermoFisher Scientific™, Waltham, MA, USA) and the medium in the transwell was changed after every second day. Differentiation of Caco-2 cells occurred after 15-17 days of incubation. After reaching confluency, the transepithelial electrical resistance across the Caco-2 cell monolayer was measured using an EndOhm TEER cup (World Precision Instruments, Sarasota, FL, USA) connected to the EVOM^2 Epithelial Voltohmmeter (World Precision Instruments, Sarasota, FL, USA) to determine the barrier integrity of the cells. With preliminary tests, the cells were considered confluent when TEER was higher than 500 Ω/cm^2. The TEER values were regularly noted for every 2 hours for the next 12 hours, and the final reading was taken at 24 hours. Resistance values are calculated using Equation 4.

$$\text{TEER } (\Omega/cm^2) = Raw\ TEER\ value\ (\Omega) \times surface\ area\ of\ insert\ (cm^2) \quad \text{(Eq. 4)}$$

Statistical analysis

All the experiments were replicated at least three times. The results were expressed as mean ± standard error mean. One-way ANOVA (Analysis of variance) was used to determine the effect of FP2 and RH on the various biochemical and physicochemical properties at α = 0.05. For multiple comparisons, Tukey's post hoc test was employed to determine significant differences between means of these three sample groups (i.e., FP2 and RH) ($P<0.05$) using the IBM SPSS statistical software (Version 26). Graphs were analysed and created using ORIGIN software (OriginPro, 64-bit, 2020) and Microsoft Excel (Version 2111, 64-bit, 2021).

Results of Phase 2 (data collected and processed by Anubhavi Singh, Massey University, Palmerston North, New Zealand*, and Dr Raise Ahmed, AgResearch Ltd., Palmerston North, New Zealand*)

Figure 21 shows the bioaccessible rutin available in FP2 (added to banana milk) obtained during *in vitro* intestinal digestion phase. As can be seen in this figure (Figure 21), no significant differences ($p>0.05$) were seen for the bioaccessibility of rutin in the FP2 powder incorporated in banana milk at different time points of the simulated intestinal digestion. This indicates that almost half of the rutin from this product can be

bioaccessible after the gastric phase. This is a substantial improvement in the bioaccessibility of rutin as this hydrophobic flavonoid has a very poor bioaccessibility (and bioavailability) in its untreated (raw/commercial) form. We also tested the *in vitro* of rutin in control (i.e., RH) using the same method, and found that no rutin from RH was bioaccessible at this time. This was not surprising as due to insolubility and sedimentation (being highly crystalline), all of RH was withdrawn during sampling at the early stages of digestion. Therefore, this invention has solved the bioaccessibility challenge of RH, because of the substantial improvement in its dispersibility and solubility, as a result of the dramatic decrease in its crystallinity.

The results of the particle size analysis and zeta potential (surface charge) of digested fortified banana milk containing FP2, digested fortified banana milk containing RH, and unfortified banana milk are shown below in Tables 4, 5 and 6, respectively. We monitored the changes in these parameters over 240 minutes under simulated gastric and intestinal conditions (120 minutes each). First of all, there were significant differences among the particle size of different time points for each milk during both stages of the simulated digestion, which was expected. Interestingly, the size of the particles in the sample containing FP2 at any time point was larger than the sample containing no rutin (Table 6) or the sample containing RH. This can be beneficial for rutin delivery as it may result in the delayed release of rutin (protection during gastric digestion). In the case of the banana milk containing RH, the results may not be reliable (and comparable) enough as the added rutin sedimented at the bottom of the digestion unit due to insolubility of RH in an aqueous medium.

There were also significant differences among the zeta potential values of different time points for each milk during both stages of the simulated digestion. This was expected because, as digestion progresses, the surface of the particles is disrupted. Additionally, there were significant differences in the zeta potential values of different samples for the same specific time points, further indicating changes in the properties of the particles after the application of the process of the current invention. It appeared that the addition of FP2 to banana milk could have a slight effect on its zeta potential, especially at the beginning of the digestion, which could be related to the change in the surface charge of the particles. Nevertheless, what matters in the case of this experiment is whether FP2 could protect rutin in the gastric phase and deliver it to the intestinal phase. This is confirmed by the results presented in Figure 21, where almost half of the rutin in the FP2 powder was bioaccessible at the end of simulated intestinal digestion. In contrast, no rutin from RH was bioaccessible at this time, as due to insolubility and sedimentation all of it was withdrawn during sampling at the early stages of digestion. Therefore, it can confidently be stated that the process of the invention results in an improved product

with respect to the delivery of rutin and other hydrophobic flavonoids, including behaviour under simulated digestion system, and consequently, final *in vitro* bioaccessibility.

Table 4: Particle size (D [4,3]-volume weighted mean (µm)), specific surface area (m^2/gm) and zeta potential (mV) of digested fortified banana milk containing FP2.

Sample - FP2	D [4,3] (µm)	Zeta potential (mV)	Specific surface area (m^2/gm)
Gastric 0 mins	369.36 ± 19.12^{a}	4.30 ± 0.20^{a}	0.19 ± 0.01^{e}
Gastric 30 mins	181.39 ± 3.97^{b}	3.35 ± 0.13^{a}	0.28 ± 0.01^{a}
Gastric 60 mins	186.11 ± 9.93^{b}	2.66 ± 0.16^{a}	0.23 ± 0.01^{c,d}
Gastric 120 mins	169.81 ±2.74^{b}	2.39 ± 0.19^{a}	0.26 ± 0^{a,b}
Intestinal 0 mins	371.68 ± 16.16^{a}	-13.55 ± 0.47^{b,c}	0.21 ± 0.01^{d,e}
Intestinal 30 mins	345.88 ± 32.11^{a}	-11.5 ± 0.29^{b}	0.24 ± 0.25^{b,c}
Intestinal 60 mins	353.15 ± 10.48^{a}	-17.33 ± 2.14^{c}	0.27 ±0.01^{a}
Intestinal 120 mins	369.59 ± 10.70^{a}	-11.32 ± 1.53^{b}	0.27 ± 0.02^{a}

Data represents the mean of three replicates with error bars corresponding to the standard error of the mean. Values in the three columns that do not share the same letters are significantly different ($p \leq 0.05$).

Table 5: Particle size (D [4,3]-volume weighted mean (µm)), specific surface area (m^2/gm) and zeta potential (mV) of digested fortified banana milk containing RH.

Sample - RH	D [4,3] (µm)	Zeta potential (mV)	Specific surface area (m^2/gm)
Gastric 0 mins	274.61 ± 8.74^{a}	-1.64 ± 0.15^{a}	0.13 ± 0^{d}
Gastric 30 mins	279.72 ± 8.322^{a}	-1.52 ± 0.12^{a}	0.16 ± 0^{d}
Gastric 60 mins	138.62 ± 32.05^{b}	-1.37 ± 0.14^{a}	0.22 ± 0.02^{c}
Gastric 120 mins	95.09 ± 18.14^{c,d}	-1.88 ± 0.15^{a}	0.21 ± 0.01^{c}
Intestinal 0 mins	73.04 ± 10.59^{d,e}	-15.88 ± 0.48^{b}	0.41 ± 0.02^{b}
Intestinal 30 mins	60.06 ± 2.68^{e}	-15.8 ± 0.61^{b}	0.43 ± 0.01^{b}
Intestinal 60 mins	104.22 ± 23.28^{c}	-15.2 ± 0.77^{b}	0.44 ± 0.03^{a,b}
Intestinal 120 mins	78.25 ± 15.33^{c,d,e}	-16.1 ± 0.41^{b}	0.48 ± 0.05^{a}

Data represents the mean of three replicates with error bars corresponding to the standard error of the mean. Values in the three columns that do not share the same letters are significantly different ($p \leq 0.05$).

Table 6: The particle size (D [4,3]-volume weighted mean (µm)), specific surface area (m^2/gm) and zeta potential (mV) of digested unfortified banana milk.

Sample- Milk	D [4,3] (µm)	Zeta potential (mV)	Specific surface area (m^2/gm)
Gastric 0 mins	96.97 ± 10.04^{a}	-1.09 ± 0.05^{a}	0.19 ± 0^{b}
Gastric 30 mins	80.20 ± 9.45^{a,b}	-1.06 ± 0.04^{a}	0.21 ± 0.02^{b}
Gastric 60 mins	60.67 ± 8.41^{b,c}	-1.13 ± 0.05^{a}	0.26 ± 0.01^{b}
Gastric 120 mins	55.66 ± 5.51^{c,d}	-1.15 ± 0.06^{a}	0.27 ± 0.01^{b}
Intestinal 0 mins	64.23 ± 17.13^{b,c}	-15.85 ± 0.49^{b}	0.66 ± 0.06^{b}
Intestinal 30 mins	54.17 ± 16.73^{c,d}	-15.48 ± 0.42^{b}	6.47 ± 4.23^{a}
Intestinal 60 mins	74.6 ± 12.33^{b,c}	-15.46 ± 0.30^{b}	7.96 ± 0.62^{a}
Intestinal 120 mins	39.35 ± 2.75^{d}	-14.8 ± 0.40^{b}	0.44 ± 0^{b}

Data represents the mean of three replicates with error bars corresponding to the standard error of the mean. Values in the three columns that do not share the same letters are significantly different ($p \leq 0.05$).

7. Acknowledgments

The New Zealand High-Value Nutrition National Science Challenge (New Zealand) supported this work. The authors would like to acknowledge the help and support received from the following people for the collection and process of some of the data in this specification: Dr Hamid Gharanjig, Anubhavi Singh, and Matthijs Nieuwkoop (Riddet Institute, Massey University, Palmerston North, New Zealand), and Dr Raise Ahmed (AgResearch Ltd., Palmerston North, New Zealand). We thank Professor Geoffrey B. Jameson (Massey University, Palmerston North, New Zealalnd) for his scientific advice and support during XRD analysis. We acknowledge the scientific and technical support from the staff at the Manawatu Microscopy and Imaging Centre (MMIC) and the MacDiarmid Institute for Advanced Materials and Nanotechnology, Massey University, Palmerston North, New Zealand.

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What we claim is:

1. A spray-dried powder comprising a hydrophobic flavonoid and a soluble, edible phosphate salt.

2. A spray-dried powder of claim 1 wherein the hydrophobic flavonoid has a hydrophobicity of about 2 to about 4 and/or is soluble in aqueous solution at high pH, preferably above 7.

3. A spray-dried powder of claim 1 wherein the hydrophobic flavonoid is selected from the group consisting of rutin, naringenin, quercetin, curcumin, hesperidin, alpha-naphthoflavone (ANF), beta-naphthoflavone (BNF), catechin and catechin derivatives, chrysin, luteolin, myricetin, and anthocyanins.

4. A spray-dried powder of claim 3 wherein the hydrophobic flavonoid is selected from the group consisting of rutin, naringenin, curcumin, hesperidin and catechin.

5. A spray-dried powder of any one of claims 1-4 wherein the soluble, edible phosphate salt is a sodium, potassium or ammonium phosphate salt.

6. A spray-dried powder of claim 5 wherein the soluble, edible phosphate salt is selected from the group consisting of K_2HPO_4, tetra potassium diphosphate, and sodium triphosphate.

7. A spray-dried powder of any one of claims 1-6 which comprises about 1 to about 70 wt% edible phosphate salt, preferably about 3 to 50 wt%, more preferably about 5 wt% phosphate salt.

8. A spray-dried powder of any one of claims 1-7 which has a flavonoid concentration of about 2 to about 70 wt%, preferably about 20 to about 50 wt%, more preferably about 33 wt%.

9. A spray-dried powder of any one of claims 1-8 which has a flavonoid loading capacity (LC) of about 5 to about 90%, preferably about 10 to about 70%, more preferably about 25 to about 35%, and most preferably, about 30 to about 35%.

10. A spray-dried powder of any one of claims 1-9 which has a mass ratio of phosphate salt:flavonoid of about 20:1 to about 1:10, preferably about 15:1 to about 1:7 and more preferably about 10:1 to about 1:6.

11. A spray-dried powder of any one of claims 1-10 further comprising a macromolecular compound selected from a protein, a polysaccharide, a lipid and a non-ionic surfactant, preferably a protein, more preferably NaCas.

12. A spray-dried powder of any one of claims 1-11 in which the hydrophobic flavonoid is about 10x, 20x, 30x, 40x, 50x, 100x, 200x, 300x or 400x more dispersible in aqueous solution than the same raw/untreated, solid flavonoid.

13. A spray-dried powder of any one of claims 1-12 which is completely dispersed in aqueous solution when present at a concentration of at least about 8 wt%, preferably at about 12 wt%.

14. A spray-dried powder of any one of claims 1-13 in which the hydrophobic flavonoid is about 10x, 15x, 20x, 30x, 40x, 50x, 60x, 70x, 80s, 90x, or 100x more soluble in aqueous solution than the same raw (untreated) flavonoid.

15. A process for producing a spray-dried powder comprising a hydrophobic flavonoid and a soluble, edible phosphate salt, the process comprising the steps of:

(a) adding a hydrophobic flavonoid to an aqueous solution of a soluble, edible phosphate salt which has a starting pH of about 7.1 to about 10,

(b) stirring the mixture at a temperature of about 20 to about 85°C until the hydrophobic flavonoid has dissolved, while maintaining the pH at about the starting pH;

(c) adjusting the pH to be about 7.0 to about 7.5; and

(d) spray-drying the mixture to provide the powder product.

16. A process according to claim 15 in which the concentration of phosphate salt in the aqueous solution of step (a) is about 0.5 to about 10% (w/v), preferably about 3 to about 7% (w/v), more preferably about 5% (w/v).

17. A process according to claim 15 or claim 16 in which the amount of hydrophobic flavonoid added in step (a) is an amount that results in a concentration of about 0.1 to about 10% (w/v) hydrophobic flavonoid, preferably about 3 to about 6% (w/v).

18. A process according to any one of claims 15 to 17 in which a macromolecular compound selected from the group consisting of a protein, a polysaccharide, a lipid, and a non-ionic surfactant is added to the solution of dissolved hydrophobic flavonoid produced in step (b).

19. A process according to claim 18 wherein the concentration of macromolecular compound added to the solution in step (b) is about 0.1 to about 7% (w/v), preferably about 0.5 to about 2.5% (w/v), more preferably about 0.3 to about 1.5% (w/v).

20. A process according to claim 18 or claim 19 wherein protein is added to the solution of dissolved hydrophobic flavonoid produced in step (b), preferably NaCas.

21. A spray-dried powder produced by the process according to any one of claims 15 to 20 or a food product comprising a spray-dried powder of any one of claims 1-14 and 21, preferably where the food product is a transparent or semi-transparent food or beverage.

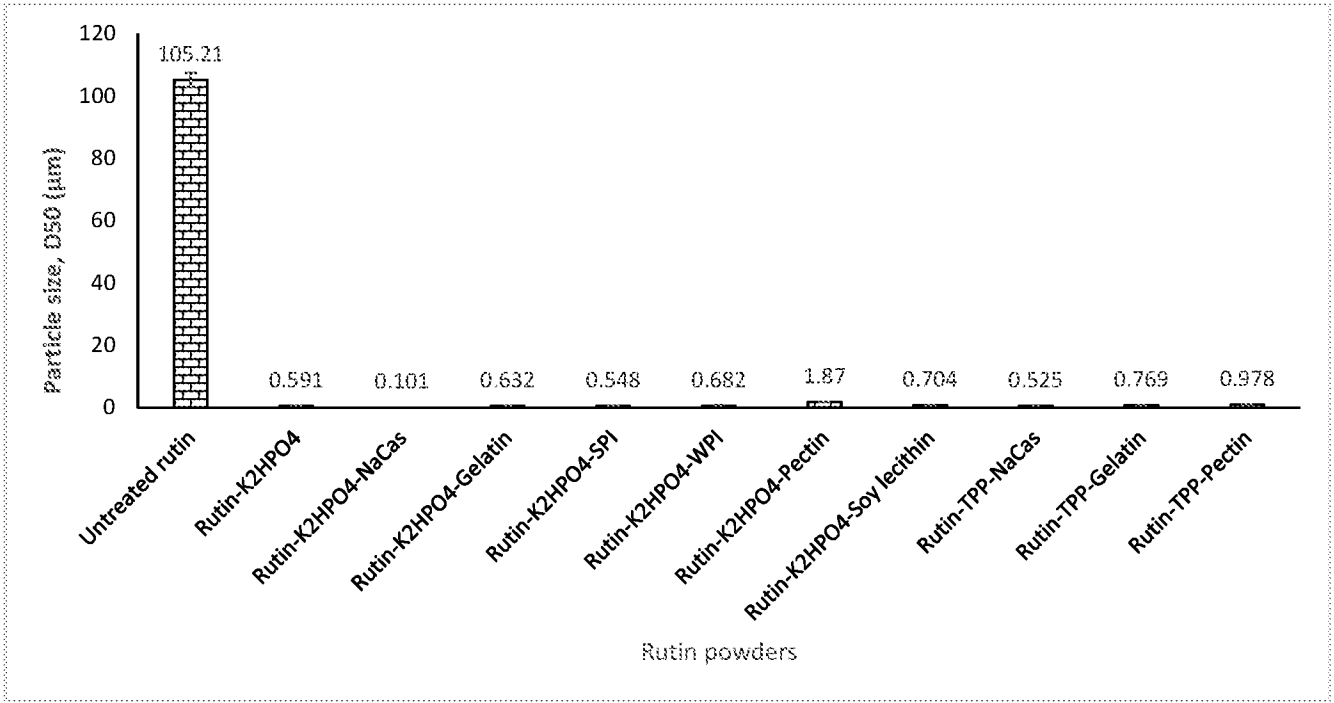

Figure 1

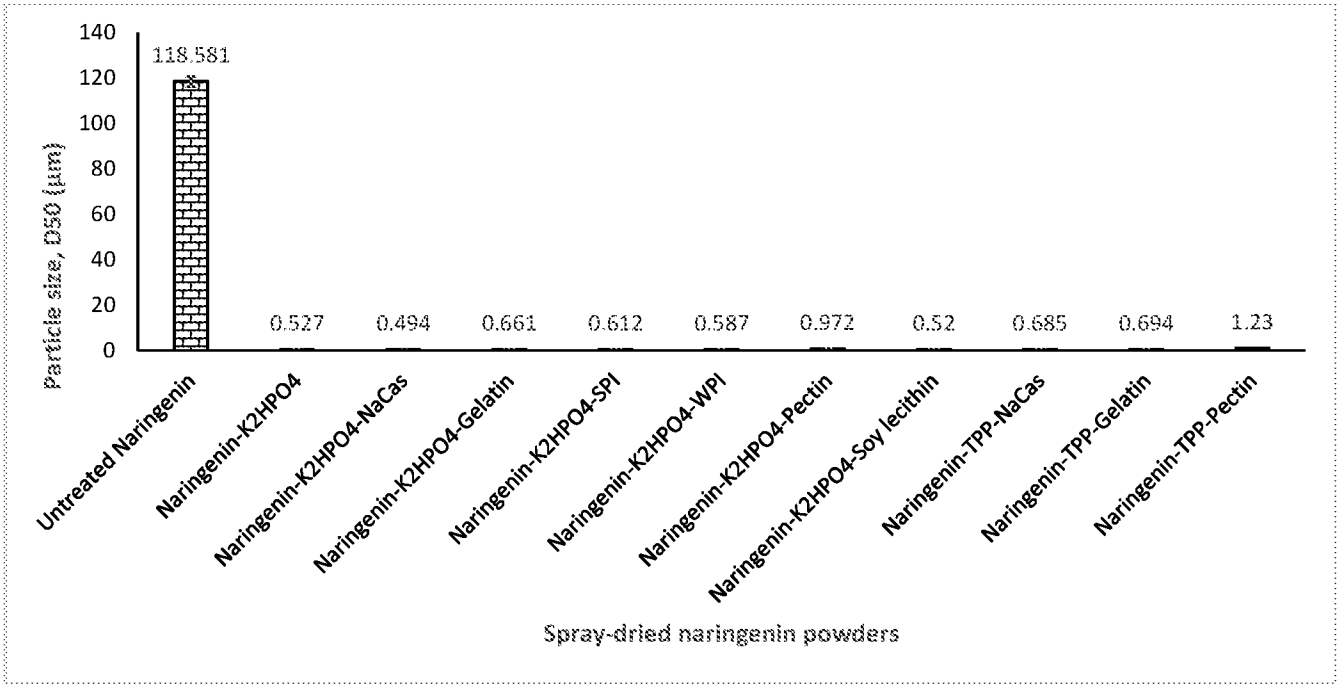

Figure 2

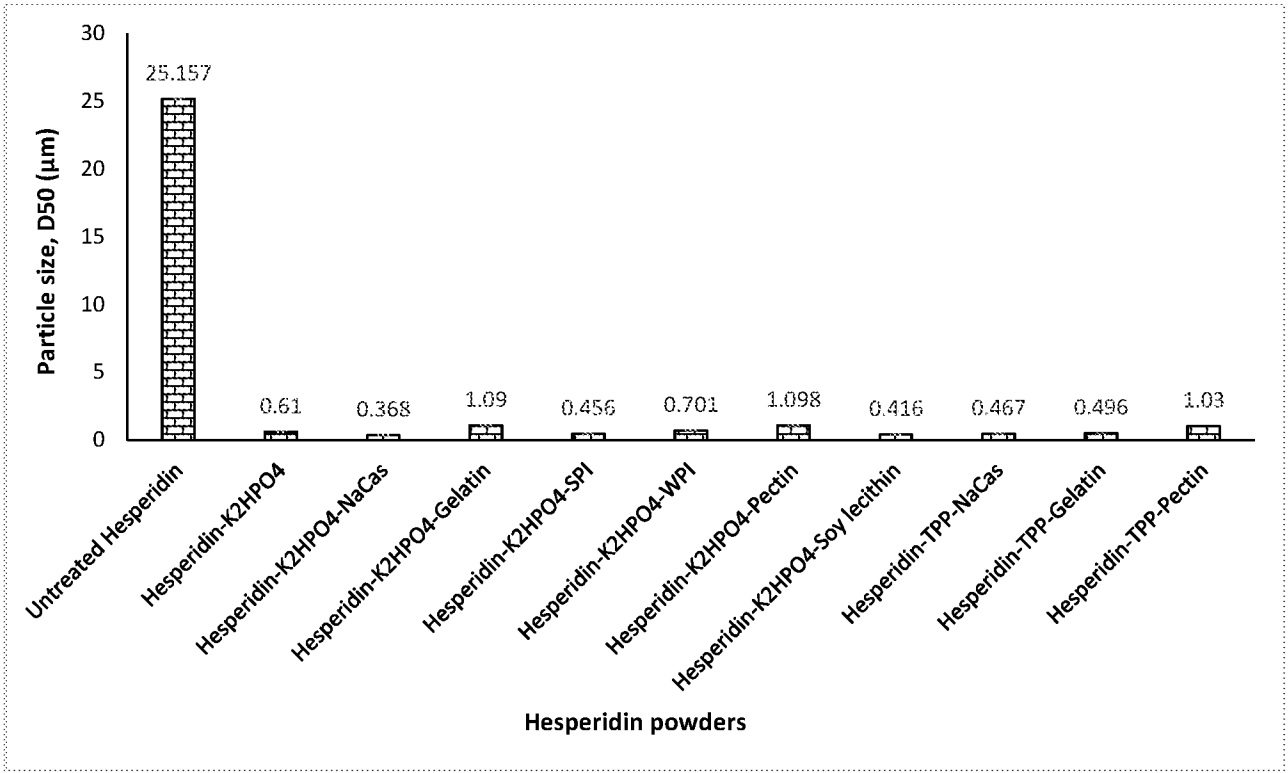

Figure 3

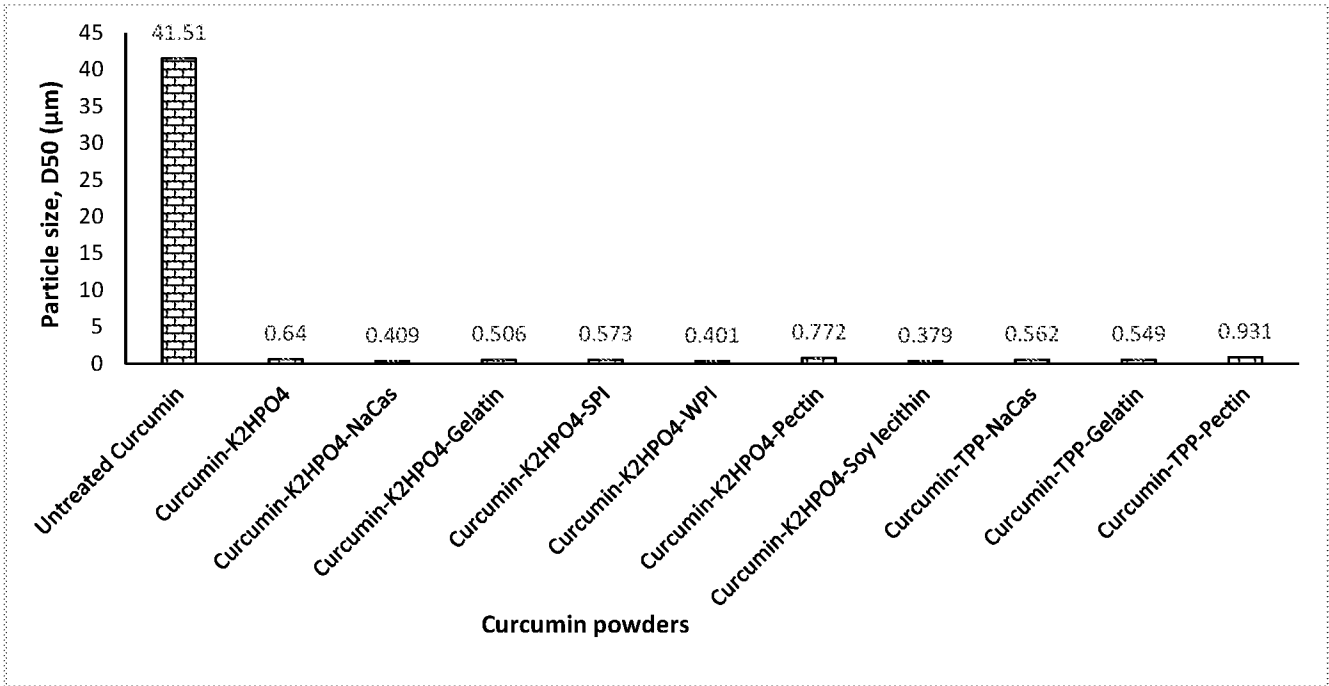

Figure 4

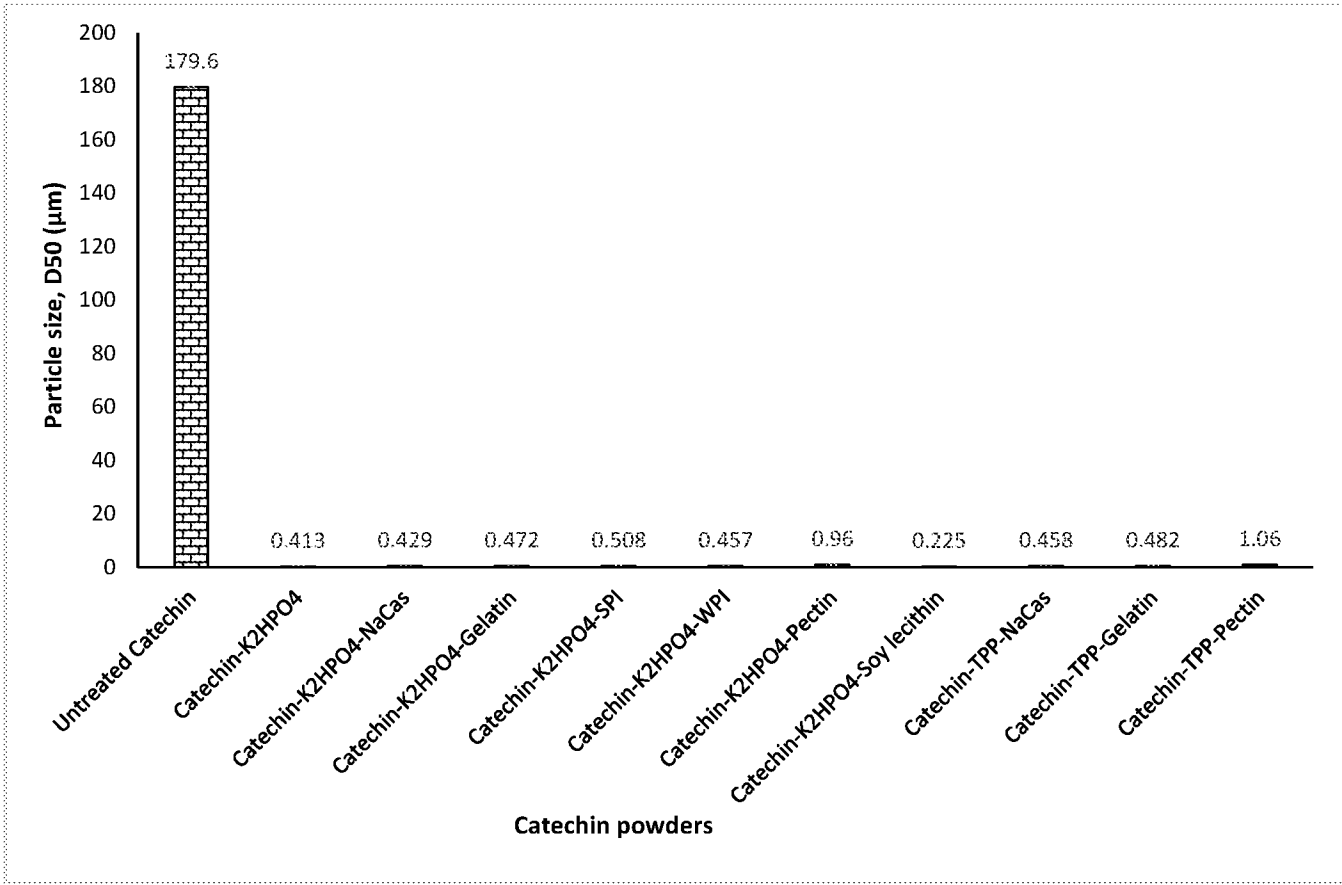
Particle size, D50 (µm)
200
180
160
140
120
100
80
60
40
20
0
179.6
0.413
0.429
0.472
0.508
0.457
0.96
0.225
0.458
0.482
1.06
Untreated Catechin
Catechin-K2HPO4
Catechin-K2HPO4-NaCas
Catechin-K2HPO4-Gelatin
Catechin-K2HPO4-SPI
Catechin-K2HPO4-WPI
Catechin-K2HPO4-Pectin
Catechin-K2HPO4-Soy lecithin
Catechin-TPP-NaCas
Catechin-TPP-Gelatin
Catechin-TPP-Pectin
Catechin powders

Figure 5

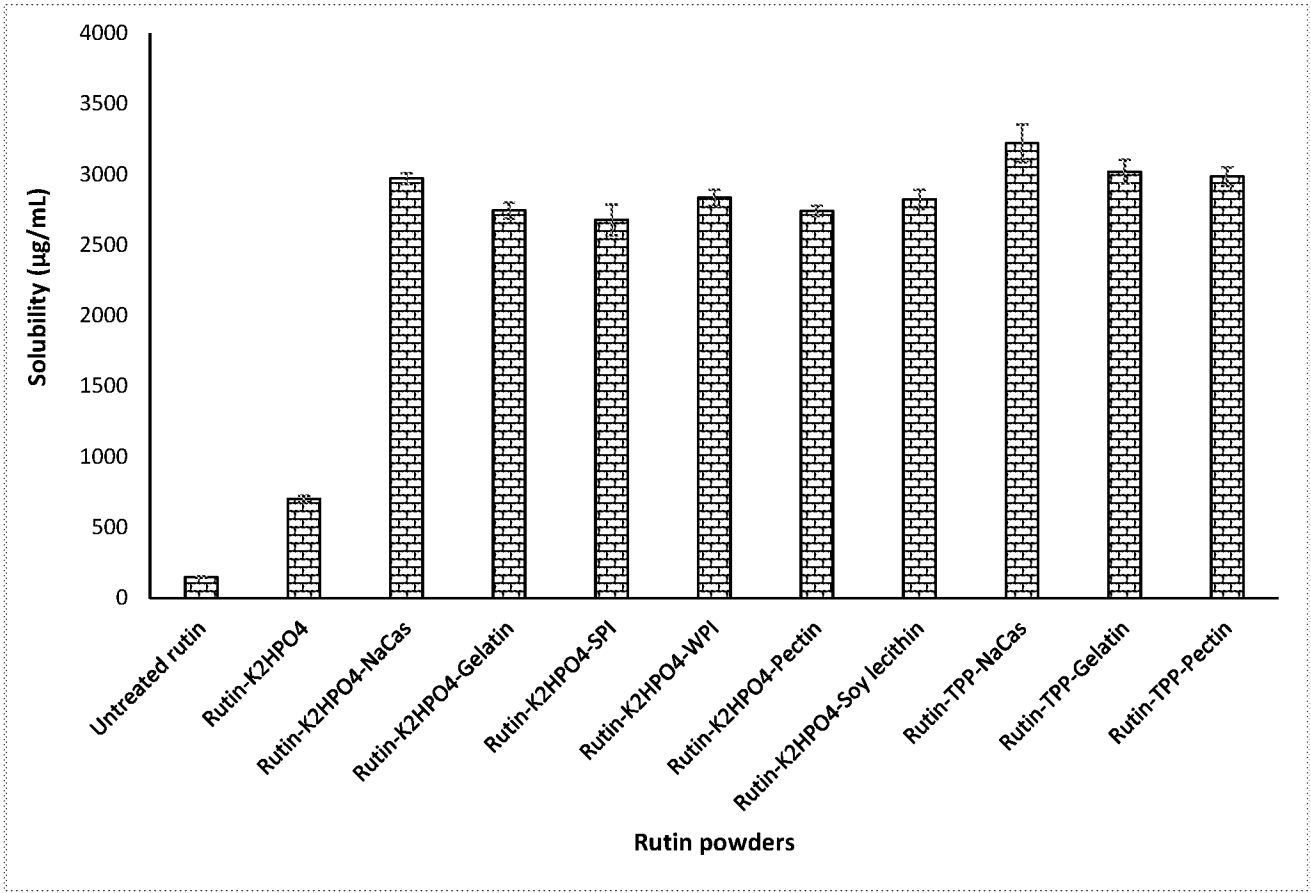
4000
3500
3000
2500
2000
1500
1000
500
0
Solubility (µg/mL)
Untreated rutin
Rutin-K2HPO4
Rutin-K2HPO4-NaCas
Rutin-K2HPO4-Gelatin
Rutin-K2HPO4-SPI
Rutin-K2HPO4-WPI
Rutin-K2HPO4-Pectin
Rutin-K2HPO4-Soy lecithin
Rutin-TPP-NaCas
Rutin-TPP-Gelatin
Rutin-TPP-Pectin
Rutin powders

Figure 6

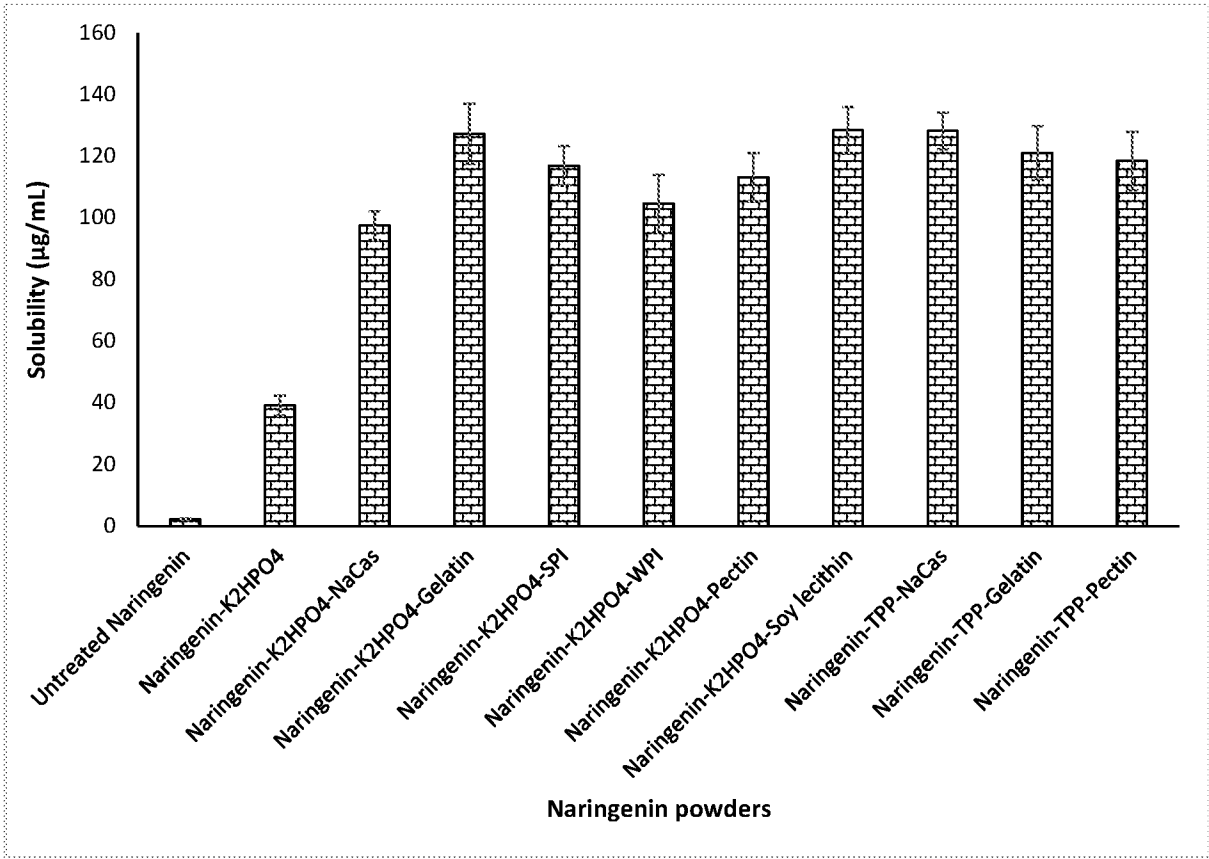
160
140
120
100
80
60
40
20
0
Solubility (µg/mL)
Untreated Naringenin
Naringenin-K2HPO4
Naringenin-K2HPO4-NaCas
Naringenin-K2HPO4-Gelatin
Naringenin-K2HPO4-SPI
Naringenin-K2HPO4-WPI
Naringenin-K2HPO4-Pectin
Naringenin-K2HPO4-Soy lecithin
Naringenin-TPP-NaCas
Naringenin-TPP-Gelatin
Naringenin-TPP-Pectin
Naringenin powders

Figure 7

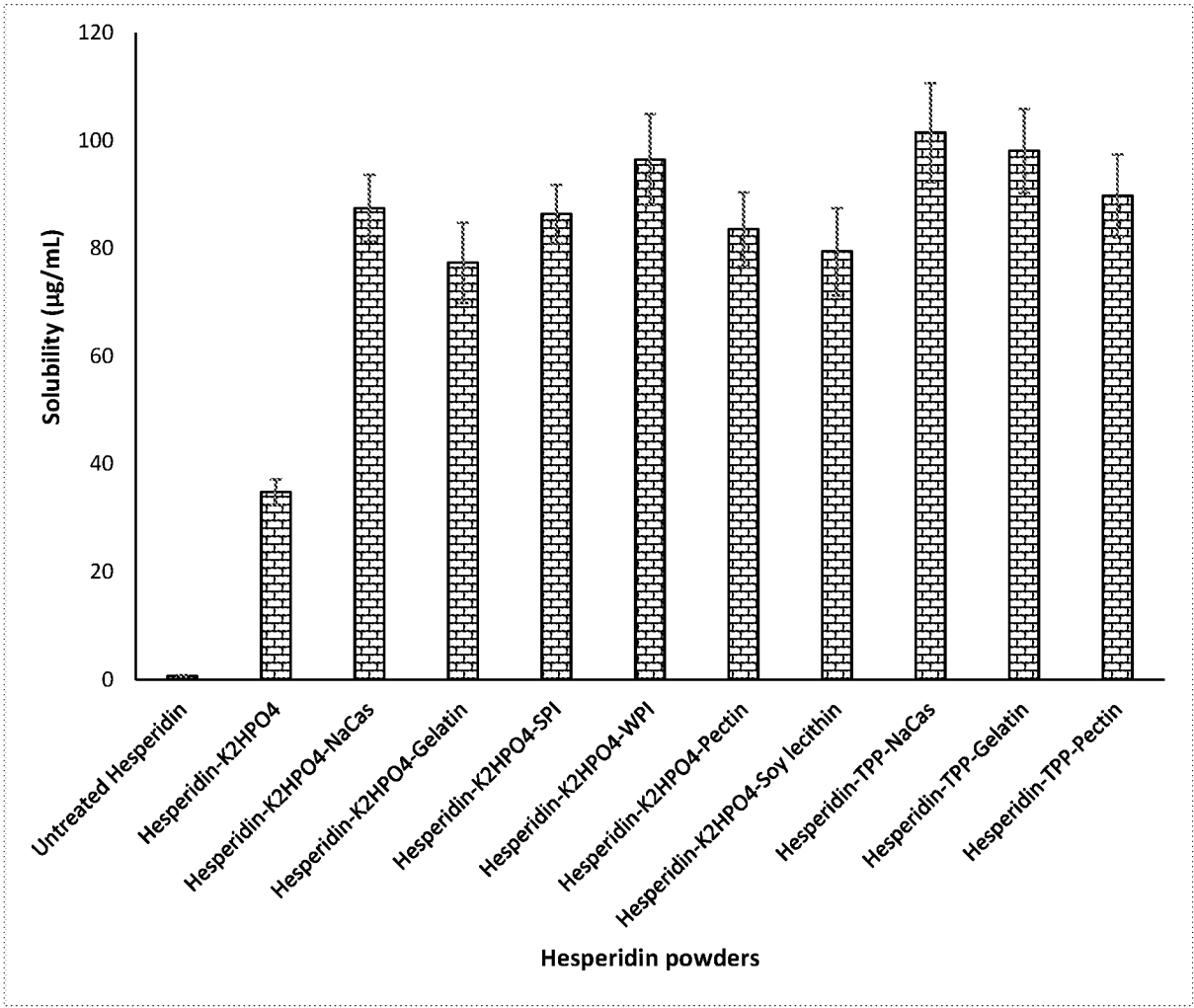
120
100
80
60
40
20
0
Solubility (µg/mL)
Untreated Hesperidin
Hesperidin-K2HPO4
Hesperidin-K2HPO4-NaCas
Hesperidin-K2HPO4-Gelatin
Hesperidin-K2HPO4-SPI
Hesperidin-K2HPO4-WPI
Hesperidin-K2HPO4-Pectin
Hesperidin-K2HPO4-Soy lecithin
Hesperidin-TPP-NaCas
Hesperidin-TPP-Gelatin
Hesperidin-TPP-Pectin
Hesperidin powders

Figure 8

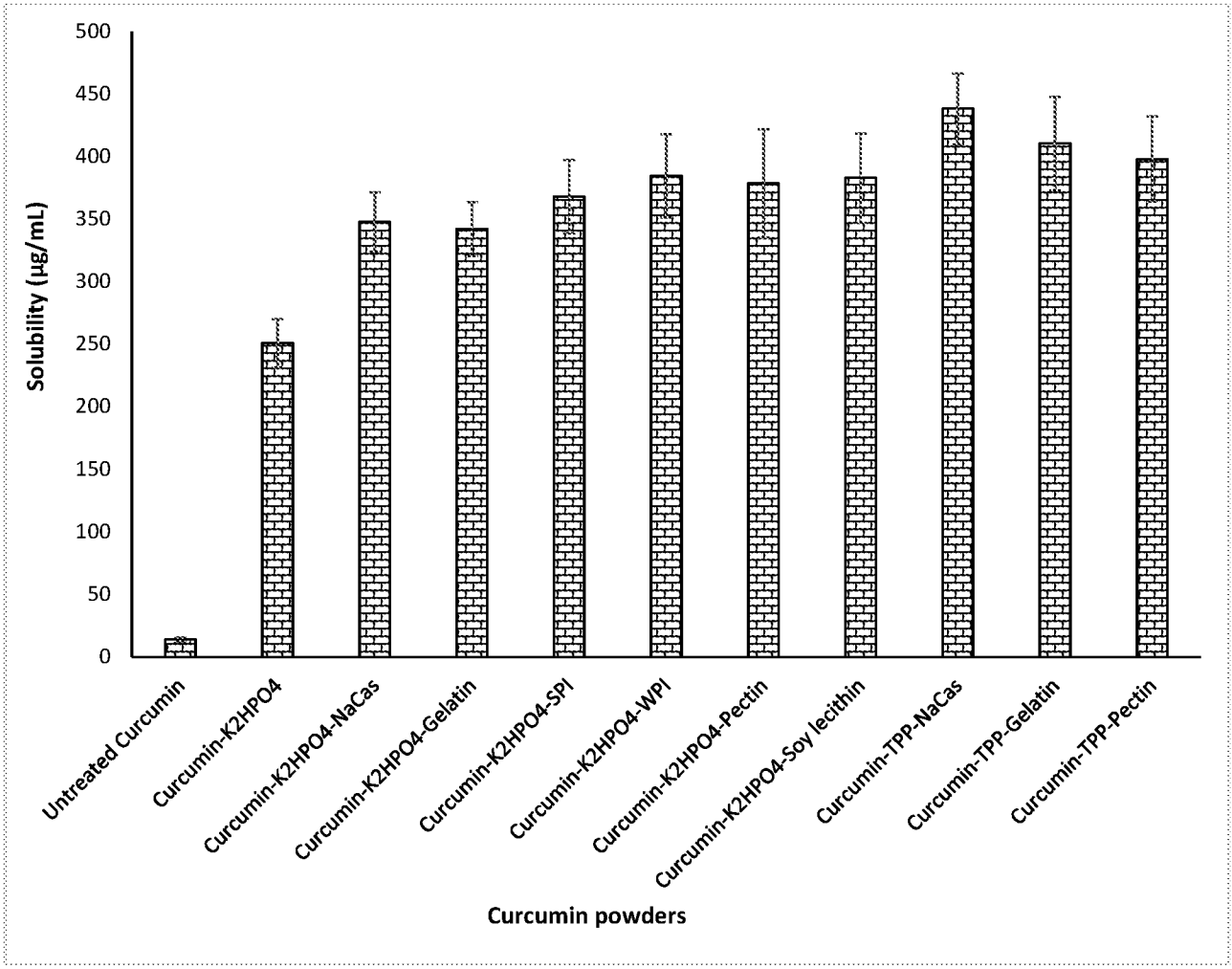
500
450
400
350
300
250
200
150
100
50
0
Solubility (µg/mL)
Untreated Curcumin
Curcumin-K2HPO4
Curcumin-K2HPO4-NaCas
Curcumin-K2HPO4-Gelatin
Curcumin-K2HPO4-SPI
Curcumin-K2HPO4-WPI
Curcumin-K2HPO4-Pectin
Curcumin-K2HPO4-Soy lecithin
Curcumin-TPP-NaCas
Curcumin-TPP-Gelatin
Curcumin-TPP-Pectin
Curcumin powders

Figure 9

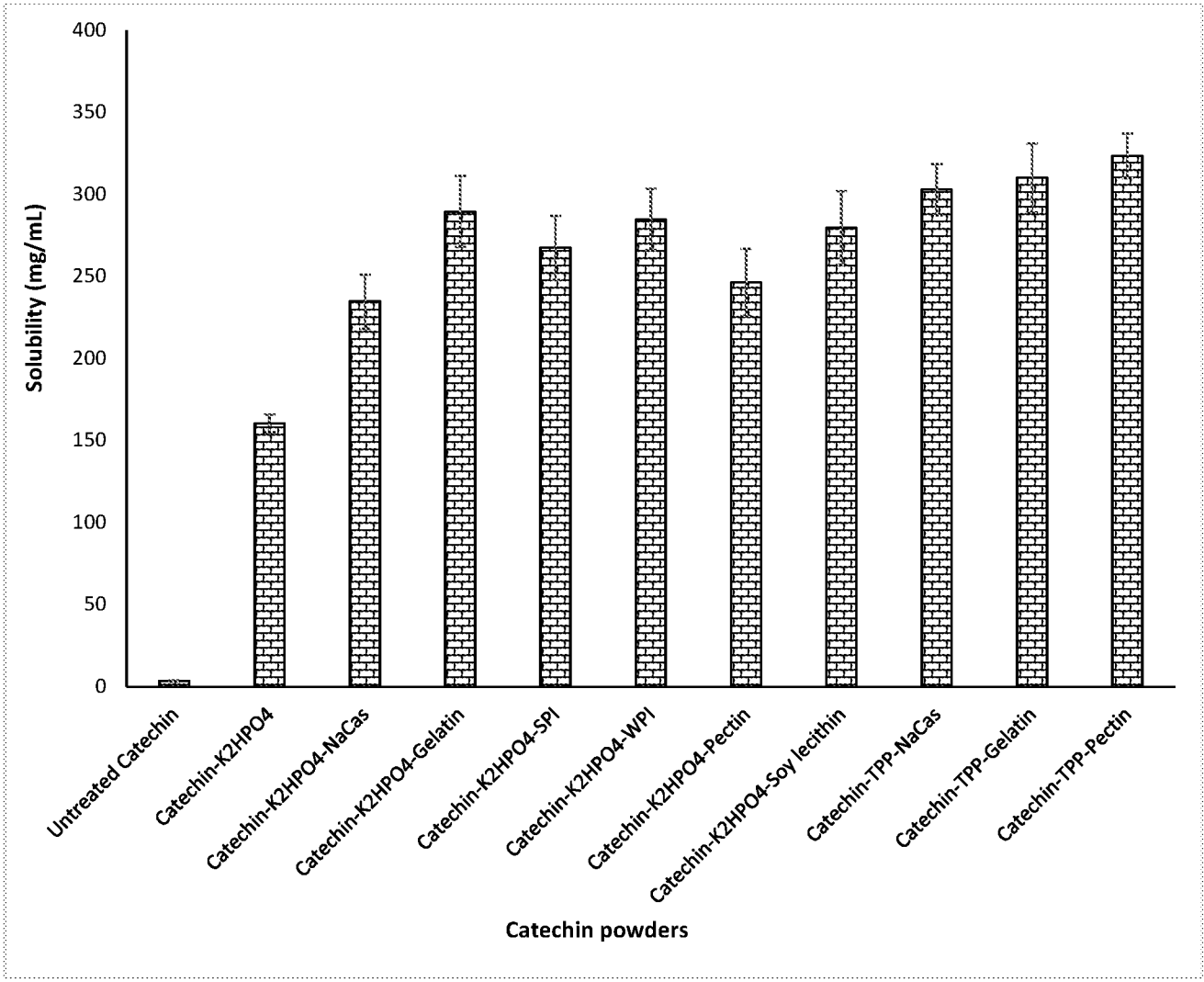
400
350
300
250
200
150
100
50
0
Solubility (mg/mL)
Untreated Catechin
Catechin-K2HPO4
Catechin-K2HPO4-NaCas
Catechin-K2HPO4-Gelatin
Catechin-K2HPO4-SPI
Catechin-K2HPO4-WPI
Catechin-K2HPO4-Pectin
Catechin-K2HPO4-Soy lecithin
Catechin-TPP-NaCas
Catechin-TPP-Gelatin
Catechin-TPP-Pectin
Catechin powders

Figure 10

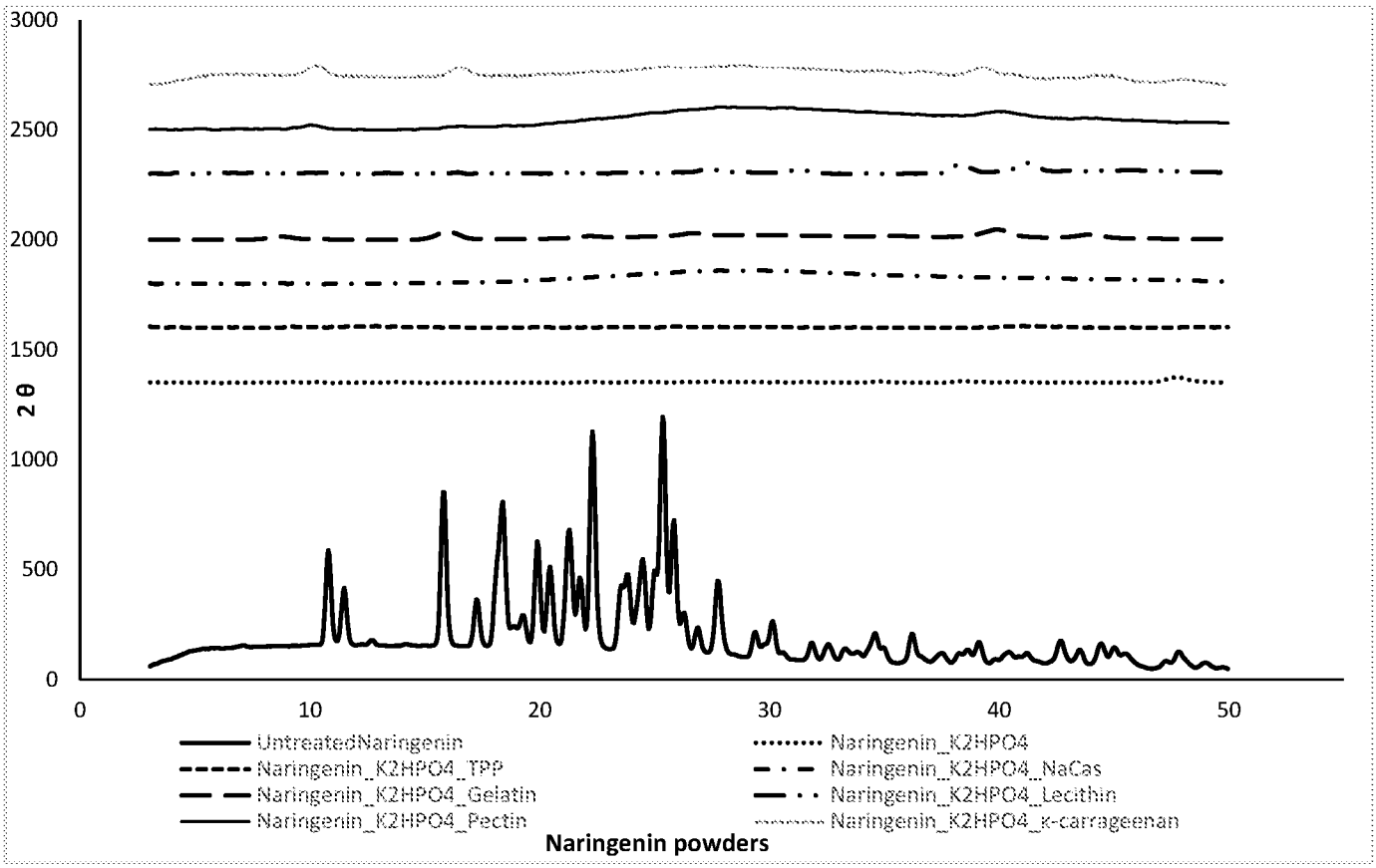
3000
2500
2000
1500
1000
500
0
2θ
0
10
20
30
40
50
UntreatedNaringenin
Naringenin_K2HPO4
Naringenin_K2HPO4_TPP
Naringenin_K2HPO4_NaCas
Naringenin_K2HPO4_Gelatin
Naringenin_K2HPO4_Lecithin
Naringenin_K2HPO4_Pectin
Naringenin_K2HPO4_κ-carrageenan
Naringenin powders

Figure 11

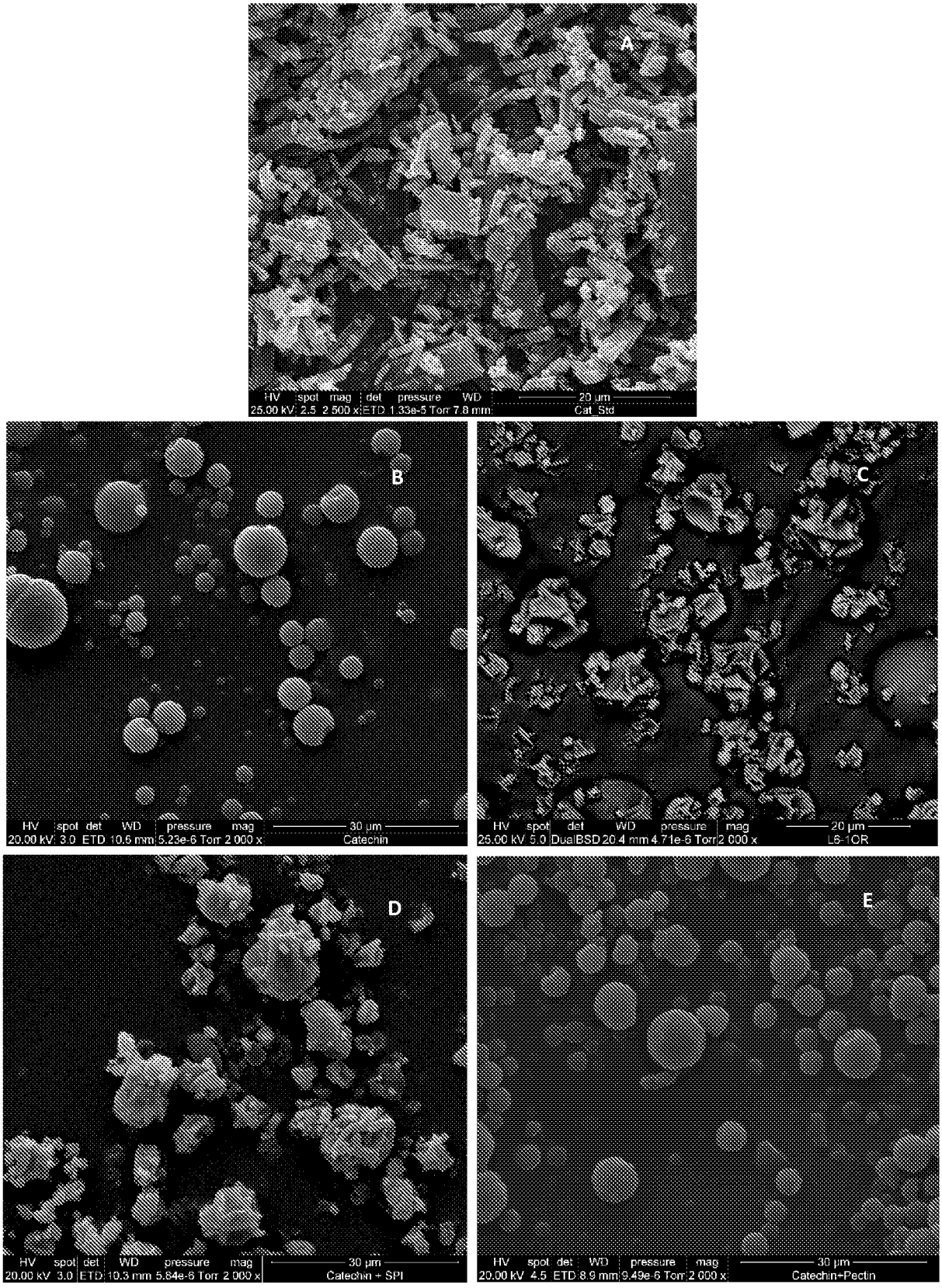
A
HV spot mag det pressure WD 20 µm
25.00 kV 2.5 2 500 x ETD 1.33e-5 Torr 7.8 mm Cat Std
B
HV spot det WD pressure mag 30 µm
20.00 kV 3.0 ETD 10.6 mm 5.23e-6 Torr 2 000 x Catechin
C
HV spot det WD pressure mag 20 µm
25.00 kV 5.0 DualBSD 20.4 mm 4.71e-6 Torr 2 000 x L6-1OR
D
HV spot det WD pressure mag 30 µm
20.00 kV 3.0 ETD 10.3 mm 5.84e-6 Torr 2 000 x Catechin + SPI
E
HV spot det WD pressure mag 30 µm
20.00 kV 4.5 ETD 8.9 mm 9.49e-6 Torr 2 000 x Catechin+Pectin

Figure 12

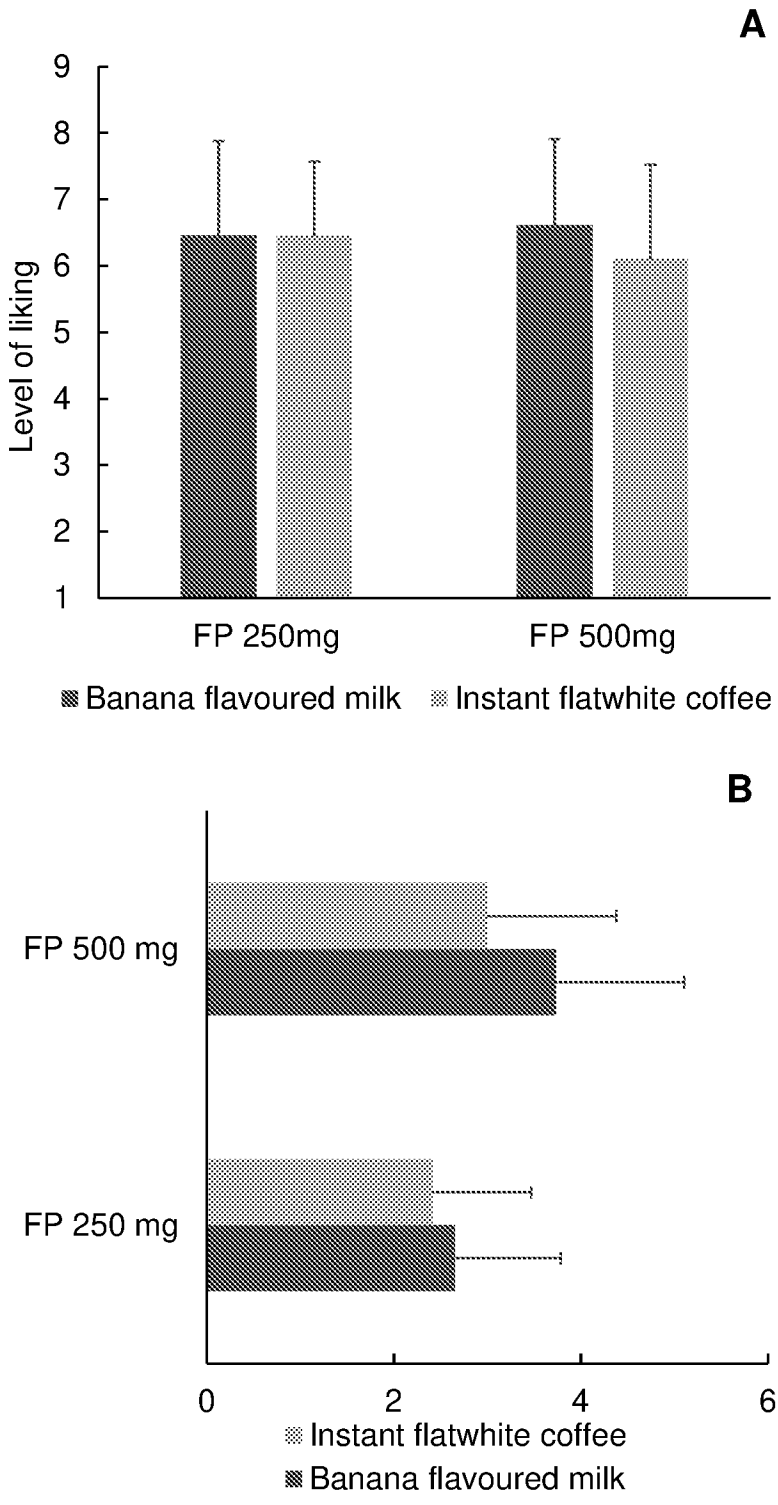
A
9
8
7
6
5
4
3
2
1
Level of liking
FP 250mg
FP 500mg
Banana flavoured milk
Instant flatwhite coffee
B
FP 500 mg
FP 250 mg
0
2
4
6
Instant flatwhite coffee
Banana flavoured milk

Figure 13

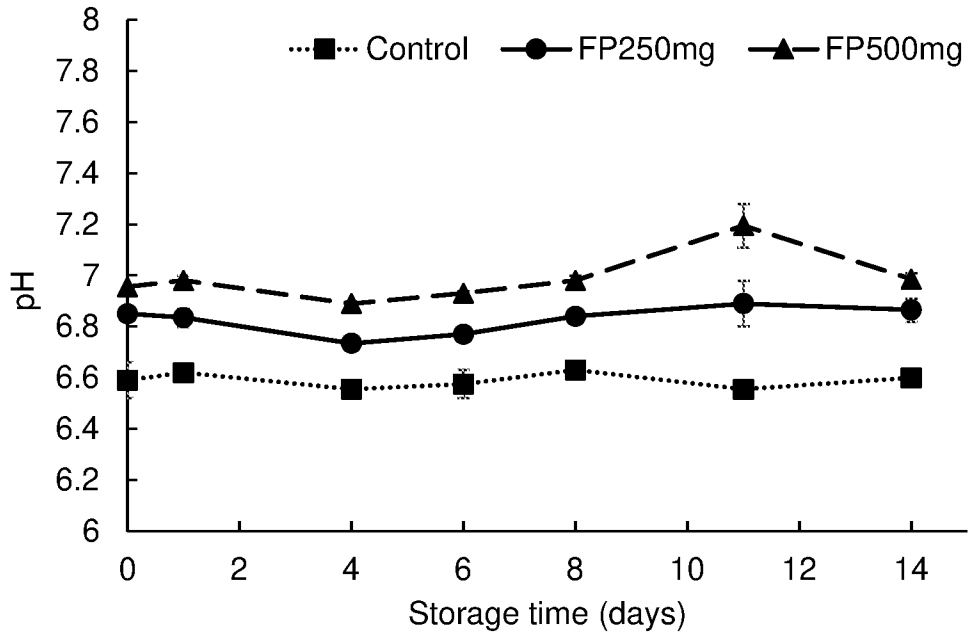

Figure 14

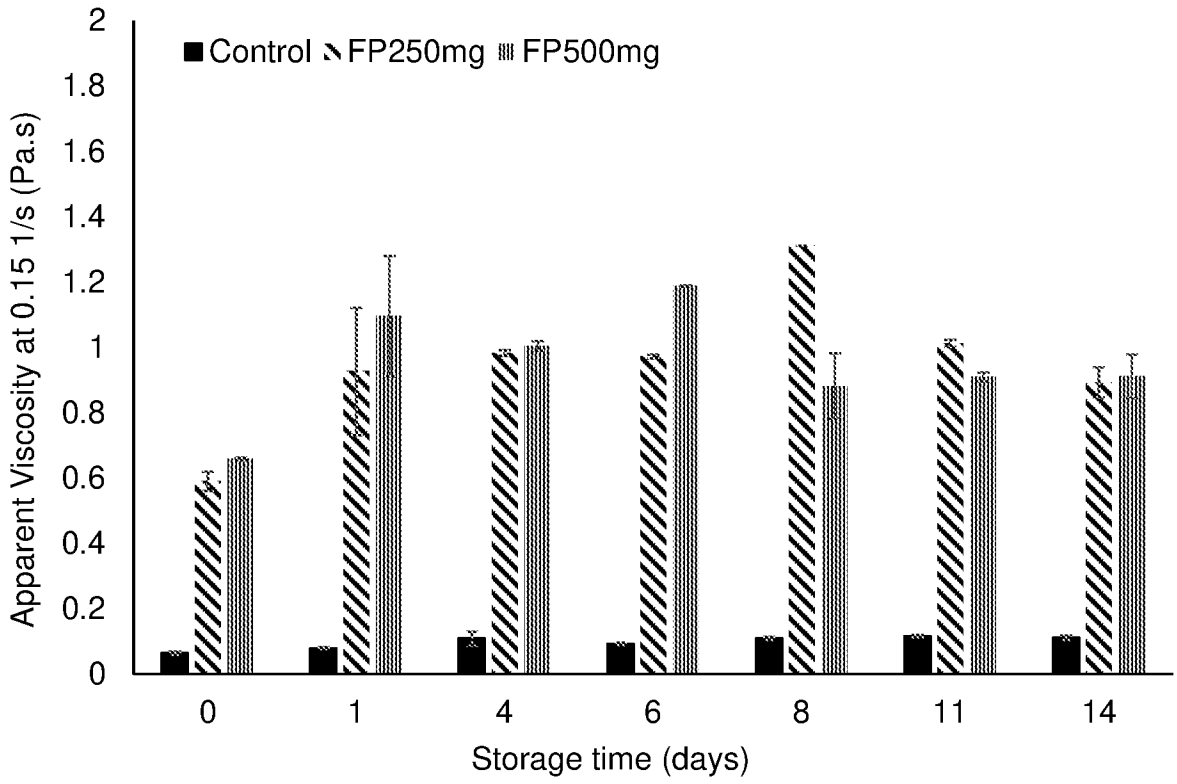

Figure 15

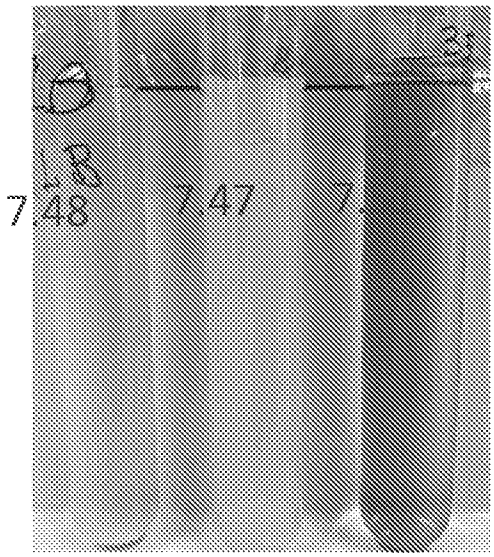

Figure 16

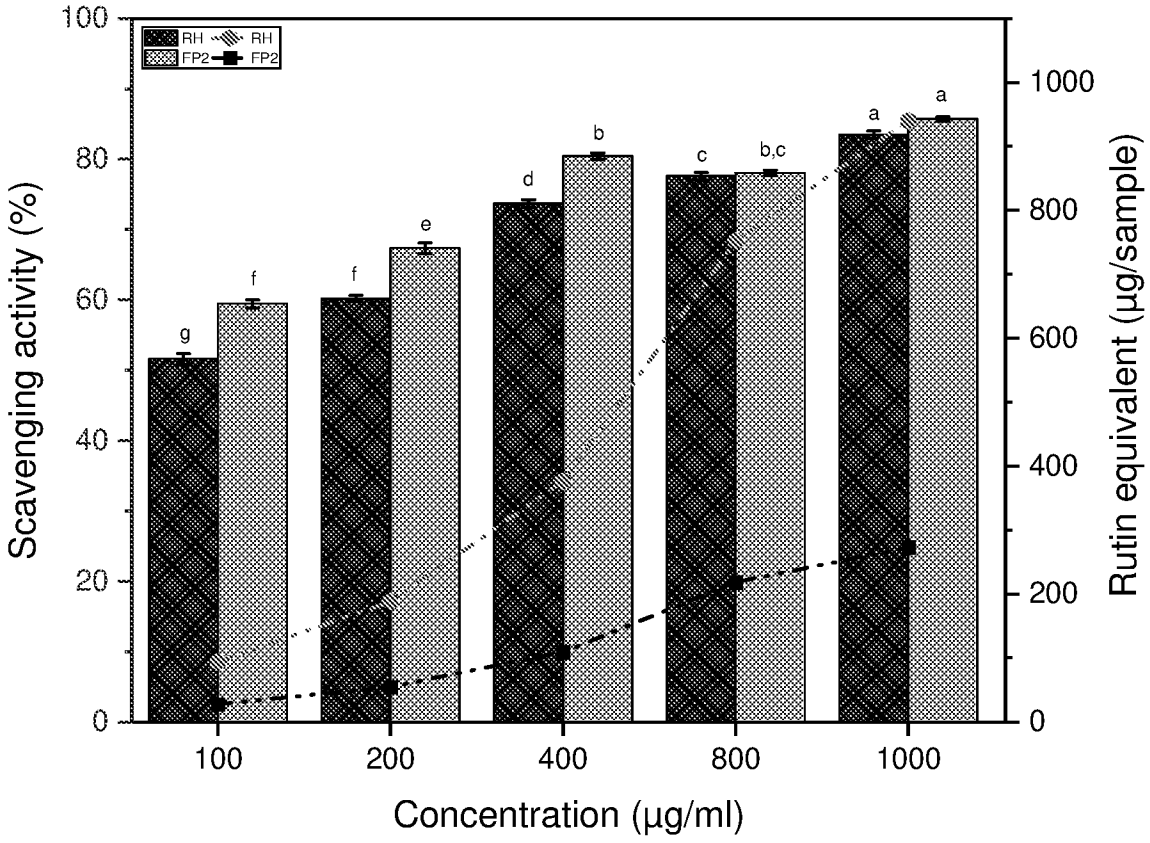

Figure 17

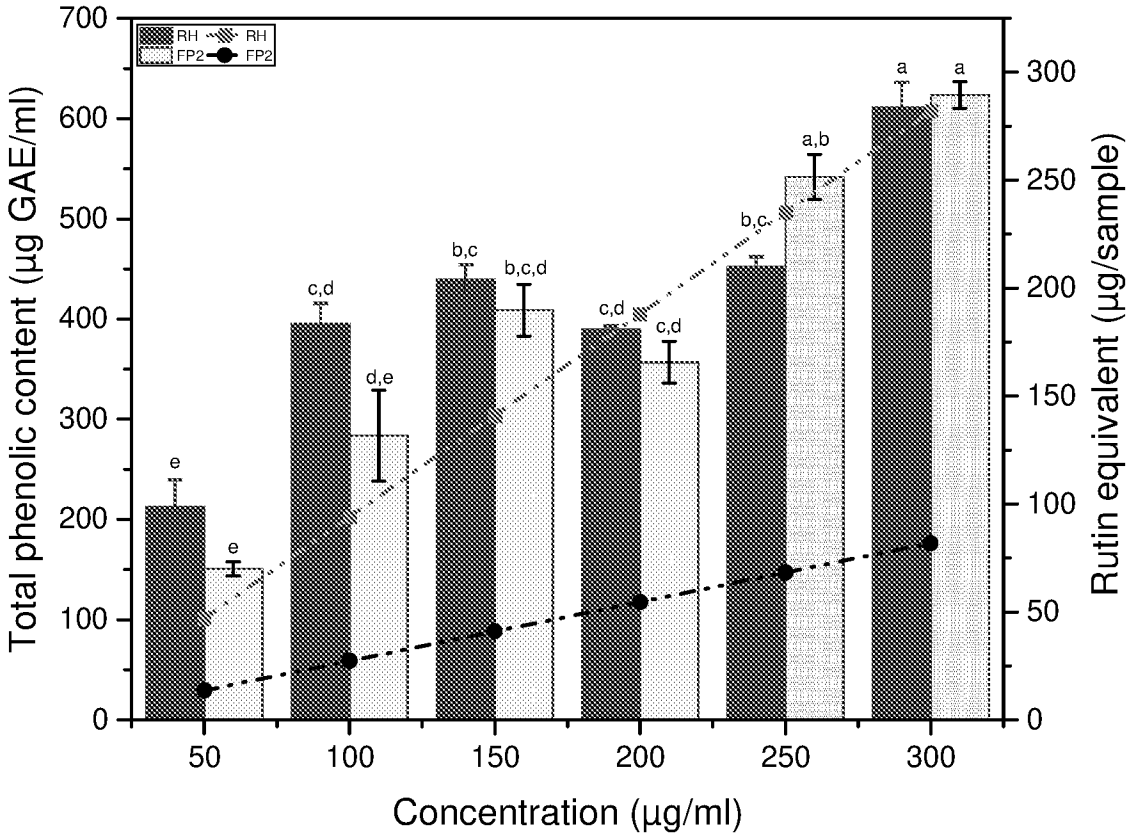
RH
RH
FP2
FP2
Total phenolic content (µg GAE/ml)
Rutin equivalent (µg/sample)
Concentration (µg/ml)
0
100
200
300
400
500
600
700
0
50
100
150
200
250
300
e
e
c,d
d,e
b,c
b,c,d
c,d
c,d
b,c
a,b
a
a

Figure 18

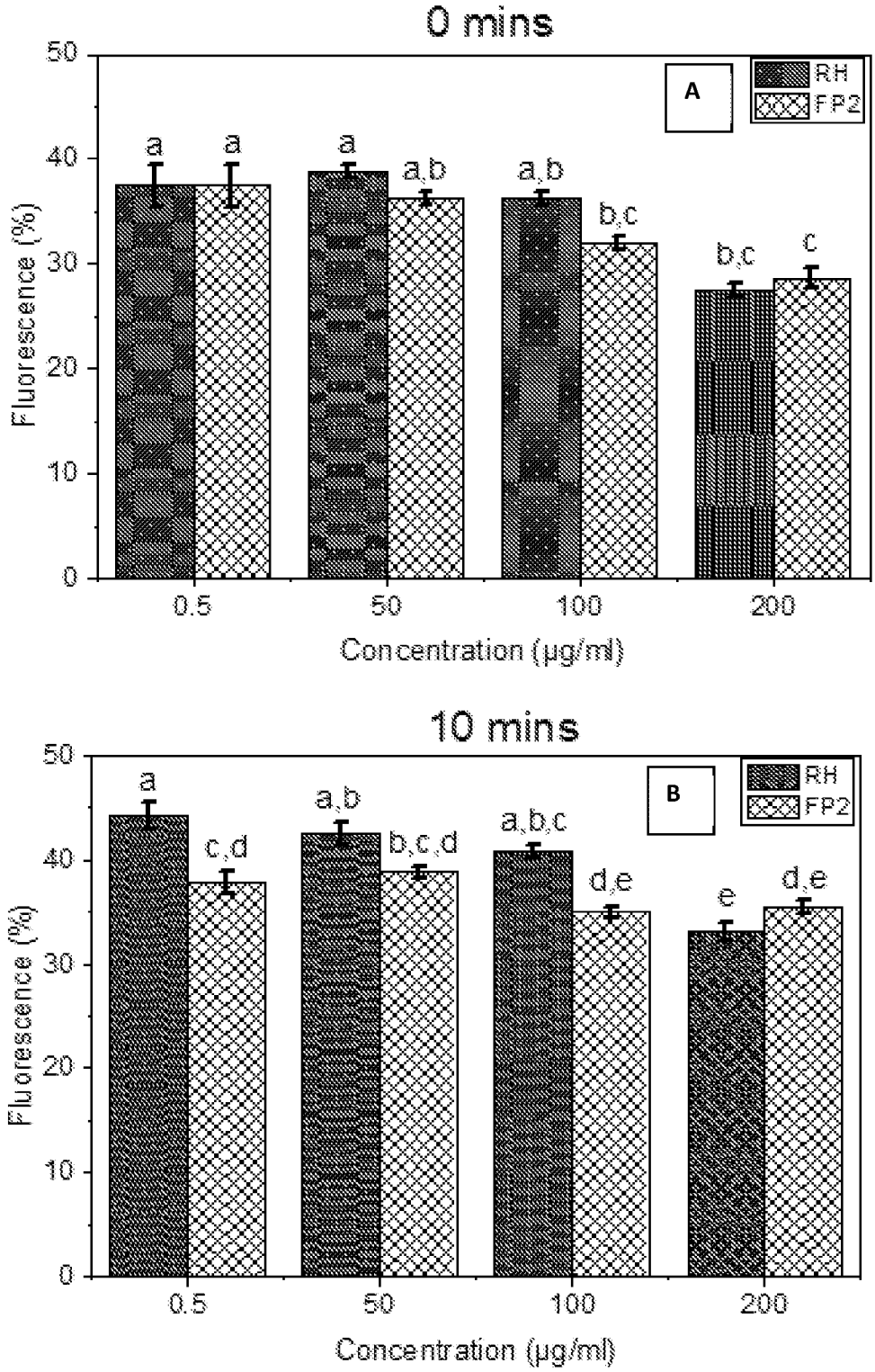
0 mins
A
RH
FP2
Fluorescence (%)
Concentration (µg/ml)
0.5
50
100
200
a
a
a
a,b
a,b
b,c
b,c
c
10 mins
B
RH
FP2
Fluorescence (%)
Concentration (µg/ml)
a
c,d
a,b
b,c,d
a,b,c
d,e
e
d,e

Figure 19

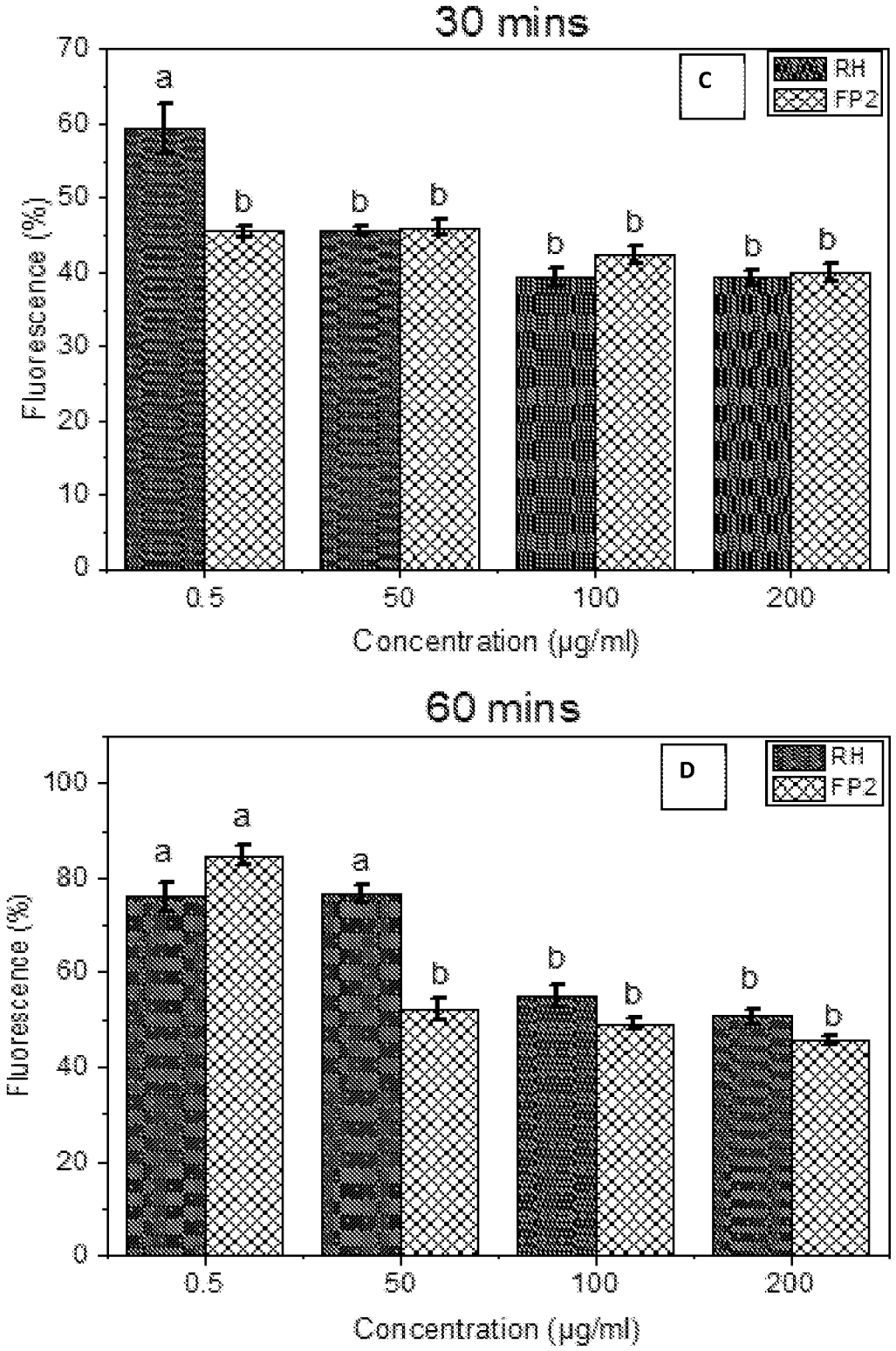

Figure 19 (cont)

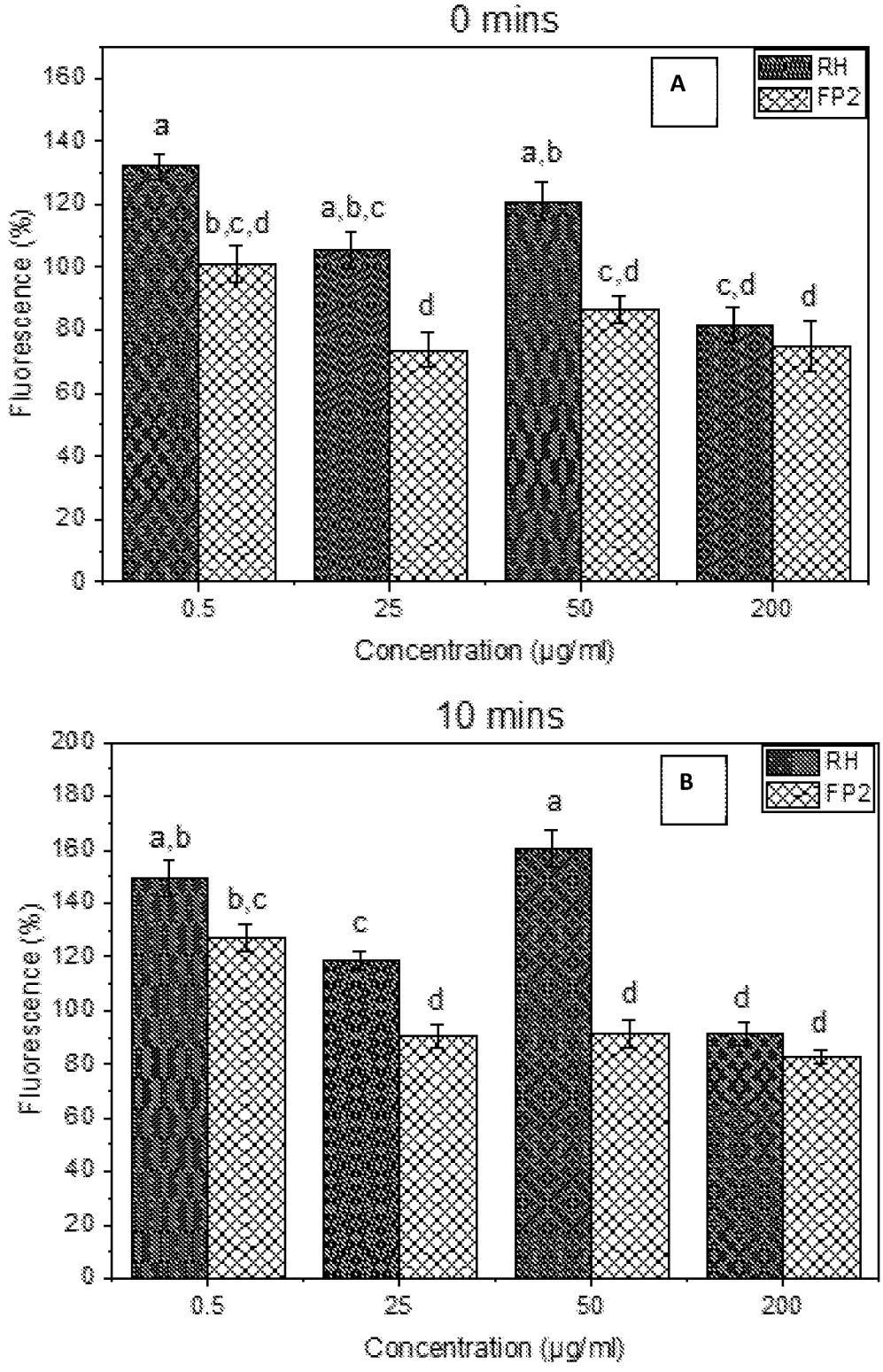
0 mins
A
RH
FP2
Fluorescence (%)
Concentration (µg/ml)
0.5
25
50
200
a
b,c,d
a,b,c
d
a,b
c,d
c,d
d
10 mins
B
RH
FP2
Fluorescence (%)
Concentration (µg/ml)
a,b
b,c
c
d
a
d
d
d

Figure 20

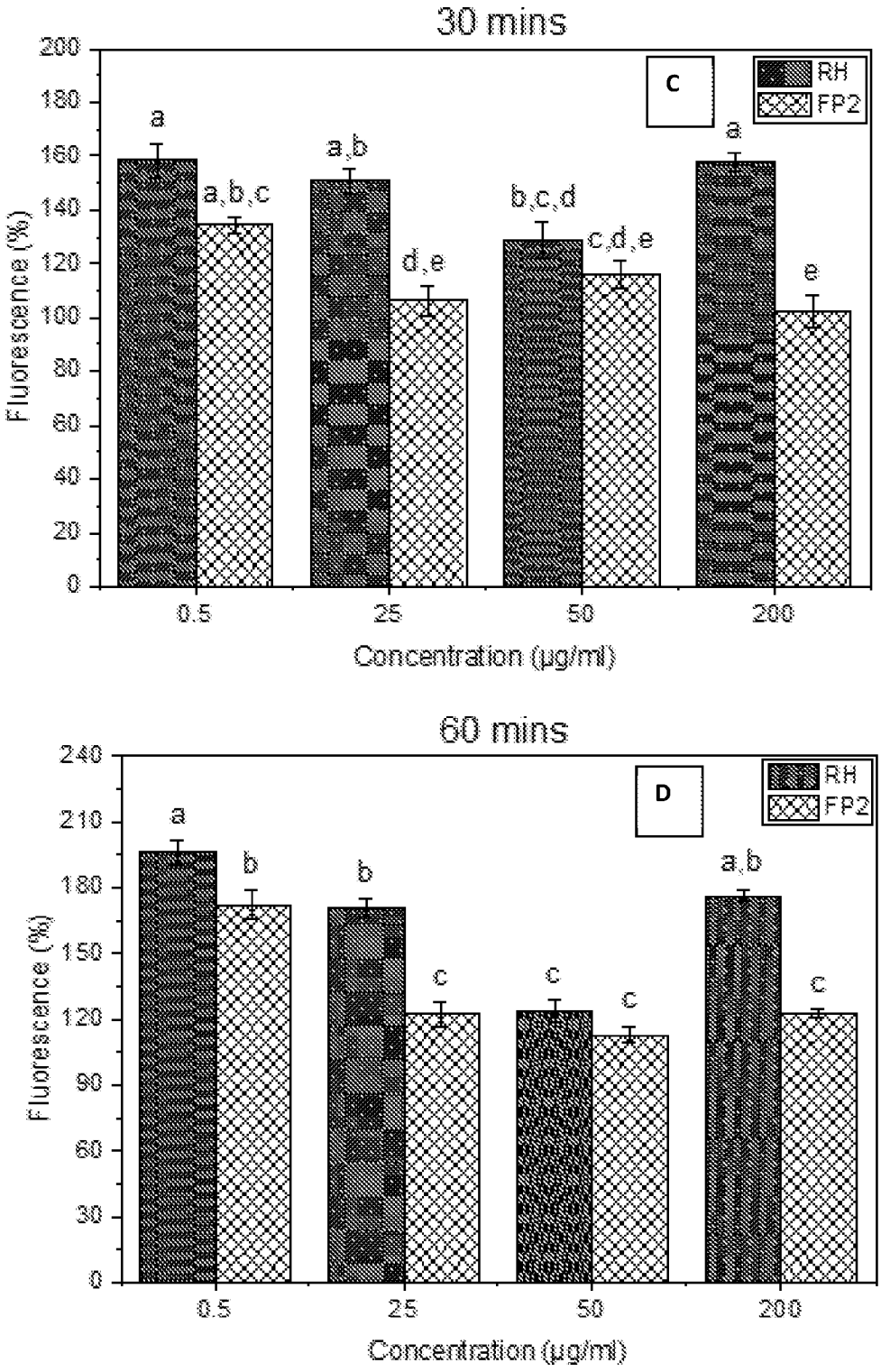

Figure 20 (cont)

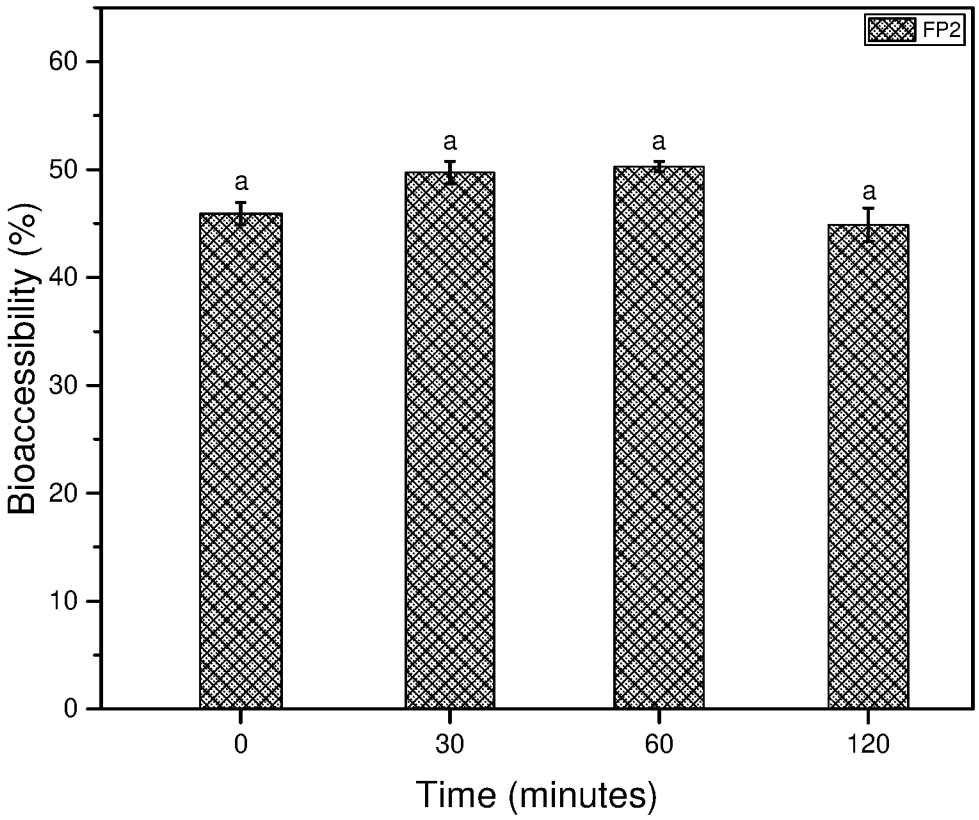
FP2
Bioaccessibility (%)
0
10
20
30
40
50
60
a
a
a
a
0
30
60
120
Time (minutes)

Figure 21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2022/050697

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/7048 (2006.01) ***A61K 9/14 (2006.01)*** ***A61K 31/12 (2006.01)*** ***A61K 31/353 (2006.01)*** ***A61K 47/02 (2006.01)*** ***A61K 47/42 (2017.01)*** ***A61P 39/06 (2006.01)*** ***A23L 33/105 (2016.01)***

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PATENW (EPOQUE); CAPLUS, EMBASE, MEDLINE, BIOSIS, FSTA (STN); Keywords: Flavoplus, rutin, rutoside, quercetin-3-O-rutinoside, sophorin, 153-18-4, naringenin, naringetol, salipurol, salipurpol, 480-41-1, quercitin, quercetin, sophoretin, meletin, xanthaurine, quercitol, quertine, 117-39-5, 6151-25-3, curcumin, diferuloylmethane, 458-37-7, hesperidin, 520-26-3, alpha-naphthoflavone, ANF, 604-59-1, beta-naphthoflavone, BNF, 6051-87-2, catechin, cianidanol, cyanidanol, catechinic acid, catechuic acid, dexcyanidanol, 7295-85-4, 154-23-4, 18829-70-4, 88191-48-4, chrysin, 480-40-0, luteolin, luteolol, digitoflavone, flacitran, 491-70-3, myricetin, myricitol, myricetol, myricitin, cannabiscetin, 529-44-2, anthocyanin, aurantinidin, 25041-66-1, cyanidin, 13306-05-3, 528-58-5, delphinidin, 13270-61-6, 528-53-0, europinidin, 19077-87-3, pelargonidin, 134-04-3, malvidin, 643-84-5, peonidin, 134-01-0, petunidin, 1429-30-7, rosinidin, 4092-64-2, vitamin P, phosphate, K2HPO4, phosphoric acid dipotassium salt, TPP, 7758-11-4, 16788-57-1, 7601-54-9, 10101-89-0, spray dry, spray dried, spray dehydrate, flavonoid, flavanone, flavan-3-ol, A61K9/14, A61K9/0095, and like terms. Patentscope, AUSPAT, PubMed, NOSE, INTESS databases: Inventor and Applicant name searches – MASSEY; RASHIDINEJAD; ACEVEDO; SINGH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 March 2022	09 March 2022

Name and mailing address of the ISA/AU	**Authorised officer**
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INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/IB2022/050697**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2020/095238 A1 (MASSEY UNIVERSITY) 14 May 2020 Claims 1, 2, 4, 6, 7, 9, 12 to 21; page 4 line 8, page 5 lines 29 to 31, page 6 lines 16 to 18, page 7 lines 1 to 12; Examples 17, 18	1 to 21
X	BABITHA, V. et al. "MODULATION OF PHARMACEUTICAL AND PHARMACOKINETIC ATTRIBUTE OF CURCUMIN ENCAPSULATED IN CHITOSAN USING SPRAY DRYING TECHNIQUE" World Journal of Pharmacy and Pharmaceutical Sciences (2020) Vol.9 No.11, pages 1262 to 1280 Abstract, Introduction, Experimental section, Results and Discussion	1 to 21
X	PARIZE, A.L. et al. "EVALUATION OF CHITOSAN MICROPARTICLES CONTAINING CURCUMIN AND CROSSLINKED WITH SODIUM TRIPOLYPHOSPHATE PRODUCED BY SPRAY DRYING" Quimica Nova (2012) Vol.35 No.6, pages 1127 to 1132 Abstract, Introduction, Experimental, Results and Discussion, Conclusions	1 to 21
X	PHAN, A.N.Q. et al. "Efficient Method for Preparation of Rutin Nanosuspension Using Chitosan and Sodium Tripolyphosphate Crosslinker" Journal of Nanoscience and Nanotechnology (2019) Vol.19, pages 974 to 978 Abstract, Introduction, Experimental Details, Results and Discussion, Conclusion	1 to 21
X	NAYAK, C.A. et al. "Effect of Selected Additives on Microencapsulation of Anthocyanin by Spray Drying" Drying Technology (2010) Vol.28, pages 1396 to 1404 Abstract, Introduction, Materials and Methods, Results and Discussion, Conclusion	1 to 21
X	CN 105250244 A (SOUTHWEST UNIVERSITY) 20 January 2016 & English language machine translation provided by EPO Claims 1, 5; Examples 1 to 4	1 to 21
X	CN 109852646 A (CHENGDU WAGOTT BIO-TECH CO LTD) 07 June 2019 & English language machine translation provided by EPO Embodiment 1	1 to 10, 12 to 17, 21

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/IB2022/050697**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2020/095238 A1	14 May 2020	WO 2020095238 A1	14 May 2020
		AU 2019376902 A1	03 Jun 2021
		CN 113163834 A	23 Jul 2021
		EP 3876754 A1	15 Sep 2021
		JP 2022506801 A	17 Jan 2022
CN 105250244 A	20 January 2016	CN 105250244 A	20 Jan 2016
CN 109852646 A	07 June 2019	CN 109852646 A	07 Jun 2019

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.