Literature review of health claims for Rubus

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RB10002

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Literature Review and Health Claims for Rubus (RB10002)

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EXECUTIVE SUMMARY

Horticulture Australia Limited (HAL), in conjunction with the Rubus Industry Advisory Committee is looking to determine and promote the health benefits of Rubus. Broadly, this project involves a nutritional and regulatory review of four Rubus varieties (Raspberry, Blackberry, Boysenberry, Loganberry), to enable the Rubus Industry to develop nutrition messages and content claims for the fruit.

This project aimed to:

1. Review the scientific literature related to Rubus and the health benefits of the bioactives and key nutrients. Where sufficient studies exist, to relate the outcomes of the scientific studies to government policy documents in relation to health claims.
2. Provide possible health claims that could be made for Rubus.

Nutrient data was obtained for Australia and New Zealand (where Australian data was absent) and a review of the scientific literature using defined search terms in the Web of Science and Scopus databases for the years 2001-2011 was conducted.

Scientific review

The primary components of Rubus are the water soluble vitamins. A review of Australian and New Zealand nutrient composition databases revealed that Rubus can range from 184-293kJ energy, 0.3-0.7% total fat content, 1.1-1.5% protein, 4.9-7.5% carbohydrate and also contain 34-63mg/100g folate and 9-38mg/100g vitamin C. Many publications have addressed the nutrient content of the berries individually with 24 identified in this project relating to raspberries, 15 for blackberries, 3 for boysenberries and 2 for loganberries separate from those addressing health in this project. The most commonly identified nutrients were the phenolic compounds with ellagic acid the specific bionutrient.

Health claims

The following types of claims could be used immediately for Rubus Berries:
http://www.foodstandards.gov.au/thecode/industryuserguides/overviewoffoodlabel1267.cfm

Raspberry

- *Raspberries are high in fibre* or *Raspberries are a good source of fibre*
- *Raspberries are a source of folate and manganese*
- *Raspberries are high in vitamin C* or *Raspberries are a good source of vitamin C*
- Combined statement: *Raspberries are high in/good source of vitamin C and fibre*

Blackberry

- *Blackberries are high in fibre* or *Blackberries are a good source of fibre*
- *Blackberries are a source of folate, beta-carotene, manganese and vitamin E*
- *Blackberries are high in vitamin C* or *Blackberries are a good source of vitamin C*
- Combined statement: *Blackberries are high in/good source of vitamin C and fibre*

Boysenberry

- *Boysenberries are high in fibre* or *Boysenberries are a good source of fibre*
- *Boysenberries are a source of vitamin C and niacin*
- *Boysenberries are high in folate or Boysenberries are a good source of folate*
- Combined statement: *Boysenberries are high in/good source of folate and fibre*

Loganberry

- *Loganberries are high in fibre* or *Loganberries are a good source of fibre*
- *Loganberries are high in vitamin C* or *Loganberries are a good source of vitamin C*
- *Loganberries are a source of folate and niacin*

- Combined statement: *Loganberries are high in/good source of vitamin C and fibre*

Claims that can be made about the four types of rubus:

- *[insert name]berries form part of a healthy diet of fruits and vegetables.*
- *____berries are a natural source of antioxidants*
- *____berries are a natural source of antioxidants that help protect the cells from oxidative damage*
- *____berries is a natural source of antioxidants vitamins C that helps protect the cells from oxidative damage*
- *A natural source of the antioxidants – to help keep your body healthy*
- *Antioxidants in ____berries help protect the body against free radical damage*
- *____berries are high in fibre*
- *____berries are low in salt*

The following claim may be able to be used when the new Nutrition and Health Claims standard comes into force:

- *Eating a diet rich in fruits and vegetables as part of an overall healthy diet may reduce risk for stroke and perhaps other cardiovascular diseases*

It is unlikely that other claims about reduced risk of serious diseases (such cancer or cardiovascular disease) could be made without substantial further research.

Recommendations

- Updated Australian analytical data for Rubus with a particular focus on boysenberries and loganberries.
- A clear portion size measure to allow health claims to be made.
- Consumer research to determine portion size preferences.
- Apply to Food Standards Australia and New Zealand in relation to eligibility to make a folate health claim http://www.foodstandards.gov.au/foodstandards/changingthecode/informationforapplicants/
- Theoretical modelling to determine how the portion size fits within a balanced diet.

BACKGROUND

Horticulture Australia Limited (HAL), in conjunction with the Rubus Industry Advisory Committee is looking to determine and promote the health benefits of Rubus. This project involved a nutritional and regulatory review of four Rubus varieties (Raspberry, Blackberry, Boysenberry and Loganberry), to enable the Rubus Industry to develop nutrition messages and content claims for the fruit.

Berries in general have long been identified as having components that provide health benefits. The genus Rubus, within the family Rosaceae, is one of the largest plant genus containing over 740 subspecies which are divided into approximately 12-15 subgenera.[1] The genus Rubus is native to six continents and can grow in varying locations. The name Rubus refers to a bramble and the health benefits have been noted as far back as the time of Hippocrates (500-370 BCE) in relation to wound healing and childbirth.[1] Australian Aborigines were identified to use large quantities of raspberries for their laxative effect. In the present day the health benefits of Rubus extend to a number of conditions with specific nutrients and bioactive compounds targeted, of which anthocyanins are the most commonly identified.

OBJECTIVES

1. Characterise the nutritional content of Rubus and the extent of scientific research on potential health benefits.
2. Develop recommendations for priorities of the nutritional components in Rubus for potential health claims.
3. Establish guidelines for the Rubus industry on the regulations governing nutrient claims for Rubus and provide suggested areas for further research.

METHODS

1. Literature Review and Recommendations

A review of food composition databases was conducted to determine the existing composition information for Rubus (Raspberry, Blackberry, Boysenberry, Loganberry).

Nutrient data was obtained for:

- Australia
- New Zealand

A review of the scientific literature using defined search terms was conducted to produce a summary of the research on key nutrient and bioactive components of Rubus, namely

a. Raspberry
b. Blackberry
c. Boysenberry [hybrid of a, b]
d. Loganberry [hybrid of a, b, c]

and their health benefits. Using the search terms raspberr*, blackberr*, boysenberr*, loganberr* and rubus the Web of Science and Scopus databases (2001-2011) were searched to obtain English language publications, scientific journal articles and reviews relating to the health benefits of these berries.

The search retrieved 410 documents from Scopus and 137 from Web of Science. Of these 91 were duplicate references. The abstracts of the remaining 456 articles were read to determine suitability and full text articles obtained for those related to the work of this project. Articles specifically focussed on the composition of the berries, antioxidant capacity of the nutrients alone, bioavailability and metabolism of the nutrients, botanical characteristics of the plants, inedible components of the plant and genetic variability of the plant were excluded from the review. Human cellular studies, mouse, rat and hamster animal studies and human participant studies were included. Studies that specifically addressed the nutrient composition of the berries were excluded from the health review though the data was compiled as an adjunct to the food composition data.

All relevant information from each included study was summarised in a tabular format, including Rubus variety; study design; health impact and nutrients affected. Any review articles were printed and used to provide a narrative summary alongside any human and animal studies found. Relevant publications obtained by Horticulture Australia Limited in July 2010 were also included in the review.

An evaluation of the overall findings, grouped by Rubus variety and health condition as well as the array of scientific evidence was determined.

On the basis of these findings, recommendations for the key nutritional components that should be promoted by the Rubus industry were developed based on key nutrients and bioactive components deemed to have the greatest impact (quality and quantity) from the literature review.

2. Regulatory Review and Opportunities

Guidelines from following organisations were reviewed and in combination with the nutritive properties of Rubus, opportunities for label claims were compiled.

- National Food Authority – Guidelines for Food Labelling
- National Food Authority – Code of Practice: Nutrient Claims in Labels and in Advertisements
- Australian Food and Grocery Council – Code of Practice for the Provision of Food Information on Food Labels
- Food Standards Australia New Zealand – Proposal P293 Nutrition, Health and Related Claims,
- Australian Competition and Consumer Commission - Food descriptors guideline to the Trade Practices Act

2.1 NUTRIENT CONTENT OF RUBUS

Rubus are a low energy fruit comprised primarily of natural carbohydrate and dietary fibre, with the main sugar form being fructose. They contain minimal dietary protein and very minor quantities of dietary fatty acids. Rubus contain negligible amounts of cholesterol and long chain omega-3 fatty acids and are also low in natural sodium, though this is dependent on the region of growth. The micronutrient composition sees levels of vitamin C, potassium and folate levels that are able to provide a substantial contributions to the recommended dietary intakes for Australian adults and children. Blackberries also contain higher amounts of beta-carotene and retinol equivalents than Raspberries, Loganberries and Boysenberries, though Blackberries and Raspberries both provide dietary sources of magnesium as well. Table 1 provides the nutrient composition for Raspberries, Blackberries, Boysenberries and Loganberries from Australian sources and New Zealand sources, where Australian data was unavailable.

Table 1: Nutrient composition of fresh Rubus per 100g

Nutrient		Raspberry[2a]	Blackberry[2b]	Boysenberry[3]	Loganberry[4c]
Energy, including dietary fibre	kJ	225	211	184	292.8
	kcal	54	50	44	70
Moisture	g	84.6	84.2	85	84.5
Protein	g	1.2	1.4	1.1	1.5
Fat	g	0.3	0.3	0.7	0.3
Dietary fibre	g	6.1	6.1	3	8.1
Fructose	g	3.8	3.9		
Glucose	g	3.1	3.6		
Sucrose	g	0.1	0		
Maltose	g	0	0		
Lactose	g	0	0		
Total sugars	g	7	7.5	7.1	4.9
Starch	g	0.3	0	0.1	
Mannitol	g	0.1			
Sorbitol	g	0			
Available carbohydrate, without sugar alcohols	g	7.3	7.5		
Available carbohydrate, with sugar alcohols	g	7.4	7.5	7.2	4.9
Lactic acid	g	0	0.1		
Malic acid	g	0.1	0.2		
Acetic acid	g	0	0		
Citric acid	g	2.4	0.4		
Quinic acid	g	0	0		
Calcium (Ca)	mg	28	30	24	27
Copper (Cu)	mg	0.104	0.16		
Fluoride (F)	µg	0	0		
Iron (Fe)	mg	0.6	0.42	0.8	0.6
Magnesium (Mg)	mg	22	30		21
Manganese (Mn)	mg	0.565	0.55		
Phosphorus (P)	mg	37	29	19	26
Potassium (K)	mg	169	114	150	161
Selenium (Se)	µg	1.2	2	0.1	

Sodium (Na)	mg	1	0	3	1
Sulphur (S)	mg	14	16		
Zinc (Zn)	mg	0.36	0.24	0.5	0.3
Iodine				0.2	
Thiamin (B1)	mg	0.037	0.02	0.01	0
Riboflavin (B2)	mg	0.027	0.03	0.02	0
Niacin (B3)	mg	0.36	0.3		0.8
Niacin Equivalents	mg	0.56	0.53	1.1	1.1
Pantothenic acid (B5)	mg	0.39	0.35		
Pyridoxine (B6)	mg	0.05		0.01	
Biotin (B7)	μg	5.7	1.4		
Folate, natural	μg	34	36		
Total folates	μg	34	36	63	34
Dietary folate equivalents	μg	34	36		
Alpha carotene	μg	0	0		
Beta carotene	μg	28	150		
Cryptoxanthin	μg	0	340		
Beta carotene equivalents	μg	28	320	301	25
Retinol	μg	0	0		
Retinol equivalents	μg	5	53	50	4
Vitamin C	mg	32	38	9	22
Alpha tocopherol	mg	0.8	1.4		
Vitamin E	mg	0.77	1.4		
Total polyunsaturated fatty acids (%)	%T	46.2			
Total polyunsaturated fatty acids (g)	g	0.1	0	0.5	
Total long chain omega 3 fatty acids (mg)	mg	0		0	
Cholesterol	mg	0	0	0	

[a] *Rubus idaeus 1995 and 1998,* [b] *Rubus fruticosus 1995,* [c] *1992,1981*

The phenolic compounds are the most commonly studied phytonutrient of Rubus with the differing sub-types addressed in many studies as well. The phenolic composition changes dramatically throughout the growing and ripening stage of Rubus and the types of individual compounds studied also vary widely. Anthocyanins are the most common sub-type of bionutrient studied. This class has been found to provide the red, blue or purple colouring of the berries depending on the overall pH of the fruit. For raspberries (table 2) the total anthocyanin content appears to vary from 2.43mg/100g[5] to 1113.1mg/100g.[6] Anthocyanins are a type of flavonoid compound and therefore, when total flavanol content is presented without a further breakdown, these figures may include the anthocyanin content as well. Raspberries are particularly rich in cyanidin glycosides, are unique for their high ellagitannin content and are one of the main dietary sources of ellagitannins.[7-8] Epidemiological evidence supports anthocyanins for health purposes.[9] Of the individual types of phenolic compounds studied, the one available in the greatest abundance appears to be gallic acid. This phenolic acid has strong antioxidant properties and as a result is often used as a marker (gallic acid equivalents, GAE) when extracts are used for further studies (see section 2.2; Appendix).

Very few studies address other nutrients of Rubus. Those that were identified in this report include phytonutrients[10] which have been identified to have an oestrogenic effect on the body in particular with post menopausal women. The phytonutrient sub-types mimic the actions of natural oestrogen in the body. These nutrients were studied for raspberries but the quantities identified were minute. The values identified for the natural sugars found in raspberries were not dissimilar to those of the Australian food composition databases shown in table 1. The values identified for the percentage fatty acids for raspberries were largely different to those found in the Australian database indicating the importance of using country specific nutrient data.

The natural sugar levels for blackberries (table 3) were seen to be higher for the Australian food composition data than those of the published studies found for this report.[11-12] Similarly, the values for protein were also higher for the Australian data and the values for vitamin C were comparable for one of the two studies found.[11] The anthocyanins were similarly studied for blackberries though cyanidin 3-glucoside appeared to be the compound identified in the greatest abundance. Again, a powerful antioxidant, this anthocyanin is suggested to be higher in blackberries due to the dark colouring of the fruit. The total anthocyanin content appears to vary from 11.08mg/100g[5] to 1660mg/100g[13] showing a general trend toward increased anthocyanin content in blackberries compared to raspberries.

Loganberries were only analysed in two studies. The first addressed the mean anthocyanin content, finding it to be 1126ug/g[14] and the second the mean total phenolic content 429mg/100g.[12] Boysenberry analysis found the mean anthocyanin content of the fruit to be 1514ug/g[14] with proanthocyanins from the juice reaching 1.14mg/100g and 76.3mg/100g from the seeds.[15] The mean phenolic content of boysenberry was higher than for loganberries, reaching 536mg/100g.[12] The total flavanol content was assessed at 1.94mg/100g from the juice and 12.8mg/100g from the seed with the total flavanols analysed reaching 3.26mg/100g for the juice and again higher at 25.9mg/100g for the seed.[15] These limited studies support the findings from the food composition databases in which New Zealand data was used in the absence of Australian data and both datasets similarly contained many gaps in the nutritional data.

Table 2: Summary of nutrient content values for Raspberry from scientific publications

Study	Content
Natural sugars (carbohydrates)	
Cekic et al 2010[16]	Fructose 32.2g/kg, glucose 24.3g/kg, 9.1g/kg sucrose
Gulcin et al 2011 [17]	Total soluble sugars 9.68-11.66%,
Milivojevic et al 2011[18]	Glucose 38.3±1.33mg/g, fructose 31.5±1.14mg/g, sucrose 6.9±0.53mg/g, citric acid 0.15±0.01mg/g, 0.08±0.01mg/g
Total fats and fatty acids	
Ceik et al 2009[19]	% lipid 0.40-0.63%, C14:0 nd-0.33%, C16:0 4.93-9.14%, C18:0 nd-1.18%, total saturated fatty acids 5.97-9.73%, C16:1 0.54-1.47%, C18:1 10.78-14.60%, C20:1 0.19-0.45%, C24:1 nd-0.17%, total monounsaturated fatty acids 12.09-15.82%, C18:2 42.18-52.61%, C18:3 18.40-24.10%, C20:3 0.72-1.69%, total polyunsaturated fatty acids 66.99-75.91%
Protein	
Halbwirth et al 2009[20]	Protein 0.15-1.00ug/uL
Minerals	
Hegedus et al 2008 [21]	Aluminium 1.02-1.17mg/100g, Boron 0.16-0.21mg/100g, Barium 0.03-0.06mg/100g, Calcium 29.88-39.90mg/100g, Copper 0.09-0.11mg/100g, Iron 0.52-0.62mg/100g, Potassium 153.42-171.84mg/100g, Magnesium 17.56-22.17mg/100g, Mangenese 0.16-0.28mg/100g, Sodium 3.86-5.06mg/100g, Phosphorus 29.18-35.06mg/100g, Zinc 0.27-0.29mg/100g
Vitamins	
Straksjo et al 2003[22]	Folate 46ug/100g
Beekwilder et al 2008[23]	Alpha-tocopherol 4.3mg/kg, delta-tocopherol 5.1mg/kg, gamma-tocopherol 5.8mg/kg
Bordes et al 2010[24]	Vitamin C 1014±30nmol/g
Gulcin et al 2011 [17]	Alpha-tocopherol 3.1-8.9mg/kg, ascorbic acid 2.4-5.34mg/kg
Mullen et al 2002 [25]	Vitamin C 672±11 (fresh), 671±14 (frozen)
Carotenoids	
Beekwilder et al 2008[23]	Lutein 2.8mg/kg, beta-carotene <0.01mg/kg, alpha-caotene 0.44mg/kg, beta-ionone 1.72mg/kg, alpha-ionone 0.62mg/kg (stage 5/ripe)

Marinova et al 2007[26]	Lutein 320±37ug/100g, Zeaxanthin 11±2.6ug/100g, beta-cryptoxanthin 5.9±1.5ug/100g, alpha-carotene 24±1.9ug/100g, beta-carotene 9.3±3.3ug/100g, total carotenoids 370ug/100g
Anthocyanins (flavonoids)	
Hager et al 2008[6]	Total monomeric anthocyanins 1113.1±30.7mg/100g (fresh), 425±9.9mg/100g (juice non pasturised), 342±14.2 (juice pasturised), 14.0±0.6mg/100g (sediment)
Heinonen et al 2007[27]	Cyanidin-3-glucoside 15.6%, cyaniding-3-sophoroside 5.3%, cyaniding-3-arabinose 59.4%, pelargonidin-3-glucoside 0.9%, perlargonidin-3-rutinoside 0.7%, perlargonidin-3-sophoroside 2.5%
Jakobek et al 2007 [5]	Total anthocyanins 242.90±3mg/kg
Jacobek et al 2009 [28]	cy-3-sopho 620.1±4mg/kg, cy-3-glu 152.1±1, total anthocyanins 772.2±5mg/kg
Liu et al 2002[29]	Anthocyanin 0.17-57.60mg/100g
Mullen et al 2002 [25]	Total anthocyanin 1037±8nmol/g (fresh), 1049±14 (frozen) nmol/g, total anthocyanins 70±21g/100g (fresh), 782±31g/100g (frozen),
Proteggente et al 2002[30]	Cy-3-glu 5.66±0.18mg/100g, cy-3-rut 0.82±0.02mg/100g, cy-3-sop 22.42±0.60mg/100g, p-coumaric glucose 2.87±0.17mg/100g
Scalzo et al 2008[14]	Mean anthocyanin 1126-3814,ug/g
Sparzak et al 2010[31]	Cy-3-O-soph 22.74-80.03mg/100g, cy-3-O-2G-O-glu 9.88-53.09mg/100g, cy-3-O-glu 15.02-76.12mg/100g, total anthocyanin 76.22-277.06mg/100g
Phenolic compounds	
Bordes et al 2010[24]	lambertanin C 322±4nmol/g, sanguiin H-6 1030±107nmol/g, ellagic acid 11±1nmol/g [phenolic compounds]
Cekic et al 2010[16]	Total phenolics average 2046ug GAE/g
Gulcin et al 2011 [17]	Total phenolic acids 5.83-26.66%, , caffeic acid 2.41-5.31mg/kg, ferulic acid 4.90mg/kg, syringic acid 0.1-7.32mg/kg, ellagic acid 1.01-10.92mg/kg, gallic acid 3.0-14.6mg/kg
Hager et al 2010[32]	Total ellagitannin 23.34±2.01mg/100g (fresh), 35.16±8.18mg/100g (quick frozen), 39.48±1.42 (canned in syrup), 44.39±1.18mg/100g (canned in water), 32.24±6.39 (pureed), 10.41±0.39 (non-clarified juice), 6.39±0.51 (clarified juice)
Jakobek et al 2007 [5]	Total polyphenols 1256.16±133mg/kg
Levj et al 2010[33]	Catechin 11.10±0.95mg/L, epicatechin 13.6±1.11mg/L, total flavonols 24.6mg/L, quercitin 1.18±0.15mg/L, chlorogenic acid 20.20±2.15mg/L, caffeic acid 14.20±0.95mg/L, p-coumaric acid 8.96±0.71, ferulic acid 4.19±0.12, gallic acid 13.10±1.02mg/L, p-hydroxybenzoic acid 19.10±1.15mg/L, ellagic acid 163.20±7.25mg/L, total phenolic compounds 268.70mg/L
Liu et al 2002[29]	Total phenolics 3.59-512mg/100g, 63.5-103.4mg/100g
Maatta et al 2004[34]	p-coumaric acid 1-2mg/kg, p-coumaroyl esters 7-13mg/kg, caffeoyl esters 1-6mg/kg, galloyl esters 5mg/kg, ellagitannins 977-1560mg/kg, ellagic acid 12-112mg/kg, catechin 2 mg/kg, epicatechin 3-11mg/kg, quercitin-3-glu 1-5mg/kg
Mullen et al 2002 [25]	Total phenolics 3383±230g/100g (fresh), 3321±103g/98g, ellagic acid 3.5±0.1g/100g (fresh), 3.9±0.1 g/111g (frozen)
Flavanols	
Gulcin et al 2011 [17]	quercitin 80.0mg/kg
Bordes et al 2010[24]	quercetin-3-O-glucoside 28±4nmol/g
Gulcin et al 2011 [17]	Total flavonoids 1.77-6.09%, quercitin 80.0mg/kg, catechol 2.12-12.1mg/kg, progallol 2.02-9.7mg/kg, vanillin 3.0-4.41mg/kg, p-coumaric acid 67.03-2792.60mg/kg,
Jacobek et al 2009 [28]	p-coumaric acid 0.5±0.1mg/kg, ferulic acid 0.9±0.1mg/kg, ellagic acid 22.7±1.8mg/kg, catechin 14.9±1.3mg/kg, epicatechin 18.1±1.5mg/kg, quercetin 0.3±0.1mg/kg, total flavonols 57.4±4.9,
Samappito et al 2010[35]	Catechin 125.63±1.08mg/100g, epicatchin 74.61±2.35mg/100g, rutin 20.45±0.44mg/100g, myricetin 0.74±0.11mg/100g, resveratrol 5.88±0.10mg/100g, quercetin 6.92±0.11mg/100g, naringenin 0.90±0.01mg/10g, kaempferol 0.34±0.11mg/100g
Phytoestrogens	
Bartkiene et al 2011[10]	Enterodiol 191.9nmol/g, Enterolactone 55.6 nmol/g, Total enterolignans 227.9nmol/g (dry weight)

Table 3: Summary of nutrient content values for Blackberries from scientific publications

Study	Content
Natural sugars (carbohydrates)	
Cisse et al 2009[11]	Glucose 228±10g/kg, fructose 228±10g/kg, sucrose 8±1g/kg
Hassimoto et al 2008 [12]	Moisture 90.88-93.25%, carbohydrate 3.07-5.92%, glucose 1.69-2.60g/100g, fructose 1.15-2.11g/100g, sucrose nd-0.7g/100g, total soluble sugars 2.75-4.78g/100g
Protein	
Halbwirth et al 2009[20]	Protein 0.12-0.72ug/uL
Vitamins	
Cisse et al 2009[11]	Ascorbic acid 44±2mg/100g [antioxidants]
Hassimoto et al 2008 [12]	Vitamin C 9.9-15.6mg/100g [antioxidants]
Carotenoids	
Marinova et al 2007[26]	Lutein 270±33ug/100g, zeaxanthin 29±0.8ug/100g, beta-cryptoxanthin 30±3.7ug/100g, alpha-carotene 9.2±0.7ug/100g, beta-carotene 100±13ug/100g, total carotenoids 440ug/100g
Anthocyanins (flavonoids)	
Cisse et al 2009[11]	Cyaniding 3-glucoside 1189±55mg/L, d
Hassimoto et al 2008 [12]	Anthocyanins 116.0-194.0mg/100g
Jakobek et al 2007 [5]	Total anthocyanins1108.87±6mg/kg
Jakobek et al 2009 [28]	Total anthocyanins 976.5±5mg/kg, cy-3-glu 976.5±5mg/kg
Mertz et al 2007[36]	cy-3-glu 380-680mg/100g, cy-3-rut nd-630mg/100g, lambertianin 520-598mg/100g, sanguiin 420-2450mg/100g
Mi et al 2004[37]	Cyanidin 3-glucoside 852.1-1583.3mg/kg, cy-3-rut 13.4-138.1mg/kg, cy-3-xyl 46.0-178.1mg/kg, cy-3-mal 23.8-55.0mg/kg, cy-3-dio 35.7-193.4mg/kg, total anthocyanins 1143-2415mg/kg
Reyes-Carmona et al 2005[13]	Total anthocyanins 8.0-16.6mg/g
Scalzo et al 2008[14]	Mean anthocyanin 1499ug/g
Siriwoharn et al 2004[38]	Total anthocyanins 131-221 mg Cy-3-glu/100g (ripe)
Phenolic compounds	
Connor et al 2005* [39]	Total phenolic mean content 373-513mg/100g
Hassimoto et al 2008 [12]	Total phenolics 341-499mg/100g
Jakobek et al 2007 [5]	Total polyphenol 2484.87±234mg/kg [polyphenols]
Mi et al [40]	Total pheolics 2922-4440mg/kg
Reyes-Carmona et al 2005[13]	Total phenols 14.2-35.1umol CE/g [phenolic compounds]
Siriwoharn et al 2004[38]	Total phenolics 903-960 mg GAE/100g (ripe) [phenolic compounds]
Vasco et al 2009[41]	Ellagitannins and ellagic acid derivatives 3547±659mg EAE/kg [phenolic compounds]
Zadernowski et al 2005[42]	Total phenolic acids 3255.2±160.0mg/kg
Flavanols	
Hassimoto et al 2008 [12]	Quercitin 7.8-19.0mg/100g, cyanidin 91.0-157.0mg/100g, epicatechin 20.7-63.0mg/100g, total flavonoid 123.29-233.34mg/100g [flavanols]
Jakobek et al 2009 [28]	ellagic acid 19.5±0.7mg/kg, catechin 1.9±0.1mg/kg, epicatechin 24.6±1.35mg/kg, quercetin 1.8±0.2mg/kg, total flavonols

	63.8±3.4
Mertz et al 2007[36]	Gallic acid 0.46-1.8mg/100g, caffeoyl esters 1-2.6mg/100g, p-coumaroyl esters 4.2-6.3mg/100g, feruloyl esters 1.54-3.8mg/100g, epicatechin 5.1-6.3mg/100g, ellagic acid nd-2mg/100g, quercitin glucurnide 51-57mg/100g [phenolic compounds].
Mi et al [40]	Quercitin-3-rut 7.3-20.2mg/kg, quercitin 3-gal 9.0-49.3mg/kg, quercetin 3-meth 4.1-17.2mg/kg, quercetin 3-glu 15.1-41.5mg/kg, quercetin 3-b-galac 12.8-41.9mg/kg, quercetin 3-gluco 3.9-4.5mg/kg, quercetin 1.89.6mg/kg, total flavonols 99-150mg/kg
Zadernowski et al 2005[42]	Gentisic acid 136.6±10.8mg/kg, gallic acid 89.0±5.0mg/kg, o-pyrocatechuic acid 0.2±0.1mg/kg, protocatchiuic acid 129.5±10.5mg/kg, salicylic acid 524.1±79.0mg/kg, vanillic acid 45.1±5.3mg/kg, caffeic acid 105.5±4.0mg/kg, m-coumaric acid 596.6±75.1mg/kg, 3,4-dimethylxycinnamic acid 501.9±7.3mg/kg, fruilic acid 55.3±7.5mg/kg, hydroxycaffeic acid 627.6±75.0mg/kg, sinapic acid 627.6±7.0mg/kg

* study contains nutrient analyses from New Zealand grown berries

2.2 LITERATURE REVIEW: RUBUS AND HEALTH

The literature search revealed an abundance of studies (34) relating to Cancer for the Rubus types addressed in this project. Of those studies, 23 were focussed on raspberries and 11 were focussed on blackberries. Other health conditions addressed were Ageing (2 raspberry, 1 blackberry, 1 boysenberry), Inflammation (3 raspberry, 3 blackberry), Wound healing (1 raspberry), Oxidative stress (1 boysenberry), Vascular disease (1 raspberry, 1 blackberry), Obesity (1 raspberry), Osteoporosis (1 raspberry), Fluorosis (1 blackberry), Oesophagitis (1 raspberry), Liver failure (1 raspberry), Kidney disease (1 raspberry). The types of studies identified saw no studies related to Loganberries and only two studies related to Boysenberries. This suggests that these varieties may have been grouped with blackberries due to their hybrid nature or they are understudied in the literature overall. It is felt that the later is most likely. The quality of the studies identified, were also of a low quality in terms of the level of evidence provided by them. In total 37 studies were in vitro studies using cellular approaches, 15 were animal model studies and only 2 studies included humans and were limited in their size (10 subjects or less) limiting the power of any observations. A summary of the studies assessed is provided in table 5 in the Appendix of this report.

Rubus varieties have been both a food and medicine for humans throughout history. Traditional medicinal uses generally focus on the astringent action of the whole plant (fruit, stem, leaf, root) for treatments for wounds, treatment for and prevention of stomach aches, and treatment for 'stopping excess fluxes of bodily fluids', droopy eyes, mouth sores, haemorrhoids and snakebites. Raspberries, in particular have been a traditional treatment for diarrhoea, relief from morning sickness and other pregnancy/childbirth related issues.[1] The astringent raspberry was also recommended for fevers, ulcers, putrid sores of the mouth, tuberculosis, kidney stones and heavy menstrual flow.[43] The fruit, leaf and root of blackberries are traditionally used by the residents of Southern Appalacia, USA, to treat nausea and diarrhoea.[7] Despite these many traditional uses the literature of the past 10 year period did not address any of these uses.

When considering the primary nutrient composition of the Rubus types, absorption of the anthocyanins may be influenced by other foods consumed at the same meal. Alcohol has been identified to have little or even a detrimental effect, while a high fat diet may actually improve absorption.[44] It is suggested that anthocyanins absorbed from the stomach, are not circulated through the bile and long term exposure to the fruit in the longer term may be required for anthocyanins to be found in the bodily tissues.[45] Anthocyanins have been found to cross the blood-brain barrier and accumulate in areas of the brain according to different phytochemical profiles.[7] Determining dosages is difficult for any particular berry as the indication is that bioavailability is different between flavonoids and different forms of berry product, and also degradation of the nutrients are of concern.[46]

Evidence suggests further research, including a focus on nutrigenomics and nutrigenetics, is required, with well designed studies to investigate the potential for prevention and treatment of chronic disease in humans and animals.[47] The following sections provide an overview of the literature identified for specific health conditions.

2.2.1 Rubus and Cancer

This report found cancer to be the most commonly addressed health condition with raspberries the most commonly used Rubus (20 of 31 studies). The majority of these studies were supportive to the effects of raspberries or blackberries on cease of cell proliferation of cancer models. One in vitro study for breast cancer was not supportive and one in vitro study for leukaemia was not supportive for raspberry only. Colon cancer, followed by breast/mammary, lung, prostate and liver cancer were the most commonly addressed with studies for colon and breast comprising more than 50% of the studies in total. The incidence, size and multiplicity of the cancer were the most commonly addressed outcomes and when considering the levels of evidence of the studies overall, the animal studies appeared to show a slight decrease in size of tumours and multiplicity, found in both red and black raspberry and blackberry studies though only four were identified and ranged from 20 to 33 weeks in duration. Studies used powdered and frozen berry extract with the doses varying widely. The positive findings of these studies suggest that further work is needed using animal in vivo models to increase the strength of the evidence available. The array of in vitro cellular studies similarly addressed proliferation and multiplicity of the cancer cells as well as the viability.

Raspberry phytochemicals have been shown to reduce cancer cell growth in vitro by down-regulating cyclooxgenase-II (COX) expression and enzyme activity. A review paper identified the use of black raspberries in a topical manner four times daily in relation to oral lesions. The study felt that the COX expression and inhibition of cell death was seen after a 6 week period.[45] Following on from the COX-II mechanism further animal studies support the use of berry extract in a cancer cell model. [48] The results of a study using red raspberries demonstrated that the extract can decrease hepatic lesions and nodule incidence in a Diethylnitrosamine (DEN, carcinogen) induced rat model, in a dose-dependent manner. For a period of 20 weeks the low (0.75g), medium (1.0g) and high (3.0g/kg/d) of fresh red raspberry were fed to the rats. The high dose red raspberry extract showed decreased hepatocellular adenocarcinoma, a type of highly malignant tumour, which is also the main cause of death in hepatic cancer patients. No toxicity was found from administration of the berries. The authors suggest that the red raspberry may be useful for chemoprevention or therapeutics in relation to hepatic cancer and that the liver metabolism of the anthocyanins is related to its effect.

An animal study with rats investigating black raspberry, ellagic acid and mammary cancer in comparison with blueberry determined that the level of ellagic acid in this black raspberry diet was about 8 times less than pure ellagic acid, yet it yielded the same response to the cancer.[49] The ellagic acid on its own was able to produce the greatest result in reduction of tumour volume (75%), followed closely by the black raspberry (>69%), while the blueberry was only able to reduce the volume by 40%. This suggested that ellagic acid is more bioavailable in black raspberry or that other components that are present in the berries such as anthocyanins and flavanoids may work together to provide better protection. The blueberries tested, are also high in anthocyanins but lower in ellagic acid and were able to produce moderate reductions in the oestrogen mammary carcinogenicity as well as providing a significant reduction in morbidity. Another study using black raspberries investigated the colon cancer rat model. Powdered berry was used at 2.5%, 5% and 10% concentration mixed into the animals diets by weight. Total tumour multiplicity declined up to 71% and tumour burden declined by 75%, though the finding was not significant. The use of whole fruit in comparison to single nutrients was felt to affect the results. Studies addressing calcium, ellagic acid, beta-sitosterol, and ferulic acid are tested in much higher levels that those found in the berry powder used in this study, however the existence of multiple protective compounds in the berries was suggested to provide multiple chemopreventive effects.

The final animal study identified in this review tested an isolated bionutrient, Cyanidin-3-glucoside, from blackberries.{Ding, 2006 #300} The extract was used topically in a skin cancer mouse model in which the skin was pre-treated topically with the anthocyanin 30 minutes before applying the carcinogen for 21 weeks. The application was found to reduce the number of non-malignant and malignant skin tumours when compared with the control group. The anthocyanin used in this study represents over 80% of the anthocyanins found in blackberries and also suggests a chemopreventive role. Unfortunately the application within this study was topical only and further studies would be required to determine if oral consumption has the same preventive capacity.

2.2.2 Rubus and Ageing

Although berries in general have been studied widely for their effects on ageing,[46] the Rubus berries addressed in this review have not been addressed as thoroughly. Two animal studies were identified one using black raspberries and the other blackberries. Both followed eight weeks in duration. The raspberry study targeted age related oxidative stress and compared the outcomes with other traditional medicines including Chinese lizard's tail and Korean red ginseng.[50] The diets of young and old rats were standardised and the extract added in place of sucrose. The balance between oxidative stress and defence was measured as this may have an impact on a number of health conditions including cardiovascular disease and cancer. This was assessed using lipid oxidation, protein oxidation and DNA damage. At 1% concentration a decrease in the plasma 8-iso-PGF2-alpha (a free radical formed from lipid peroxidation) concentration was identified though no significant dose dependant relationship was identified for the raspberry extract. The extract also increase the levels of circulating plasma alpha-tocopherol indicating that it may preserve the levels of circulating antioxidants or reduce the levels of lipid peroxide in the body. The biomarker studied for the protein oxidation was found to be similar for both the young and the older rats though no significant results were found for the raspberry extract. Similar outcomes were also identified for the DNA damage analyses. The dose of extract given to the rats was calculated to be equivalent to 5-10g of extract given to humans.

The second rat study used a modified version of the NIH-31 diet with 2% blackberry extract added to it. The control group received the same but with corn in place of the extract. [51] A range of psychomotor tests were employed which included rod walking, wire suspension, plank walking, incline screen and accelerating rotarod to test behaviours including balance, coordination, muscle tone, fatigue, stamina and reflexes. A range of cognitive tests were also employed including a Morris water maze. This age sensitive test can detect spatial learning, working and reference memory and navigation. The rats fed the blackberry extract performed better for the activities for the behavioural tests. There was no difference between the groups for the cognitive tests, though an improvement in memory, double that of the control group was observed. The authors suggest that the anti-inflammatory and antioxidant properties of the blackberry may work on the pathways in the brain to improve behaviour. The authors report of another study in which the anthocyanins from the blackberries were able to enter the rat brains within 15 days of consumption of a 1.5% blackberry diet[52] and excretion of the anthocyanins in the urine after 8 days of a 20% blackberry diet.

2.2.3 Rubus and Inflammation

Several *in vitro* studies have suggested anti-inflammatory properties but often these results are not translated to *in vivo* studies. This may be due to low bioavailability of the elligitannins and ellagic acid.[44, 47] Six studies relating to inflammation were identified for this report. One animal study using a rat model was identified, testing acute lung inflammation,[53] Using the extract from 2kg fresh ripe blackberries and given in doses of 10 and 30mg/kg intraperitoneally (injected into the body cavity) 30 minutes before injection with carrageenan to induce pleurisy. Biomarkers of peroxidation were taken as well as lung biopsies. The chemical was found to create significant damage to the lungs with oedema also prominent. Biomarkers of peroxidation were evident after chemical treatment though decreased in a dose dependant manner when the blackberry anthocyanin extract was employed. The authors propose that the mechanism of action for the protective nature of the extra is via a reduction in the peroxynitrite formation by removing the oxygen free radical before it can react with nitrous oxide.

2.2.4 Rubus and conditions of the Metabolic syndrome

Black raspberry may be effective in reducing vascular endothelial growth factor and raspberry phytochemicals have shown potential to improve glucose control in people with diabetes by inhibiting carbohydrate digestion.[45] The high antioxidant content of Rubus also flags it in relation to oxidative stress. A rat study using a chow based, soy based and fish oil based diet and given either 2 or 10% of boysenberry extract.[54] Biomarkers of oxidative damage, plasma oxidative status as well as plasma antioxidant status were assessed. The soy diet saw a reduction in the MDA biomarker for both doses of extract, no differences were seen for the chow diet and the fish oil diet saw an increase in the MDA biomarker for those fed the 2% dose. The authors suggest this to be a pro-oxidant effect. Similarly, those on the fish oil diet also saw a higher total plasma antioxidant capacity than both the chow and soy diets before the addition of the boysenberry extract and no change after it was added. The plasma vitamin E content was again found to be the highest for the fish oil and lowest for the soy diet with a significant increase in plasma vitamin E seen after the addition of 10% treatment. These findings suggest that the composition of the whole diet need to be considered for boysenberry extract to work effectively as an antioxidant.

The antioxidant function in relation to atherosclerosis tested in a hamster model against other high antioxidant beverages. The raspberry juice as a dose of 0.6g berry/mL was tested for its effect on the aortic fatty streak, plasma total cholesterol, HDL cholesterol, LDL cholesterol and VLDL cholesterol. The animals were fed the juice by gavage daily for 12 weeks to an amount equating to 275 ml/day for a 70 kg human. The study found the fatty streak to be reduced the most by the raspberry juice in comparison to the other juices tested but less than the black and green teas tested. These findings were not accompanied by lower plasma cholesterol levels seeing no differences between groups. Another study using a mouse model also investigated the impact of raspberries on cholesterol levels though under obesogenic conditions.[55] This study aimed to investigate the effects of purified anthocyanins obtained from freeze dried black raspberry powder on the development of obesity. The composition of the anthocyanins obtained were found to be 17.94% Cyanidin-3-glucoside, 22.55% Cyanidin 3-sambubioside-5-rhamnoside, 57.99% Cyanidin 3-rutinoside and 1.52% Pelargonidin 3-rutinoside totalling 4.16mg/g of extract. The mice were placed on a control (10% energy from fat), low fat + 10% extract and compared against blueberry, strawberry, grape and a high fat (45% energy from fat) control, high fat+10% extract from each of the berry types. Body weight, total triglyceride, total cholesterol, and glucose were measured alongside serum cytokines, TNF-alpha and plasminogen activator inhibitor. The mice on the high fat diet were found to gain weight in comparison to the control mice, though when the raspberry extract was added and also when the grape extract was added the weight gain exceeded that of the high fat control independent of energy or food intake changes. Both of these berries do have higher levels of Cyanidin-3-glucoside than the other berries which may have impacted upon the findings. It has been suggested that acylated cyanidins are not absorbed as well as glycoside cyanidins. Such findings for weight gain were not seen for the low fat diet. Serum triglyceride levels were also increased for both the low and high fat diets for raspberry and blueberry only, Serum total cholesterol, glucose and insulin levels were not significantly different for either of the diets or the berries tested. The authors conclude that the rate of absorption, other nutrient interactions within the berry and form of the anthocyanins in the berry may be reasons for the results.

2.2.5 Rubus and Other health conditions

There are few reports of raspberries or blackberries causing allergies. This may be due to general low allergenicity of the berry, the relatively small amounts consumed or the infrequency of consumption. Inhalation of frozen raspberry powder has been found to cause occupational asthma.[56] A study also found an anti-allergic response of rats when given black raspberry extract at a 0.001-1g/kg dose.[57] Topic application of red raspberry extract to combat hair growth and skin elasticity in persons suffering with alopecia found increases hair growth when applied for a period of 5 months and skin elasticity after 2 weeks at 0.01% concentration.[58] Raspberries were given to rats with induced oesophagitis at a level of 2.5%w/w.[59] There was found to be no increased in lipid peroxidation levels and the addition of the supplement was not able to reduce the oesophagitis or the effects on antioxidant enzymes beyond 2 weeks. Red raspberries were also studied for their role in liver damage [60] and kidney function [61]

The impact of blackberry juice in fluorosis was also investigated in a rat model.[62] A dose of 1.6g/kg body weight was administered to the rats for a period of 5 weeks. Total blood lipids, total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides were measured from the rat livers to determine the level of sodium fluoride induced free radical generation. The blackberry juice resulted in decreases in the enzyme activity resulting from fluoride intoxication. The lipid and cholesterol levels were not affected by the blackberry juice on its own, though in the presence of induced fluorosis, the juice was able to return the levels to their normal levels as fluorosis had caused a negative response to all levels tested. The authors recommend avoidance of sodium fluoride in food and water to avoid the peroxidation resulting from its consumption.

2.3 OVERVIEW OF CURRENT AUSTRALIAN REGULATIONS

2.3.1 Food Standards Australia New Zealand

Australia regulates health, nutrition and related claims for food, made both on labels and in advertising, through an integrated system involving agencies at a national and state level and industry codes of practice. State governments police the compliance of the food standards developed by the bi-national statutory agency, Food Standards Australia and New Zealand (FSANZ)[63].

These food standards cover:

- general labelling provisions about the ingredients in foods – Standard 1.2.4
- the elements in the mandatory nutrition information panel (NIP) that must appear on all packaged food as well as regulations on additional voluntary and claims about the presence or absence of nutrients – Standard 1.2.8
- the special labelling requirements for foods requiring pre-market approval (eg novel foods, foods with genetically modified ingredients, or irradiated foods) – Standards 1.5.1-3
- health claims for foods - Standard 1.1A.2

Currently in Australia and New Zealand nutrient content claims are permitted provided that the food item meets the requirements. These claims are:

1. a 'source' claim: the food provides at least 10% of recommended dietary intake (RDI) per serve (eg. *Loganberries are a source of folate*).
2. a 'good source/high in' claim: the food provides at least 25% of recommended dietary intake (RDI) per serve (eg. *Raspberries are a good source/high in vitamin C*).

FSANZ has defined the RDI values for labelling purposes in Standard 1.1.1.

General Level Health Claims - do not reference a biomarker (eg, blood cholesterol or blood pressure) or a serious disease or condition and include content claims, function claims, enhanced function claims and risk reduction claims that reference a non-serious disease or non-serious condition. There are three types of general level claims:

a) Nutrient Function Claims – which describe the biological role of a food or energy or a nutrient [or a biologically active substance] in normal growth, development, maintenance and other like functions in the body (e.g. "*Iron helps transport oxygen around the body")*

b) Enhanced Function Claims - which describe the biological role of a food or energy or a nutrient [or a biologically active substance] beyond normal growth, development, maintenance and other like functions of the body (e.g., "*XX can help improve concentration")*

C) Risk Reduction Claims in relation to a non-serious disease or condition - which describe the biological role of a food or energy or a nutrient [or a biologically active substance] in significantly reducing the risk of developing a non-serious disease or condition (e.g., "*XX may help reduce the risk of acne")*

In the original proposed new framework, food companies would not need pre-approval to make general level claims but must use either a FSANZ model list of approved statements, or provide suitable scientific texts or dietary guidelines to support the claim, or must hold scientific evidence to substantiate such claims and produce this evidence, on request, for enforcement agencies.

The following sites provide guidance as to the sort of general level claims that are likely to be acceptable:

- Dietary Guidelines for Australian Adults
  National Health and Medical Research Council.
  http://www.nhmrc.gov.au/publications/synopses/dietsyn.htm
- Generic Health Claims

Joint Health Claims Initiative (JHCI), United Kingdom
http://www.jhci.org.uk/

- Health Claims in the Labelling and Marketing of Food Products, The Food Sector's Code of Practice Swedish Nutrition Foundation.
  http://www.snf.ideon.se/snf/en/rh/Healh_claims_FF.htm
- Health Claims that meet Significant Scientific Agreement (SSA)
  Food and Drug Administration, USA
  http://www.fda.gov/food/labelingnutrition/labelclaims/healthclaimsmeetingsignificantscientificagreementssa/default.htm
- Health Claims based on an Authoritative Statement of a Scientific Body
  Food and Drug Administration, USA
  http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodLabelingNutrition/ucm056975

High level claims[64] – are claims which reference a biomarker or a serious disease or condition and include biomarker maintenance claims, biomarker enhancement claims and risk reduction claims which reference a serious disease or condition

a) Biomarker Maintenance Claims – describe the biological role of a food or energy or a nutrient [or a biologically active substance] in maintaining a normal level of a recognized biomarker (e.g., *helps maintain normal blood cholesterol*)
b) Biomarker Enhancement Claims – describe the biological role of a food or energy or a nutrient [or a biologically active substance] in reducing or increasing the level of a recognized biomarker (e.g., *helps reduce cholesterol*)
c) Risk Reduction Claims which reference a serious disease or condition - describe the biological role of a food or energy or a nutrient [or a biologically active substance] in significantly reducing the risk of developing a serious disease or condition (e.g., *helps reduce the risk of cardiovascular disease*)

2.3.2 Australian Competition & Consumer Commission (ACCC)

The ACCC has responsibility for enforcement of the Trades Practices Act, which generally prohibits any form of false or misleading claims in relation to all products on sale or in advertising, including food. The ACCC has developed guidelines related to a number of issues concerning food in their publication *Food descriptors guideline to the Trade Practices Act*[65]. However, the ACCC takes a wide view of evidence as to whether a claim on labels or in advertising is misleading, and impressions provided by graphics and design can be taken into account, not just the accuracy of specific text. The ACCC does not necessarily accept guidelines from FSANZ when deciding what are false or misleading representations. For example, previously FSANZ had allowed claims of 'fat free' on products that contained minimal levels of fat (<0.15%). The ACCC has taken a more rigorous interpretation, and decided that unless a product contains zero fat, claims of 'fat free' would be false and misleading.

2.3.3 Australian Food and Grocery Council (AFGC)

The AFGC has produced a voluntary code of practice on the provision of information on food products[66]. This incorporates a code of practice on nutrient claims that was earlier developed by the National Food Authority, which sets the levels of nutrients required in foods to be able to make claims about high, low, reduced or increased levels of nutrients, or comparisons between products[67]. In general products need to have a difference of at least 25% from comparison products to be able to make claims of nutritional superiority.

The fact that this guide is only voluntary has led to a considerable amount of non-compliance – particularly for claims about "% fat free". As a result, it is proposed that all claims for nutrient content will be included in black letter law of the new food standards on nutrition, health and related claims.

2.4 HEALTH CLAIMS

2.4.1 Nutrient Claims

The current Food Standards Code summarises the regulations on nutrient claims in Standards 1.2.8 (FSANZ 2002) with explanatory guidance about this in the Users Guide [68 69]. The definition of a **nutrition claim** is:

a representation that states, suggests, or implies that a food has a nutritional property whether general or specific and whether expressed affirmatively or negatively, and includes a reference to:

energy, or
salt, sodium or potassium, or
amino acids, carbohydrate, cholesterol, fat, fatty acids, fibre, protein, starch, or sugars, o
vitamins or minerals, or
any other nutrient, or
a biologically active substance.

A nutrient claim (and values declared in the Nutrition Information Panel) must be based on the "average" level of the component in that product. This can be determined in a variety of ways:

a) Laboratory analysis
b) Food composition tables or databases, or
c) Calculation from the actual ingredients used in a product (eg a recipe)

It is likely that other nutrients (such a phosphorus, zinc or calcium) would not be present in the levels needed to make a claim.

Nutrient claims relate to particular nutrients that are recognised as essential for normal health. They may be quantitative (eg, high in fibre, low in salt) or qualitative (eg, polyunsaturated). Other nutrition claims relate to bioactive substances that may offer particular health benefits (eg, isoflavones), or the general physiological effects of the food (eg, glycaemic index).

In addition to nutrition claims, there are a number of nutrition related product description claims that describe the presence or absence of additives (eg, free of artificial colours), ingredients (eg,lactose free, GM free), make environmental claims (eg, organic, free range) or provide qualitative descriptions of the food (eg, wholegrain, natural).

Table 4: Comparison of nutrient data with recommended dietary intake (RDI) values for 100g serving

Nutrient		RDI*	Blackberry	%RDI	Raspberry	%RDI	Boysenberry	%RDI	Loganberry	%RDI
Energy	kJ			211		225		184		292.8
Saturated fat	g	**1.5**	0.00	0	6.67	0.1		T	0.00	
Dietary fibre	g	**3**	**203.33**	6.1	**203.33**	6.1	**100**	3	**270.00**	8.1
Magnesium	mg	320	9.38	30	6.88	22	0	-	6.56	21
Phosphorus	mg	1000	2.90	29	3.70	37	1.9	19	2.60	26
Sodium	mg	**120**	0.00	0	0.83	1	2.5	3	0.83	1
Copper	mg	3	0.00	-	0.00		0	-	0.00	-
Manganese	mg	5	**11.00**	0.55	**11.30**	0.57	0	-	0.00	--
Zinc	mg	12	2.00	0.24	3.00	0.36	4.17	0.5	2.50	0.3
Vitamin C	mg	40	**95.00**	38	**80.00**	32	**22.5**	9	**55.00**	22
Thiamin	mg	1.1	1.82	0.02	3.36	0.04	0.91	0.01	0.00	0
Riboflavin	mg	1.7	1.76	0.03	1.59	0.03	1.18	0.02	0.00	0
Niacin	mg	10	5.30	0.53	5.60	0.56	**11**	1.1	**11.00**	1.1

Pantothenate	mg	5	7.00	0.35	7.80	0.39	0	-	0.00	-
Vitamin B6	mg	1.6	0.00	-	3.13	0.05	0.63	0.01	0.00	-
Folate Equiv.	µg	200	**18.00**	36	**17.00**	34	**31.5**	63	**17.00**	34
Retinol Eq.	µg	750	7.07	53	0.67	5	6.67	50	0.53	4
Beta-carotene Eq.	µg	750	**20.00**	150	3.73	28	0		0.00	
Vitamin E	mg	10	**14.00**	1.4	7.70	0.77	0		0.00	

*Values shown in bold are those considered a source of the nutrient at minimum. * from standard 1.1.1*

Table 5: Comparison of nutrient data with recommended dietary intake (RDI) values for 150g serving

Nutrient		RDI*	Blackberry	%RDI	Raspberry	%RDI	Boysenberry	%RDI	Loganberry	%RDI
Energy	kJ		316.5		337.5		276		439.2	
Saturated fat	g	**1.5**	0	0.00	0.15	10.00	-	-	0	0.00
Dietary fibre	g	**3**	9.15	**305.00**	9.15	**305.00**	4.5	**150**	12.15	**405.00**
Magnesium	mg	320	45	**14.06**	33	**10.31**	0	0	31.5	9.84
Phosphorus	mg	1000	43.5	4.35	55.5	5.55	28.5	2.85	39	3.90
Sodium	mg	**120**	171	-	253.5	-	225	-	241.5	-
Copper	mg	3	0	0.00	1.5	1.25	4.5	3.75	1.5	1.25
Manganese	mg	5	0	0.00	0	0.00	0	0	0	0.00
Zinc	mg	12	0.83	**16.50**	0.85	**16.95**	0	0	0	0.00
Vitamin C	mg	40	0.36	3.00	0.54	4.50	0.75	6.25	0.45	3.75
Thiamin	mg	1.1	57	**142.50**	48	**120.00**	13.5	**33.75**	33	**82.50**
Riboflavin	mg	1.7	0.03	2.73	0.06	5.05	0.015	1.36	0	0.00
Niacin	mg	10	0.05	2.65	0.04	2.38	0.03	1.76	0	0.00
Pantothenate	mg	5	0.80	7.95	0.84	8.40	1.65	**16.5**	1.65	**16.50**
Vitamin B6	mg	1.6	0.53	**10.50**	0.59	**11.70**	0	0	0	0.00
Folate Equiv.	µg	200	0	0.00	0.08	4.69	0.015	0.94	0	0.00
Retinol Eq.	µg	750	54	**27.00**	51	**25.50**	94.5	**47.25**	51	**25.50**
Beta-carotene Eq.	µg	750	79.5	**10.60**	7.5	1.00	75	10	6	0.80
Vitamin E	mg	10	225	**30.00**	42	5.60	0	0	0	0.00

*NB: Portion references taken from Australian Guide to Healthy Eating (1 cup). Values shown in bold are those considered a source of the nutrient at minimum. * from standard 1.1.1*

Antioxidants

- *Blackberries, Raspberries, Boysenberries and Loganberries are a natural source of antioxidants*

Aside from specific vitamin and mineral claims, a number of products on the market are now making general antioxidant claims (eg, "*rich in antioxidants*"). Such claims are not regulated within current Food Standards or the voluntary Code of Practice on Nutrient Claims, but they would fall under the category of claims about "bioactive substances" that will be referred to in the proposed new standard on health claims. In this standard it is proposed that manufacturers would need to define for themselves which components are antioxidants and the daily reference quantity of each substance that is recommended, and then products would need to contain at least 10% of that level per serve to justify a claim. If claims were to be made that a foods was a source of bioactive substances such as lutein and/or zeaxanthin then the marketer would need to be able to have scientific evidence for a recommended daily intake.

Some of the more traditional nutrients in Rubus also have antioxidant properties (vitamin C), and this too could be used to substantiate the antioxidant properties of Rubus. Alternatively data on the analysed total antioxidant capacity of Rubus could be relied upon (see Table 2), although it would be better to use more specific analytical data on particular bioactive substances – such as the carotenoids lutein and zeaxanthin - if possible.

2.4.2 Health Claims

The following comments summarise the status of some potential types of claims that might be made, although many claims would need to be able to be substantiated with nutritional analysis information which is largely lacking for Rubus. Where the term Rubus has been used generally, for the work of this project this refers to the Rubus group assessed namely, Raspberry, Blackberry, Loganberry and Boysenberry.

Folate
One of the pre-approved FSANZ nutrient function claims is that "Folate is necessary for normal blood formation", and under this rubus could use this now, provided the folate level was declared in the NIP and contained more than 20 µg/serve (10% RDI in Food Standards Code).
Both content and nutrient function claims could be made:

- *A source of folate*
- *Folate is necessary for normal blood formation*
- *Folate is a B group vitamin that is essential for normal cell growth and development*

Boysenberries, in particular, are considered to be a high source of folate (>25% of RDI per serve).

Dietary guideline claims

- *Rubus form part of a healthy diet of fruits and vegetables. Eating a diet rich in fruits and vegetables as part of an overall healthy diet may reduce risk for stroke and perhaps other cardiovascular diseases*

The first sentence is fine; it is consistent with Dietary Guidelines for Australians [70] and is permitted now as normal nutritional advice of a general nature.

The second sentence contravenes current regulations, even though it is a well established fact. However it is one of the claims that has been pre-approved for use in the proposed new health claims standards and could be used once the new standard came into force.

Antioxidant Function Claims

- *Rubus are a natural source of antioxidants which may reduce the risk of cancer*

Any claim about reducing the risk of a serious disease (like cancer or cardiovascular disease) is currently prohibited and would require pre-approval by FSANZ when the new standard comes into law. It is unlikely that there is sufficient clinical evidence yet to substantiate this specific claim adequately. Claims about antioxidants and their normal function in the body would be permissible, provided there was analytical data to demonstrate a reasonable amount present in a single serve. Such claims could be worded as Nutrient Function Claims as follows:

- *Rubus are a natural source of antioxidants vitamins C and E that help protect the cells from oxidative damage*
- *A natural source of the antioxidants – to help keep your body healthy*
- *Antioxidants in Rubus help protect the body against free radical damage.*

Other Claims
There are a number of possible nutrients or nutrient function claims that could be relevant to rubus berries within current regulations, for example:

- *All the goodness of one serve of fruit*
- *All natural*

Furthermore, although most fruits are naturally low in salt and cholesterol, such claims are compelling for consumers and there is no reason not to highlight the fact with claims such as:

- *Low in salt*

Final Note
This review did not assessed whether any of the suggested claims have sufficient scientific substantiation to support the claim. The FSANZ substantiation guidelines should be followed if further claims are to be made though a substantial body of scientific evident is required to support such applications.

2.5 CONCLUSIONS AND RECOMMENDATIONS

The nutrient composition for Rubus were obtained from the existing Australian food composition databases though data for boysenberries could only be obtained from New Zealand. The Australian data is from pre 1990 and therefore considered to be old data. To support with promoting the profile of Rubus within the Australian context, it is recommended that more recently analytical data be obtained and submitted to Food Standards Australian New Zealand for inclusion in the food composition database. Such data will not only provide current day insight into the nutrient composition but it will also allow for theoretical studies to be conducted within which Rubus fruit may be modelled into a healthy diet in place of other fruit to see the impact this may have on the nutrient outcomes. Similarly, analytical data for boysenberries will provide more accurate data for Australian use overall as this is presently not available. Furthermore, in order to make health claims a specified portion size is required for a food product. For this project the serving of 150g equating to approximately 1 cup of berries has been used based on the Australian Guide to Healthy Eating. This figure was calculated based on the approximation of 300kJ for a serving of fruit. The energy content of the Rubus berries addressed in their project varies and therefore an adjusted serving size may be used. Consumer research into appropriate portion sizes and portion sizes currently consumed would be recommended to support this work. Theoretical modelling can then also be employed to determine how Rubus best fit within the diet. Once such work was been conducted human studies are required to build the evidence base overall for Rubus.

REFERENCES

1. Hummer KE. Rubus pharmacology: Antiquity to the present. HortScience. 2010;45(11):1587-1591.
2. Food Standards Australia New Zealand. NUTTAB 2010 Online version. 2007; http://www.foodstandards.gov.au/consumerinformation/nuttab2010/nuttab2010onlinesearchabledatabase/onlineversion.cfm. Accessed 28 July, 2011.
3. Crop and Food Research. the 8th Edition Concise Food Composition Tables. 2008. http://www.crop.cri.nz/home/products-services/nutrition/foodcompdata/fcd-products/fcd-food-comp-tables.php.
4. Australia New Zealand Food Authority. AUSNUT - Australian Food and Nutrient Database. Canberra: ANZFA; 1999.
5. Jakobek L, Šeruga M, Medvidović-Kosanović M, Novak I. Antioxidant activity and polyphenols of Aronia in comparison to other berry species. Agriculturae Conspectus Scientificus. 2007;72(4):301-306.
6. Hager A, Howard LR, Prior RL, Brownmiller C. Processing and storage effects on monomeric anthocyanins, percent polymeric color, and antioxidant capacity of processed black raspberry products. Journal of Food Science. 2008;73(6):H134-H140.
7. Cavender A. Folk medical uses of plant foods in southern Appalachia, United States. Journal of Ethnopharmacology. 2006;108(1):74-84.
8. Badjakov I, Nikolova M, Gevrenova R, Kondakova V, Todorovska E, Atanassov A. Bioactive compounds in small fruits and their influence on human health. Biotechnology and Biotechnological Equipment. 2008;22(1):581-587.
9. Horobowicz M, Kosson R, Grzesuik AD, Henryk. Anthocyanins of fruits and vegetables - their occurrence, analysis and role in human nutrition. Vegetable Crops Research Bulletin. 2008;68:5-22.
10. Bartkiene E, Juodeikiene G, Basinskiene L, Liukkonen KH, Adlercreutz H, Kluge H. Enterolignans enterolactone and enterodiol formation from their precursors by the action of intestinal microflora and their relationship with non-starch polysaccharides in various berries and vegetables. LWT - Food Science and Technology. 2011;44(1):48-53.
11. Cisse M, Vaillant F, Acosta O, Claudie DM, Dornier M. Thermal degradation kinetics of anthocyanins from blood orange, blackberry, and roselle using the arrhenius, eyring, and ball models. Journal of Agricultural and Food Chemistry. 2009;57(14):6285-6291.
12. Hassimotto NMA, Da Mota RV, Cordenunsi BR, Lajolo FM. Physico-chemical characterization and bioactive compounds of blackberry fruits (Rubus sp.) grown in Brazil. Ciencia e Tecnologia de Alimentos. 2008;28(3):702-708.
13. Reyes-Carmona J, Yousef GG, Martínez-Peniche RA, Lila MA. Antioxidant capacity of fruit extracts of blackberry (Rubus sp.) produced in different climatic regions. Journal of Food Science. 2005;70(7):S497-S503.
14. Scalzo J, Currie A, Stephens J, McGhie T, Alspach P, Horticulture Food Res Inst New Z. The anthocyanin composition of different Vaccinium, Ribes and Rubus genotypes. Biofactors. 2008;34(1):13-21.
15. Furuuchi R, Yokoyama T, Watanabe Y, Hirayama M. Identification and Quantification of Short Oligomeric Proanthocyanidins and Other Polyphenols in Boysenberry Seeds and Juice. Journal of Agricultural and Food Chemistry. Apr 2011;59(8):3738-3746.
16. Çekiç Ç, Özgen M. Comparison of antioxidant capacity and phytochemical properties of wild and cultivated red raspberries (Rubus idaeus L.). Journal of Food Composition and Analysis. 2010;23(6):540-544.
17. Gülçin I, Topal F, Çakmakçi R, Bilsel M, Gören AC, Erdogan U. Pomological Features, Nutritional Quality, Polyphenol Content Analysis, and Antioxidant Properties of Domesticated and 3 Wild Ecotype Forms of Raspberries (Rubus idaeusL.). Journal of Food Science. 2011;76(4):C585-C593.
18. Milivojević J, Maksimović V, Nikolić M, Bogdanović J, Maletić R, Milatović D. chemical and antioxidant properties of cultivated and wild fragaria and rubus berries. Journal of Food Quality. 2011;34(1):1-9.
19. Celik F, Ercisli S. Lipid and fatty acid composition of wild and cultivated red raspberry fruits (Rubus idaeus L.). Journal of Medicinal Plant Research. 2009;3(8):583-585.
20. Halbwirth H, Waldner I, Miosic S, Ibanez M, Costa G, Stich K. Measuring flavonoid enzyme activities in tissues of fruit species. Journal of Agricultural and Food Chemistry. 2009;57(11):4983-4987.

21. Hegedus A, Balogh E, Engel R, et al. Comparative nutrient element and antioxidant characterization of berry fruit species and cultivars grown in hungary. HortScience. 2008;43(6):1711-1715.
22. Strålsjö L, Åhlin H, Witthöft CM, Jastrebova J. Folate determination in Swedish berries by radioprotein-binding assay (RPBA) and high performance liquid chromatography (HPLC). European Food Research and Technology. 2003;216(3):264-269.
23. Beekwilder J, van der Meer IM, Simic A, et al. Metabolism of carotenoids and apocarotenoids during ripening of raspberry fruit. Biofactors. 2008;34(1):57-66.
24. Borges G, Degeneve A, Mullen W, Crozier A. Identification of flavonoid and phenolic antioxidants in black currants, blueberries, raspberries, red currants, and cranberries. Journal of Agricultural and Food Chemistry. 2010;58(7):3901-3909.
25. Mullen W, Stewart AJ, Lean MEJ, Gardner P, Duthie GG, Crozier A. Effect of freezing and storage on the phenolics, ellagitannins, flavonoids, and antioxidant capacity of red raspberries. Journal of Agricultural and Food Chemistry. 2002;50(18):5197-5201.
26. Marinova D, Ribarova F. HPLC determination of carotenoids in Bulgarian berries. Journal of Food Composition and Analysis. 2007;20(5):370-374.
27. Heinonen M. Antioxidant activity and antimicrobial effect of berry phenolics - A Finnish perspective. Molecular Nutrition and Food Research. 2007;51(6):684-691.
28. Jakobek L, Šeruga M, Šeruga B, Novak I, Medvidović-Kosanović M. Phenolic compound composition and antioxidant activity of fruits of Rubus and Prunus species from Croatia. International Journal of Food Science and Technology. 2009;44(4):860-868.
29. Liu M, Li XQ, Weber C, Lee CY, Brown J, Liu RH. Antioxidant and antiproliferative activities of raspberries. Journal of Agricultural and Food Chemistry. 2002;50(10):2926-2930.
30. Proteggente AR, Pannala AS, Paganga G, et al. The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. Free Radical Research. 2002;36(2):217-233.
31. Sparzak B, Merino-Arevalo M, Vander Heyden Y, et al. HPLC analysis of polyphenols in the fruits of Rubus idaeus L. (Rosaceae). Natural Product Research. 2010;24(19):1811-1822.
32. Hager TJ, Howard LR, Prior RL. Processing and storage effects on the ellagitannin composition of processed blackberry products. Journal of Agricultural and Food Chemistry. 2010;58(22):11749-11754.
33. Levaj B, Dragović-Uzelac V, Delonga K, Ganić KK, Banović M, Kovačević DB. Polyphenols and volatiles in fruits of two sour cherry cultivars, some berry fruits and their jams. Food Technology and Biotechnology. 2010;48(4):538-547.
34. Määttä-Riihinen KR, Kamal-Eldin A, Törrönen AR. Identification and quantification of phenolic compounds in berries of Fragaria and Rubus species (family rosaceae). Journal of Agricultural and Food Chemistry. 2004;52(20):6178-6187.
35. Samappito S, Butkhup L. Analysis of anthocyanin, flavonoids, and phenolic acid contents of ten fruits and antioxidant activity. International Journal of Fruit Science. 2010;10(3):264-280.
36. Mertz C, Cheynier V, Günata Z, Brat P. Analysis of phenolic compounds in two blackberry species (Rubus glaucus and Rubus adenotrichus) by high-performance liquid chromatography with diode array detection and electrospray ion trap mass spectrometry. Journal of Agricultural and Food Chemistry. 2007;55(21):8616-8624.
37. Mi JC, Howard LR, Prior RL, Clark JR. Flavonoid glycosides and antioxidant capacity of various blackberry, blueberry and red grape genotypes determined by high-performance liquid chromatography/mass spectrometry. Journal of the Science of Food and Agriculture. 2004;84(13):1771-1782.
38. Siriwoharn T, Wrolstad RE, Finn CE, Pereira CB. Influence of cultivar, maturity, and sampling on blackberry (Rubus L. hybrids) anthocyanins, polyphenolics, and antioxidant properties. Journal of Agricultural and Food Chemistry. 2004;52(26):8021-8030.
39. Connor AM, Finn CE, Alspach PA. Genotypic and environmental variation in antioxidant activity and total phenolic content among blackberry and hybridberry cultivars. Journal of the American Society for Horticultural Science. 2005;130(4):527-533.
40. Mi JC, Howard LR, Prior RL, Clark JR. Flavonol glycosides and antioxidant capacity of various blackberry and blueberry genotypes determined by high-performance liquid chromatography/mass spectrometry. Journal of the Science of Food and Agriculture. 2005;85(13):2149-2158.
41. Vasco C, Riihinen K, Ruales J, Kamal-Eldin A. Phenolic compounds in rosaceae fruits from Ecuador. Journal of Agricultural and Food Chemistry. 2009;57(4):1204-1212.

42. Zadernowski R, Naczk M, Nesterowicz J. Phenolic acid profiles in some small berries. Journal of Agricultural and Food Chemistry. 2005;53(6):2118-2124.
43. Patel AV, Rojas-Vera J, Dacke CG. Therapeutic constituents and actions of Rubus species. Current Medicinal Chemistry. 2004;11(11):1501-1512.
44. Prior RL, Wu X. Anthocyanins: Structural characteristics that result in unique metabolic patterns and biological activities. Free Radical Research. 2006;40(10):1014-1028.
45. Rao AV, Snyder DM. Raspberries and Human Health: A Review. Journal of Agricultural and Food Chemistry. 2010;58(7):3871-3883.
46. Willis L, Shukitt-Hale B, Joseph J. Recent advances in berry supplementation and age-related cognitive decline. Curr Opin Clin Nutr Metab Care. 2009;12(1):91-94.
47. Landete JM. Ellagitannins, ellagic acid and their derived metabolites: A review about source, metabolism, functions and health. Food Research International. 2011;44(5):1150-1160.
48. Liu Y, Liu M, Li B, et al. Fresh raspberry phytochemical extract inhibits hepatic lesion in a Wistar rat model. Nutrition and Metabolism. 2010;7.
49. Aiyer HS, Srinivasan C, Gupta RC. Dietary berries and ellagic acid diminish estrogen-mediated mammary tumorigenesis in ACI rats. Nutrition and Cancer. 2008;60(2):227-234.
50. Park SM, Choi HY, Lee EJ, et al. Dietary supplementation of red ginseng, Chinese lizard's tail, and Korean raspberry extracts alleviate oxidative stresses in aged rats. Food Science and Biotechnology. 2010;19(4):967-972.
51. Shukitt-Hale B, Cheng V, Joseph JA. Effects of blackberries on motor and cognitive function in aged rats. Nutritional Neuroscience. 2009;12(3):135-140.
52. Talavéra S, Felgines C, Texier O, et al. Anthocyanin metabolism in rats and their distribution to digestive area, kidney, and brain. Journal of Agricultural and Food Chemistry. 2005;53(10):3902-3908.
53. Rossi A, Serraino I, Dugo P, et al. Protective effects of anthocyanins from blackberry in a rat model of acute lung inflammation. Free Radical Research. 2003;37(8):891-900.
54. Barnett LE, Broomfield AM, Hendriks WH, Hunt MB, McGhie TK. The in vivo antioxidant action and the reduction of oxidative stress by boysenberry extract is dependent on base diet constituents in rats. Journal of Medicinal Food. 2007;10(2):281-289.
55. Prior RL, Wu X, Gu L, et al. Purified berry anthocyanins but not whole berries normalize lipid parameters in mice fed an obesogenic high fat diet. Molecular Nutrition and Food Research. 2009;53(11):1406-1418.
56. Battino M, Beekwilder J, Denoyes-Rothan B, Laimer M, McDougall GJ, Mezzetti B. Bioactive compounds in berries relevant to human health. Nutr. Rev. May 2009;67(5):S145-S150.
57. Shin TY, Shin HY, Kim SH, et al. Rubus coreanus unripe fruits inhibits immediate-type allergic reaction and inflammatory cytokine secretion. Natural Product Sciences. 2006;12(3):144-149.
58. Harada N, Okajima K, Narimatsu N, Kurihara H, Nakagata N. Effect of topical application of raspberry ketone on dermal production of insulin-like growth factor-I in mice and on hair growth and skin elasticity in humans. Growth Hormone and IGF Research. 2008;18(4):335-344.
59. Aiyer HS, Li Y, Liu QH, Reuter N, Martin RCG. Dietary freeze-dried black raspberry's effect on cellular antioxidant status during reflux-induced esophagitis in rats. Nutrition. 2011;27(2):182-187.
60. Lee YI, Whang KE, Cho JS, et al. Rubus coreanus extract attenuates acetaminophen induced hepatotoxicity; involvement of cytochrome P450 3A4. Biomolecules and Therapeutics. 2009;17(4):455-460.
61. Zhang Y, Zhang Z, Yang Y, Zu X, Guan D, Wang Y. Diuretic activity of Rubus idaeus L (Rosaceae) in rats. Tropical Journal of Pharmaceutical Research. 2011;10(3):243-248.
62. Hassan HA, Yousef MI. Mitigating effects of antioxidant properties of black berry juice on sodium fluoride induced hepatotoxicity and oxidative stress in rats. Food and Chemical Toxicology. 2009;47(9):2332-2337.
63. Australia New Zealand Food Authority. Users Guide to the New Foods Standards Code. Canberra: ANZFA; 2002.
64. Food Standards Australia and New Zealand. Reviews for High Level Claims2007.
65. Australian Competition and Consumer Commission. Food descriptors guideline to the Trade Practices Act. 2006; http://www.accc.gov.au/content/item.phtml?itemId=771468&nodeId=303812cdfa698071341bf9f1ac983066&fn=Food%20descriptors%20guidelines.pdf. Accessed 16 May 2008.

66. Australian Food and Grocery Council. Code of Practice for the Provision of Information on Food Products. Canberra: AFGC; 1995.
67. National Food Authority. Code of Practice. Nutrient claims in food labels and in advertisements. Canberra: National Food Authority; 1995.
68. Food Standards Australia New Zealand. User guide to Standard 1.2.8 - Nutrition Information Requirements. 2008. http://www.foodstandards.gov.au/foodstandards/userguides/nutritioninformation1406.cfm.
69. Williams P, Yeatman H, Ridges L, et al. Nutrition function, health and related claims on packaged Australian food products - prevalence and compliance with regulations. Asia Pacific Journal of Clinical Nutrition. 2006;15(1):10-20.
70. National Health and Medical Research Council. Food for Health: Dietary Guidelines for Australian Adults. Canberra: NH&MRC; 2003.
71. Fortalezas S, Tavares L, Pimpão R, et al. Antioxidant properties and neuroprotective capacity of strawberry tree fruit (Arbutus unedo). Nutrients. 2010;2(2):214-229.
72. Ghosh D, McGhie TK, Fisher DR, Joseph JA. Cytoprotective effects of anthocyanins and other phenolic fractions of Boysenberry and blackcurrant on dopamine and amyloid beta-induced oxidative stress in transfected COS-7 cells. Journal of the Science of Food and Agriculture. Aug 2007;87(11):2061-2067.
73. Bowen-Forbes CS, Mulabagal V, Liu Y, Nair MG. Ursolic acid analogues: non-phenolic functional food components in Jamaican raspberry fruits. Food Chemistry. 2009;116(3):633-637.
74. Bowen-Forbes CS, Zhang Y, Nair MG. Anthocyanin content, antioxidant, anti-inflammatory and anticancer properties of blackberry and raspberry fruits. Journal of Food Composition and Analysis. 2010;23(6):554-560.
75. Han C, Ding H, Casto B, Stoner GD, D'Ambrosio SM. Inhibition of the growth of premalignant and malignant human oral cell lines by extracts and components of black raspberries. Nutrition and Cancer. 2005;51(2):207-217.
76. Harris GK, Gupta A, Nines RG, et al. Effects of lyophilized black raspberries on azoxymethane-induced colon cancer and 8-hydroxy-2′-deoxyguanosine levels in the fischer 344 rat. Nutrition and Cancer. 2001;40(2):125-133.
77. Hogan S, Chung H, Zhang L, et al. Antiproliferative and antioxidant properties of anthocyanin-rich extract from açai. Food Chemistry. 2010;118(2):208-214.
78. Jeong JH, Jung H, Lee SR, Lee HJ, Hwang KT, Kim TY. Anti-oxidant, anti-proliferative and anti-inflammatory activities of the extracts from black raspberry fruits and wine. Food Chemistry. 2010;123(2):338-344.
79. Johnson JL, Bomser JA, Scheerens JC, Giusti MM. Effect of black raspberry (Rubus occidentalis L.) extract variation conditioned by cultivar, production site, and fruit maturity stage on colon cancer cell proliferation. Journal of Agricultural and Food Chemistry. 2011;59(5):1638-1645.
80. Jung J, Son MY, Jung S, et al. Antioxidant properties of Korean black raspberry wines and their apoptotic effects on cancer cells. Journal of the Science of Food and Agriculture. 2009;89(6):970-977.
81. Juranic Z, Zizak Z, Tasic S, et al. Antiproliferative action of water extracts of seeds or pulp of five different raspberry cultivars. Food Chemistry. 2005;93(1):39-45.
82. Kreander K, Galkin A, Vuorela S, et al. In-vitro mutagenic potential and effect on permeability of co-administered drugs across Caco-2 cell monolayers of Rubus idaeus and its fortified fractions. Journal of Pharmacy and Pharmacology. 2006;58(11):1545-1552.
83. Madhusoodhanan R, Natarajan M, Singh JVN, et al. Effect of black raspberry extract in inhibiting NF B dependent radioprotection in human breast cancer cells. Nutrition and Cancer. 2010;62(1):93-104.
84. McDougall GJ, Ross HA, Ikeji M, Stewart D. Berry extracts exert different antiproliferative effects against cervical and colon cancer cells grown in vitro. Journal of Agricultural and Food Chemistry. 2008;56(9):3016-3023.
85. Ogawa Y, Akamatsu M, Hotta Y, Hosoda A, Tamura H. Effect of essential oils, such as raspberry ketone and its derivatives, on antiandrogenic activity based on in vitro reporter gene assay. Bioorganic and Medicinal Chemistry Letters. 2010;20(7):2111-2114.
86. Ross HA, McDougall GJ, Stewart D. Antiproliferative activity is predominantly associated with ellagitannins in raspberry extracts. Phytochemistry. 2007;68(2):218-228.

87. Seeram NP, Adams LS, Zhang Y, et al. Blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry extracts inhibit growth and stimulate apoptosis of human cancer cells in vitro. Journal of Agricultural and Food Chemistry. 2006;54(25):9329-9339.
88. Smith SH, Tate PL, Huang G, et al. Antimutagenic activity of berry extracts. Journal of Medicinal Food. 2004;7(4):450-455.
89. Wu QK, Koponen JM, Mykkänen HM, Törrönen AR. Berry phenolic extracts modulate the expression of p21WAF1 and Bax but Not Bcl-2 in HT-29 colon cancer cells. Journal of Agricultural and Food Chemistry. 2007;55(4):1156-1163.
90. Xue H, Aziz RM, Sun N, et al. Inhibition of cellular transformation by berry extracts. Carcinogenesis. 2001;22(2):351-356.
91. Zunino SJ, Zhang Y, Seeram NP, Storms DH. Berry fruit extracts inhibit growth and induce apoptosis of high-risk acute lymphoblastic leukemia cells in vitro. Journal of Functional Foods. 2010;2(3):187-195.
92. Dai J, Patel JD, Mumper RJ. Characterization of blackberry extract and its antiproliferative and anti-inflammatory properties. Journal of Medicinal Food. 2007;10(2):258-265.
93. Ding M, Feng R, Wang SY, et al. Cyanidin-3-glucoside, a natural product derived from blackberry, exhibits chemopreventive and chemotherapeutic activity. Journal of Biological Chemistry. 2006;281(25):17359-17368.
94. Elisia I, Hu C, Popovich DG, Kitts DD. Antioxidant assessment of an anthocyanin-enriched blackberry extract. Food Chemistry. 2006;101(3):1052-1058.
95. Elisia I, Kitts DD. Anthocyanins inhibit peroxyl radical-induced apoptosis in Caco-2 cells. Molecular and Cellular Biochemistry. 2008;312(1-2):139-145.
96. Feng R, Bowman LL, Lu Y, et al. Blackberry extracts inhibit activating protein 1 activation and cell transformation by perturbing the mitogenic signaling pathway. Nutrition and Cancer. 2004;50(1):80-89.
97. Tate P, Kuzmar A, Smith SW, Wedge DE, Larcom LL. Comparative effects of eight varieties of blackberry on mutagenesis. Nutrition Research. 2003;23(7):971-979.
98. Kim YH, Choi JH, Rim HK, et al. 23-Hydroxytormentic acid and niga-ichgoside F1 isolated from Rubus coreanus attenuate cisplatin-induced cytotoxicity by reducing oxidative stress in renal epithelial LLC-PK1 cells. Biological and Pharmaceutical Bulletin. 2011;34(6):906-911.
99. Park JH, Oh Sm, Lim SS, et al. Induction of heme oxygenase-1 mediates the anti-inflammatory effects of the ethanol extract of Rubus coreanus in murine macrophages. Biochemical and Biophysical Research Communications. 2006;351(1):146-152.
100. Cuevas-Rodríguez EO, Dia VP, Yousef GG, et al. Inhibition of pro-inflammatory responses and antioxidant capacity of mexican blackberry (Rubus spp.) extracts. Journal of Agricultural and Food Chemistry. 2010;58(17):9542-9548.
101. Liu Z, Schwimer J, Liu D, Greenway FL, Anthony CT, Woltering EA. Black raspberry extract and fractions contain angiogenesis inhibitors. Journal of Agricultural and Food Chemistry. 2005;53(10):3909-3915.
102. Rouanet JM, Décordé K, Rio DD, et al. Berry juices, teas, antioxidants and the prevention of atherosclerosis in hamsters. Food Chemistry. 2010;118(2):266-271.
103. Serraino I, Dugo L, Dugo P, et al. Protective effects of cyanidin-3-O-glucoside from blackberry extract against peroxynitrite-induced endothelial dysfunction and vascular failure. Life Sciences. 2003;73(9):1097-1114.
104. Do SH, Lee JW, Jeong WI, et al. Bone-protecting effect of Rubus coreanus by dual regulation of osteoblasts and osteoclasts. Menopause. 2008;15(4):676-683.

APPENDIX

Table 6: Summary of scientific literature identified for Rubus (raspberry, blackberry, boysenberry and loganberry)

Study	Design (duration)	Studies/participants (age)	Source/Dose/ Nutrients	Measurement	Outcome	Disease state	Rubus	Supportive
Fortalezas et al 2010 [71]	In vitro (NR)	Human SK-N-MC neuroblastoma cell line	Freeze dried phenol and anthocyanin extract, 0, 50, 125, 175µg/mL from ground berries	Cell viability	Cell viability of 36.6% at 50µg and 27.8% at 125µg GAE/mL, though decreased for higher concentrations (8.9% at 175µg GAE/mL). Raspberry extract rescued cell survival	Ageing/Neuro-degeneration	Raspberry	Yes
Park et al 2010 [50]	Animal (8 wks)	Male Sprague-Dawley rats (10mths old, NR) Control: Male S-D (2mths old, NR)	Ethanol extracts 18% of dried weight. 0.5% Raspberry ethanol extract diet and 1.0% diet. 1:1:1 mixture of red ginseng, Chinese lizard's tail & raspberry.	Determine efficacy of 3 anti-ageing preparations in suppressing age-related oxidative stresses.	Raspberry reduced 8-IsoPGF2α and increased α-tocopherol	Ageing	(Black) raspberry	Yes
Shukitt-Hale et al 2009 [51]	Animal (8wks)	Fischer 344 rats (19mths, n=30)	2% freeze-dried blackberry extracts added to control diet	Reversal of age-related deficits in behaviour and neuronal function	Blackberry rats performed significantly better than control rats in behavioural tests for balance, fine motor co-ordination & spatial working memory. No differences in dopamine release between blackberry and control groups.	Ageing	Blackberry	Yes (motor)
Ghosh et al 2007 [72]	In vitro (48hrs)	COS-7 cells	Evaporated and freeze dried polyphenolic enriched fraction, 100-500g/mL	Cell viability	All fractions gave very significant levels of protection in cell viability.	Ageing/Neuro-degeneration	Boysenberry	Yes
Harada et al 2008 [58]	Human Study 1: (5mo), study 2: (14d)	Study 1: 10 volunteers (7 males and 3 females, (12–45 yrs, 6 males suffered from androgenetic alopecia and 4	Raspberry ketone extract, Study 1: Human: 0.01%	Study 1: Hair growth Study 2: Skin elasticity	Increasing skin elasticity and promoting hair growth Increase in dermal IGF-1 production	Alopecia	(Red) Raspberry	Yes

Study	Design (duration)	Studies/participants (age)	Source/Dose/ Nutrients	Measurement	Outcome	Disease state	Rubus	Supportive
		[1 male, 3 females] from alopecia areata) Study 2: 10 healthy female human volunteers (NR)	to scalp Study 2: 0.01% to face					
Aiyer et al 2008 [49]	Animal (24wks)	Female ACI rats (8–9 wk old, n=19)	Powdered, 2.5% w/w	Incidence of mammary tumours, size and rate of multiplication	No differences were found in tumour incidence at 24 wk; tumour volume decreased by more than 69% ($P < 0.005$) and tumour multiplicity by 37% ($P = 0.07$)	Cancer	(Black) raspberry	Yes
Bowen-Forbes et al 2009 [73]	In vitro (72hrs)	MCF-7 (breast), SF-268 (CNS), NCI H460 (lung), HCT-116 (colon) and AGS (gastric) human tumour cells	Lyophilised, powdered extract, 0.005-0.054%	Inhibition of cell proliferation from selected phytonutrients	Significant growth inhibition specific to colon tumour cells by 56% from 19a-hydroxyasiatic acid and 40% nigaichigoside F1 phytochemicals.	Cancer	(Red) raspberry	Yes
Bowen-Forbes et al 2009 [74]	In vitro (NR)	MCF-7 (breast), SF-268 (CNS), NCI-H460 (lung), HCT-116 (colon) and AGS (gastric) human tumour cells	Lyophilised, powered extract, Dose NR	Inhibition of cell proliferation from selected phytonutrients	Black raspberry inhibited colon, breast and gastric cancer cells by 11, 31, and 25%, respectively. The hexane extract of red raspberry resulted in inhibition of breast and gastric cancer cells by 17 and 22%.	Cancer	(Red, yellow and black) raspberry	Yes
Han et al 2005[75]	In vitro (24hrs)	Human oral cell lines	Frozen/freeze dried, 200μg/mL 45%, - 23%	Cell cycle regulatory proteins targeted	Inhibition of growth of premalignant and malignant but not normal human oral epithelial cell lines	Cancer	(Black) raspberry	Yes
Harris et al 2001 [76]	Animal (33wks)	F344 male Fischer rats (6–7wks, n=216) colon cancer model	Whole Freeze dried black raspberries then powdered 0, 2.5, 5 or 10% w/w	Adenocarcinoma size/Urinary 8OHdG levels	Significant decrease in aberrant crypt foci and tumour multiplicity for all groups/non-significant tumour burden observed in all groups	Cancer	(Black) raspberry	Yes
Hogan et al 2010 [77]	In vitro (72hrs)	C-6 rat brain glioma cells and MDA-468 human breast cancer cells	Anthocyanin-rich extracts, 50, 100, and 200μg/mL	Antioxidant ability and antiproliferative activity	No effect on the growth of human cells	Cancer	Raspberry	No
Jeong et al 2010 [78]	In vitro (24hrs)	HT-29 colon adenocarcinoma cell lines and human LNCaP prostate carcinoma, RAW264.7 cell lines	Fruit without or with seeds crushed were blended and wine, 20g fruit, 50, 100, 250, 500, 1000,	anti-oxidant, anti-proliferative and anti-inflammatory activities	Antioxidant activity raised when seeds included, dose dependant suppression (17-70%) >100uL for HT-29 cells and >1000uL for LNCaP cells, >84% viability with exposure to 500uL extract, >25% anti-inflammatory activity	Cancer	(Black) raspberries	Yes

Study	Design (duration)	Studies/participants (age)	Source/Dose/ Nutrients	Measurement	Outcome	Disease state	Rubus	Supportive
			2000uL extract		of extra with seed and from wines compared with control			
Johnson et al 2011 [79]	In vitro (48hrs)	HT-29 colorectal adenocarcinoma cell line	Freeze-dried extract from 75 cultivars (underripe, ripe, overripe), 0.6, 1.2mg/mL	antiproliferative activity	Cell proliferation was significantly influenced by cultivar, production site, and stage of maturity (33-82% 0.6 mg/mL, 71-118% 1.2 mg/mL), more mature fruits saw lessened inhibition	Cancer	(Black) raspberry	Yes
Jung et al 2009 [80]	In vitro (27hrs)	Human cancer cell lines, including AGS (human adenocarcinoma), HepG2 (human hepatoblastoma), and HeLa (human cervical carcinoma),	freeze-dried wine concentrates, 20, 50, 100, 500, 1000µg/mL	Cell viability	The alcoholic beverage Concentrates (4-56 mg/mL) inhibited cell growth in a dose-dependent manner. The Korean raspberry wine had this effect for all cell lines 20-30% more than other wines.	Cancer	(Black) raspberry	Yes
Juranic et al 2005 [81]	In vitro (72hrs)	Human neoplastic colon carcinoma LS174 cell	Water extract (pulp) or ground seed extracts, Maximum 67%	Antiproliferative action	Applied concentration of pulp extracts, led to a 50% decrease in number of surviving cells, antiproliferative activity of pulp extracts was more pronounced than the activity of the corresponding seeds extracts, Willamette cultivars had the strongest effect and Tulameen the weakest.	Cancer	Raspberry	Yes
Kreander et al 2006 [82]	In vitro (90mins)	Caco-2 cell line	Freeze dried berry extract and fortified ellagitannin fractions, 5kg fresh fruit, phenolic compounds, 0.01, 0.1, 1mg/mL extract	Mutagenicity, anti-mutagenicity and cyctoxicity	Neither the berry extract or the fractions of were cytoxic to the cell lines, all samples inhibited mutagenicity in the absence of metabolic activation, decreased metaprolol permeability by 50% for extracts, 80% for fractions.	Cancer	(Red) raspberry	Yes
Liu et al 2010 [48]	Animal (20wks)	Male Wistar rats (NR, N=100)	Frozen extract, 100g fresh fruit, 0.75, 1.5, 3.0g/kg body weight/d	Prevention of hepatic lesion formation	Dose-dependent inhibition on nodule formation (40.0, 25.0, and 5.0%), decreasing trend of proportions of hepatocellular	Cancer	(Red) raspberry	Yes

Study	Design (duration)	Studies/participants (age)	Source/Dose/ Nutrients	Measurement	Outcome	Disease state	Rubus	Supportive
					carcinoma masses with increasing dose (11.1, 44.4, and 88.9% reduced incidence)			
Madhusoodanan et al 2010 [83]	In vitro (72hrs)	Human MCF-7breast adenocarcinoma cells.	10mg fruit, frozen seed extract, 0.5, 1.0, 2.0µg/mL	Cell survival and activity	Dose-dependent inhibition of NFκB DNA-binding activity, inhibition of MnSOD activity for all doses, suppression of cell survival and enhanced cell death.	Cancer	(Black) raspberry	Yes
McDougall et al 2008 [84]	In vitro (72hrs)	HeLa Human cervical Cancer and CaCo-2 human colon cancer cells	Freeze dried polyphenol rich fraction from frozen fruit, 25, 50, 75µg/mL	Cell viability and proliferation	<50% (raspberry) viability of cells at 50µg/mL minimal dose dependant inhibition. (Actual values NR)	Cancer	Raspberry	Partial
Ogawa et al 2010 [85]	In vitro (NR)	MDA-kb2 human breast cancer cells	Raspberry ketone (NR)	Effects of raspberry ketone essential oil on androgen reception activity in human breast cancer cell lines	Essential oils with alkylphenol moiety act as a novel androgen receptor modulator	Cancer	(Red) raspberry	Partial
Ross et al 2006 [86]	In vitro (4d)	Human cervical cancer cells (HeLa)	Extract, unbound fraction (anthocyanin rich), LH20 bound (ellagitannin rich)	Examine the anti-proliferative effects of extracts enriched in polyphenols but no Vitamin C or carotenoids on cervical cancer cells	Extract inhibited proliferation of HeLa cells. LH-20 bound fraction more effective (EC_{50} = 13 µg/mL) than raspberry extract EC_{50} value of 17.5 lg/mL GAE but unbound less effective (EC_{50} = 67 µg/mL)	Cancer	Raspberry	Yes
Seeram, et al 2006 [87]	In vitro (48h)	Human oral, breast, colon, prostate tumour cell lines	Blackberry, black raspberry, red raspberry extracts. Tested at 25, 50, 100, and 200µg/mL concentrations	Ability of berry extracts to inhibit proliferation of a range of tumour cell lines. Ability of 200µg/mL berry extracts to stimulate apoptosis of COX-2 enzyme expressing colon cancer cell line	With increasing berry concentration, inhibition of cell proliferation increased in all cell lines with different degrees of potency. Black raspberry significantly induced apoptosis in colon cell lines 3 fold over controls. Other berry extracts increased apoptosis but not significantly	Cancer	(Black and red) raspberry	Yes
Smith et al 2004 [88]	In vitro (NR)	Aroclor 1254-induced Sprague-Dawley rat S9 liver	Fresh fruit	Anti-carcinogenic effects of ellagic acid	Fresh raspberry juice suppressed Methyl methanesulfonate (MMS) by 57% and BP by 49%.	Cancer	Raspberry	Partial
Wu et al	In vitro (24hrs)	Colon cancer HT-29 cells	Phenolic extract,	Anti-proliferation	Raspberries inhibited cell	Cancer	(Red) raspberry	Partial

Study	Design (duration)	Studies/participants (age)	Source/Dose/ Nutrients	Measurement	Outcome	Disease state	Rubus	Supportive
2007 [89]			10g frozen fruit		growth the least out of all the berries tested (20-30% at 60mg/mL).			
Xue et al [90] 2001	In vitro (7d)	Syrian hamster embryo cell model	Freeze dried extract	Anti-transformation activity (Chemopreventative activity)	Methanol extracted black raspberry decreased morphological transformation 18-84% in a dose-dependent manner	Cancer	(Black) raspberry	Yes
Zunino et al 2010 [91]	In vitro (72hrs)	SEM, RS4;11 and REH acute lymphoblastic leukeamia cell lines	Polyphenolic enriched extracts, 200g fresh fruit	Cell death and mitochondrial membrane depolorization	S phase arrest was also observed for SEM and REH cells with blackberry extract	Cancer	Raspberry	No
Bowen-Forbes et al 2009 [74]	In vitro (72hrs)	MCF-7 (breast), SF-268 (CNS), NCI-H460 (lung), HCT-116 (colon) and AGS (gastric) human tumour cells	Lyophilised, powered extract, Dose NR	Inhibition of cell proliferation from selected phytonutrients	Blackberry inhibition of colon, breast, lung, and gastric human tumour cells by 50, 24, 54 and 37%, respectively.	Cancer	Blackberry	Yes
Dai et al 2007 [92]	In vitro (72hrs)	HT-29 human colorectal cancer cells	Frozen, lyophilized puree, Total anthocyanin and phenolic content, anthocyanin composition, 5.1-37.3µg	Tumour inhibition	49.2µg of total anthocyanins/mL inhibiting HT-29 cell growth by up to 66% at 72 hours and a non-significant effect at 13.6µg	Cancer	Blackberry	Yes
Ding et al 2006[93]	In vivo and animal (21wks)	JB6 cells and male and female mice (6–9wks old, C57BL/6 crossed with DBA2) and Male nu/nu homozygous nude mice (8 wks old)	Frozen lyophilized extract, Cyanidin-3-glucoside (C3G)	TPA-induced cell transformation (cellular) and incidence of papillomas and lung carcinoma (animal)	C3G inhibited proliferation of a human lung carcinoma cell line. C3G produced a dose-dependent decrease in AP-1, NF-_B, COX-2, and TNFalpha activity/expression TPA-induced cell transformation was significantly inhibited at the concentration range from 10-40um.A decrease in the number and size of tumors per mouse, >53% inhibition of papillomagenesis. At the concentration of 40 um, the growth of lung tumour cells was	Cancer	Blackberry	Yes

Study	Design (duration)	Studies/participants (age)	Source/Dose/ Nutrients	Measurement	Outcome	Disease state	Rubus	Supportive
					completely blocked.			
Elisia et al 2006[94]	In vitro (48hrs)	human embryonic-derived intestinal cell line (INT-407)	Crude extract, 0.01-1mg/mL anthocyanin from 100g fruit	peroxyl radical-induced chemical and intracellular oxidation	Reduced fluorescence but not reduced viability of cells	Cancer	Blackberry	Yes
Elisia et al 2008 [95]	In vitro (25hrs)	Human adenocarcinoma colon cancer (Caco-2) cells	Anthocyanins extract, from 100g frozen berries, 0.8, 1.6, 3.1, 6.3, 12.5, and 25µg/ml	Oxidative damage and associated cytotoxicity	Suppression of caco-2 intracellular oxidation in a concentration-dependent manner, with an IC50 value of 6.5±0.3µg/mL. Extract alone had no effect on Caco-2 cell cycle progression.	Cancer	Blackberry	Yes
Feng et al 2004 [96]	In vitro (72hrs)	Human lung cancer cell line A549	Fresh fruit extract, 0.3 and 3mg protein/mL	Cell proliferation	Extract inhibited tumour promoter-induced carcinogenesis and the related cell signalling. A concentration-dependent reduction in resistance to the extract resulting in a deceased cell proliferation.	Cancer	Blackberry	Yes
Hogan et al 2010 [77]	In vitro (72hrs)	C-6 rat brain glioma cells and MDA-468 human breast cancer cells	Anthocyanin-rich extracts, 50, 100, and 200 µg/mL	Antioxidant ability and antiproliferative activity	No effect on the growth of human cells	Cancer	Blackberry	No
McDougall et al 2008 [84]	In vitro (72hrs)	HeLa Human cervical Cancer and CaCo-2 human colon cancer cells	Freeze dried polyphenol rich fraction from frozen fruit, 25, 50, 75µg/mL	Cell viability and proliferation	~70% (blackberry) viability of cells at 50µg/mL minimal dose dependant inhibition. (Actual values NR)	Cancer	Blackberry	Partial
Seeram, et al 2006 [87]	In vitro (48hrs)	Human oral, breast, colon, prostate tumour cell lines	Blackberry, black raspberry, red raspberry extracts. Tested at 25, 50, 100, and 200 µg/mL concentrations	Ability of berry extracts to inhibit proliferation of a range of tumour cell lines. Ability of 200 µg/mL berry extracts to stimulate apoptosis of COX-2 enzyme expressing colon cancer cell line	With increasing berry concentration, inhibition of cell proliferation increased in all cell lines with different degrees of potency. Extracts increased apoptosis but not significantly.	Cancer	Blackberry	Partial
Tate et al 2003 [97]	In vitro (48hrs)	S9 phenobarbital-induced rat liver extract	8 varieties of blackberry extract, crushed, 200µL	Anti-carcinogenic potential	All varieties suppressed the mutagenesis. <10% mutagenesis observed when mixture of carcinogen and berry extract was used, compared to the	Cancer	Blackberry	Partial

Study	Design (duration)	Studies/participants (age)	Source/Dose/ Nutrients	Measurement	Outcome	Disease state	Rubus	Supportive
					mutagenesis produced by the carcinogen alone. However, no sig effect on MMS mutagenesis.			
Zunino et al 2010 [91]	In vitro (72hrs)	SEM, RS4;11 and REH acute lymphoblastic leukemia cell lines	Polyphenolic enriched extracts, 200g fresh fruit	Cell death and mitochondrial membrane depolorization	S phase arrest was also observed for SEM and REH cells with blackberry extract	Cancer	Blackberry	Yes
Kim et al 2011 [98]	In vitro (48hrs)	Rat LLC-PK cultured renal epithelial cell line	triterpenoid glycoside niga-ichigoside F1 (NIF1) and aglycone 23-hydroxytormentic acid (23-HTA) extract, 25, 50, 100, 200μm	Cell viability	Significant suppression of induced translocation in a concentration-dependent manner, 23-HTA was found to have a greater effect on the activity of antioxidant enzymes than NIF1	Inflammation	Raspberry	Yes
Park et al 2006 [99]	In vivo (12hrs)	RAW264.7 Murine macrophages	Ripe and unripe Rubus coreanus Ethanol and aqueous extracts of both unripe (URCE 14%, URCW 31.6% and ripe fruit (RCE 49%, RCW 54.4%)	Effects of 4 extracts on lipopolysaccharide-induced inflammatory responses	No cytotoxicity was found in RAW264.7 cells from the 4 extracts. Only URCE suppressed LPS-induced nitrite production in dose-dependent way. URCE significant increase in PGE2 production. iNOS & COX-2 reduced in dose-dependent manner. Results stronger when cells preincubated with URCE	Inflammation	(Black) raspberry	Partial
Cuevas-Rodgruiges et al 2010 [100]	In vitro (48hrs)	RAW 264.7 macrophages	Freeze dried polyphenols, anthocyanins, and proanthocyanidins extract, from 100g ripe wild berries	Inhibition of pro-inflammatory responses	Proanthocyanidin-rich fraction from the domesticated noncommercial breeding line exhibited the highest NO inhibitory activities (16.1, 15.1 μM), proanthocyanidin-rich fractions from the wild Rubus adenotrichus cultivar showed the highest inhibition of iNOS expression (8.3μM), polyphenolic-rich fractions from Rubus corifolius and the domesticated noncommercial	Inflammation	Blackberry	Yes

Study	Design (duration)	Studies/participants (age)	Source/Dose/ Nutrients	Measurement	Outcome	Disease state	Rubus	Supportive
					breeding line were potent inhibitors of COX-2 expression.			
Cuevas-Rodgruiges et al 2010 [100]	In vitro (48hrs)	RAW 264.7 macrophages	Freeze dried polyphenols, anthocyanins, and proanthocyanidins extract, from 100g ripe wild berries	Inhibition of pro-inflammatory responses	Proanthocyanidin-rich fraction from the domesticated noncommercial breeding line exhibited the highest NO inhibitory activities (16.1, 15.1 μM), proanthocyanidin-rich fractions from the wild Rubus adenotrichus cultivar showed the highest inhibition of iNOS expression (8.3μM), polyphenolic-rich fractions from Rubus corifolius and the domesticated noncommercial breeding line were potent inhibitors of COX-2 expression.	Inflammation	Blackberry	Yes
Rossi et al 2003 [53]	Animal (4hrs)	Male Sprague-Dawley rats (NR)	Extract obtained from blackberry fruits (10, 30mg/kg) given 30 mins before carrageenan	Effects of anthocyanins in blackberry extract on carrageenan induced pleurisy, specifically lung injury, formation of nitrotyrosine, increases in NO production & PARS activation.	Anthocyanins at highest dose significantly reduced degree of lung injury and infiltration of polymorphonuclear leukocytes compared to controls. Production of NOx reduced in dose-dependent manner and production of PGE_2 significantly reduced in anthocyanin-treated rats.	Inflammation	Blackberry	Yes
Shin et al 2006 [57]	Animal (20mins)	Male ICR mice and male Sprague-Dawley rats (n=10/group)	From 0.001-1g/kg BW dried unripe fruits (RCF) 1hr prior to injection of allergic compound. 1g/1kg BW given anally 5, 10 and 20 mins after injection	Effect of RCF on induced local allergic reaction by anal therapy	Mortality from allergic compound RCF dose dependent, decreasing mortality with increasing dose of RCF. 100% mortality if injected 20 mins ranging to 0 mortality if injected with RCF after 5 mins. RCF inhibits mast-cell mediated anaphylaxis.	Inflammation/ Allergic reaction	(Black) raspberry	Yes
Liu et al 2005 [101]	In vitro (14d)	Human placental vein angiogenesis model	Freeze dried powder, from 908g frozen fruit,	Initiation of Angiogenesis and extent of vessel growth	At 0.075% and 0.1% w/v fractions completely inhibited angiogenic initiation and vessel	Wound healing/cancer therapy	(Black) raspberry	Yes

Study	Design (duration)	Studies/participants (age)	Source/Dose/ Nutrients	Measurement	Outcome	Disease state	Rubus	Supportive
			0.01, 0.025, 0.05, and 0.075% w/v fractions		growth. A 43, 33, 13, and 0%, inhibition of angiogenic response with increasing concentration.			
Barnett et al 2007 [54]	Animal (28d)	Male Sprague-Dawley rats (8-wk old, n=54)	Freeze dried powder extract. 2 and 10%	Biomarkers of oxidative damage to protein (carbonyls), lipids (malondialdehyde), and DNA (8-oxo-2_-deoxyguanosine urinary excretion) and plasma antioxidant status (antioxidant capacity, vitamin E)	In vivo antioxidant function, raised antioxidant status of plasma, decreased biomarkers of oxidative damage though highly modifiable by basal diet	Oxidative stress	Boysenberry	Yes
Rouanet et al 2010 [102]	Animal (12wks)	Male Golden Syrian Hamsters (n=60 male)	Equivalent of 275mL/d for 70kg human raspberry juice (also strawberry, bilberry juice, green & black tea) by gavage	Evaluated the effect of juices, green and black tea on a high fat diet	Significantly reduced aortic fatty streak areas 1.1 ± 0.2% compared to controls (water) 21.2 ± 2.7%. Lowest of all juices. No significant differences between berry groups and controls for circulating total, HDL- or non-HDL-cholesterols.	Atherosclerosis	Raspberry	Yes
Serraino et al 2003 [103]	In vitro (10 min pretreatment)	Human umbilical vein endothelial cells (huvecs)	Fruit extract with 80ppm, 40ppm or 14.5ppm cyanidin-3-O-glucoside	Juice containing cyanidin-3-O-glucoside had a protective effect against peroxynitrite-mediated endothelial dysfunction & vascular failure.	Blackberry juice & cyanidin-3-O-glucoside reduced peroxynitrite-induced oxidation (dose-dependent). Protective agains cytotoxicity in huvecs cells. Prevent peroxynitrite-mediated endothelial dysfunction & suppression of vascular contractility.	Vascular failure and endothelial dysfunction	Blackberry	Yes
Prior et al 2009[55]	Animal (91d)	Male C587BL/6J mice (21 days old, n=12 per treatment)	High fat (45% kcalfat) + 10% black raspberry powder. Low fat (10% kcals fat) + 10% Black raspberry powder.	Determine how black raspberry treatment influences serum lipids and cytokines	Significant increase in weight gain of High fat/BRB mice compared to high fat only diet mice and increased % body fat. Liver lipids elevated in high fat/BRB compared to Low fat/BRB mice. Liver cholesterol levels not altered.	Obesity	Black raspberry	No
Hassan & Yousef 2009	Animal (5wks)	Albino rats (NR, n=40	Juice, 1: untreated, 2:	Reduced hepatotoxicity and oxidative stress.	NaF induced decrease in SOD, CAT, GSH TAC and HDL-c –	Flurosis (dental)	Blackberry	Yes

Study	Design (duration)	Studies/participants (age)	Source/Dose/ Nutrients	Measurement	Outcome	Disease state	Rubus	Supportive
[62]			1.6g/kg bw black berry juice 3: 10.3mg/kg bw NaF, 4: combination	Increase/decrease in activities of superoxide dismutase (SOD), catalase (CAT) total antioxidant capacity (TAC) glutathionine (GSH)	protective against NaF-induced hepatotoxicity			
Aiyer et al 2011 [59]	Animal (2-4wks)	Male SD rats (8 wk old; n =3–5)	Freeze dried supplement, 2.5% w/w	Reflux and supplementation post oesophagoduodenal anastomosis surgery	Lower weight gain ($p<0.05$), higher oesophagitis scores ($P<0.001$), no alteration or amelioration of oesophagitis or Barrett's oesophagus. Lower lipid peroxidation at wk4, increased MDA at wk2	Reflux-induced oesophagitis	(Black) raspberry	No
Lee et al 2009 [60]	Animal (7d)	Male male Sprague-Dawley rats (NR)	Unripe fruit, 15kg fruit, 1.5-2.5% ellagic extract. 250, 500mg/kg body weight extract orally for 6d	Hepatic lipid peroxide and glutathione content and activity	Pretreatment with extract prevented the AAP-induced hepatotoxicity at 500mg by 50%, 250mg doses decreased the effect without significance. Dose dependant inhibition of membrane lipid peroxidation	Liver failure/injury	(Red) raspberry	Yes
Zhang et al 2011 [61]	Animal (1d)	Male rats (NR, N=48)	Frozen cultivated type (T), immature cultivated fruit (F), mature wild raspberry fruit (R), 2 and 5 mg/kg body weight	Diuretic activity	Highest total urine output from HCTZ +ve control group (2.50 vs 1.30 contro). Ony HCTZ sigl. Higher urine output after 1hr compared to control. Total urine volume of RM group significantly higher than control (2.32 vs 1.30) – T or F did not cause a significant increase. No significant increase was observed in water extracted groups. All 3 water raspberry extracts similar electrolyte patterns to control. R methanol group higher Na^+ than control, similar to HCTZ group. No increase in K^+ in RM group.	Renal function	Raspberry	Yes
Do et al 2008[104]	Animal (10wks)	Female Ovariectomised Wistar rats (10wks old, n=84	100, 200 and 400mg/kg extract	Biomarkers of bone metabolism	Increased osteoblast differentiation and bone resorption markers, dose	Post menopausal osteoporosis	(Black) raspberry	Yes

Study	Design (duration)	Studies/participants (age)	Source/Dose/ Nutrients	Measurement	Outcome	Disease state	Rubus	Supportive
					dependant oesteoclast apoptosis			

TAOC: Total antioxidant capacity, NR: Not reported

ANNOTATED BIBLIOGRAPHY

Aiyer, H. S., Y. Li, et al. (2011). "Dietary freeze-dried black raspberry's effect on cellular antioxidant status during reflux-induced esophagitis in rats." Nutrition **27**(2): 182-187.

Introduction: Esophageal cancer consists of two distinct types, esophageal adenocarcinoma (EAC) and squamous cell carcinoma, both of which differ significantly in their etiology. Freeze-dried black raspberry (BRB) has been consistent in its ability to modulate the biomarkers and reduce the incidence of carcinogen-induced squamous cell carcinoma in rats. In our previous studies in the esophagoduodenal anastomosis (EDA) model, we have shown that the early modulation of manganese superoxide dismutase (MnSOD) significantly correlates with the development of reflux-induced EAC in rats. In this study we looked at the short-term effects of a BRB-supplemented diet on the modulation of antioxidant enzymes in reflux-induced esophagitis. Methods: Male SD rats (8 wk old; n = 3-5) were randomized into three groups---sham-operated, fed control AIN-93M diet (SH-CD), EDA operated and fed either control diet (EDA-CD) or 2.5% (w/w) BRB diet (EDA-BRB). The effect of both reflux and dietary supplementation was analyzed 2 and 4 wk after EDA surgery. Results: Animals in the EDA groups had significantly lower weight gain and diet intake compared to SH-CD ($P < 0.05$). The sham-operated animals received an average esophagitis score of 0.1 ± 0.1; this increased significantly in EDA-CD animals to 1.8 ± 0.14 ($P < 0.001$ versus SH-CD) and in EDA-BRB group to 1.7 ± 0.06 ($P < 0.001$ versus SH-CD), with BE changes also present. However, dietary supplementation of BRB did not alter or ameliorate the grade of esophagitis or the induction of BE. BRB diet caused a 43% increase in MnSOD levels compared to EDA-CD (0.73 ± 0.16; $P = 0.09$); however, this effect was not statistically significant and at 4 wk, EDA-CD (0.58 ± 0.12) showed an increase in MnSOD expression compared to SH-CD (0.34 ± 0.01). Conclusions: In conclusion, our data suggest that dietary BRB does not increase the levels of cellular antioxidant enzymes or reduce the levels of lipid peroxidation compared to a control diet, in a short-term study of gastroesophageal reflux induction in the EDA animal model. However, it remains to be tested whether this is indicative of its ineffectiveness to inhibit reflux-induced EAC incidence over the long term.

Aiyer, H. S., C. Srinivasan, et al. (2008). "Dietary berries and ellagic acid diminish estrogen-mediated mammary tumorigenesis in ACI rats." Nutrition and Cancer **60**(2): 227-234.

Estrogen acts as a complete mammary carcinogen in ACI rats. Prevention studies in this model allowed us to identify agents that are effective against estrogen-induced mammary carcinogenesis. In this study, we investigated efficacy of dietary berries and ellagic acid to reduce estrogen-mediated mammary tumorigenesis. Female ACI rats (8-9 wk) were fed either AIN-93M diet (n = 25) or diet supplemented with either powdered blueberry (n = 19) and black raspberry (n = 19) at 2.5% wt/wt each or ellagic acid (n = 22) at 400 ppm. Animals received implants of 17β-estradiol 2 wk later, were palpated periodically for mammary tumors, and were euthanized after 24 wk. No differences were found in tumor incidence at 24 wk; however, tumor volume and multiplicity were reduced significantly after intervention. Compared with the control group (average tumor volume = 685 ± 240 mm3 and tumor multiplicity = 8.0 ± 1.3), ellagic acid reduced the tumor volume by 75% ($P < 0.005$) and tumor multiplicity by 44% ($P < 0.05$). Black raspberry followed closely, with tumor volume diminished by > 69% ($P < 0.005$) and tumor multiplicity by 37% ($P = 0.07$). Blueberry showed a reduction (40%) only in tumor volume. This is the first report showing the significant efficacy of both ellagic acid and berries in the prevention of solely estrogen-induced mammary tumors.

Barnett, L. E., A. M. Broomfield, et al. (2007). "The in vivo antioxidant action and the reduction of oxidative stress by boysenberry extract is dependent on base diet constituents in rats." Journal of Medicinal Food **10**(2): 281-289.

Dietary antioxidants are often defined by in vitro measures of antioxidant activity. Such measures are valid indicators of the antioxidant potential, but provide little evidence of activity as a dietary antioxidant. This study was undertaken to assess the in vivo antioxidant efficacy of a berry fruit extract by measuring biomarkers of oxidative damage to protein (carbonyls), lipids (malondialdehyde), and DNA (8-oxo-2′-deoxyguanosine urinary excretion) and plasma antioxidant status (antioxidant capacity, vitamin E) in rats when fed basal diets containing fish and soybean oils, which are likely to generate different levels of oxidative stress. Boysenberry (Rubus loganbaccus X baileyanus Britt) extract was used as the dietary antioxidant. The basal diets (chow, synthetic/soybean oil, or synthetic/fish oil) had significant effects on the biomarkers of oxidative damage and antioxidant status, with rats fed the synthetic/fish oil diet having the lowest levels of oxidative damage and the highest antioxidant status. When boysenberry extract was added to the diet, there was little change in 8-oxo-2′-deoxyguanosine excretion in urine, oxidative damage to proteins decreased, and plasma malondialdehyde either increased or decreased depending on the basal diet. This study showed that boysenberry extract functioned as an in vivo antioxidant and raised the antioxidant status of plasma while decreasing some biomarkers of oxidative damage, but the effect was highly modified by basal diet. Our results are further evidence of complex interactions among dietary antioxidants, background nutritional status as determined by diet, and the biochemical nature of the compartments in which antioxidants function.

Bowen-Forbes, C. S., V. Mulabagal, et al. (2009). "Ursolic acid analogues: non-phenolic functional food components in Jamaican raspberry fruits." Food Chemistry **116**(3): 633-637.

The Rubus genus produces numerous species that are known for their medicinal properties. Rubus rosifolius, called the red raspberry, grows wild in elevated regions in Jamaica. Phytochemical examination of the ethyl acetate extract of the fruit yielded eight compounds of the 19-α-hydroxyursane type: euscaphic acid (1), 1-β-hydroxyeuscaphic acid (2), hyptatic acid B (3), 19α-hydroxyasiatic acid (4), trachelosperogenin (5), 4-epi-nigaichigoside F1 (6), nigaichigoside F1 (7), and trachelosperoside B-1 (8), as confirmed by NMR spectroscopy. Inhibition of cell proliferation by these compounds were determined by using MCF-7 (breast), SF-268 (CNS), NCI H460 (lung), HCT-116 (colon) and AGS (gastric) human tumour cells. Among the human tumour cell lines assayed, only compounds 3 and 6 displayed significant growth inhibition and was specific to colon tumour cells by 56% and 40%, respectively. These ursolic acid analogues were also tested for anti-inflammatory activity using in vitro cycloxegenase-1 (COX-1) and cycloxegenase-2 (COX-2) enzyme inhibitory assays. Compounds 1, 2 and 3 showed selective COX-1 enzyme inhibitory activity (13%, 25% and 35%) at 25 μg/ml. In the lipid peroxidation (LPO) inhibitory assays, compounds 2, 4, 7 and 6 inhibited LPO by 62%, 60%, 53% and 68%, respectively, at 25 μg/ml.

Bowen-Forbes, C. S., Y. Zhang, et al. (2010). "Anthocyanin content, antioxidant, anti-inflammatory and anticancer properties of blackberry and raspberry fruits." Journal of Food Composition and Analysis **23**(6): 554-560.

Fresh or processed berries are considered to be beneficial for health by many consumers. Fruits of closely related species of plants sometimes possess strikingly different phytochemistry and biological activities. Therefore, even though similar research has been conducted on a number of Rubus berries, there is much relevance associated with the investigation of species that have not been previously studied. In the current report, the fruits of three wild Jamaica-grown species: Rubus jamaicensis, Rubus rosifolius and Rubus racemosus, and of the Michigan-grown Rubus acuminatus, Rubus idaeus cv. Heritage and Rubus idaeus cv. Golden were analyzed for their anthocyanin contents, and lipid peroxidation, cyclooxygenase enzyme and human tumor cell proliferation inhibitory activities. It was revealed that the fruits contained superior levels of anthocyanins (146-2199. mg/100. g fresh weight) to those previously reported for other raspberry and blackberry species, and their hexane, EtOAc and MeOH extracts showed good antioxidant activity, the majority of the extracts exhibiting over 50% lipid peroxidation inhibitory activity at 50μg/mL. The hexane extracts of the Jamaican Rubus spp. demonstrated moderate COX inhibitory activity (27.5-33.1%) at 100μg/mL, and exhibited the greatest potential to inhibit cancer cell growth, inhibiting colon, breast, lung, and gastric human tumor cells by 50, 24, 54 and 37%, respectively. The high anthocyanin content and biological activities of these fruits indicate that their consumption would be beneficial to health, and that they may be useful in the production of functional foods containing an efficacious dose of anthocyanins.

Cuevas-Rodríguez, E. O., V. P. Dia, et al. (2010). "Inhibition of pro-inflammatory responses and antioxidant capacity of mexican blackberry (Rubus spp.) extracts." Journal of Agricultural and Food Chemistry **58**(17): 9542-9548.

Total polyphenolic and anthocyanin- and proanthocyanidin-rich fractions from wild blackberry genotypes (WB-3, WB-7, WB-10, and WB-11), a domesticated noncommercial breeding line (UM-601), and a commercial cultivar (Tupy) were evaluated for inhibition of pro-inflammatory responses [nitric oxide (NO) production, inducible nitric oxide synthase (iNOS) expression, cyclooxygenase-2 (COX-2) expression, and prostaglandin E2 (PGE2)] in RAW 264.7 macrophages stimulated by lipopolysaccharide (LPS). At 50 μM [cyanidin-3-O-glucoside (C3G) or catechin equivalent], most fractions significantly (P < 0.05) inhibited all markers. The anthocyanin-rich fraction from WB-10 and the proanthocyanidin-rich fraction from UM-601 exhibited the highest NO inhibitory activities (IC50 = 16.1 and 15.1 μM, respectively). Proanthocyanidin-rich fractions from the wild WB-10 showed the highest inhibition of iNOS expression (IC50 = 8.3 μM). Polyphenolic-rich fractions from WB-7 and UM-601 were potent inhibitors of COX-2 expression (IC50 = 19.1 and 19.3 μM C3G equivalent, respectively). For most of the extracts, antioxidant capacity was significantly correlated with NO inhibition. Wild genotypes of Mexican blackberries, as rich sources of polyphenolics that have both antioxidant and anti-inflammatory properties, showed particular promise for inclusion in plant improvement programs designed to develop new varieties with nutraceutical potential.

Dai, J., J. D. Patel, et al. (2007). "Characterization of blackberry extract and its antiproliferative and anti-inflammatory properties." Journal of Medicinal Food **10**(2): 258-265.

Blackberries are rich in polyphenols, including anthocyanins. Polyphenols are hypothesized to have biological activities that may impact positively on human health. In these studies, an anthocyanin-rich extract from Hull blackberries grown in Kentucky was obtained and fully characterized in terms of total anthocyanin and phenolic content, polymeric color, anthocyanin composition, and total antioxidant capacity. In vitro cell culture studies showed that the blackberry extract inhibited HT-29 colon tumor cell growth in a concentration-dependent manner with 49.2 μg of total anthocyanins/mL inhibiting HT-29 cell growth up to 66% at 72 hours. Likewise, in a concentration-dependent manner, total anthocyanin concentrations in the range of 0-40 μg/mL suppressed both high-dose (10 μg/mL) and low-dose (0.1 μg/mL) lipid A-induced interleukin-12 release from mouse bone marrow-derived dendritic cells. These results suggest that Hull blackberry extract (HBE) has potent antioxidant, antiproliferative, and anti-inflammatory activities and that HBEformulated products may have the potential for the treatment and/or prevention of cancer and/or other inflammatory diseases.

Ding, M., R. Feng, et al. (2006). "Cyanidin-3-glucoside, a natural product derived from blackberry, exhibits chemopreventive and chemotherapeutic activity." Journal of Biological Chemistry **281**(25): 17359-17368.

Epidemiological data suggest that consumption of fruits and vegetables has been associated with a lower incidence of cancer. Cyanidin-3-glucoside (C3G), a compound found in blackberry and other food products, was shown to possess chemopreventive and chemotherapeutic activity in the present study. In cultured JB6 cells, C3G was able to scavenge ultraviolet B-induced .OH and O2.- radicals. In vivo studies indicated that C3G treatment decreased the number of non-malignant and malignant skin tumors per mouse induced by 12-O-tetradecanolyphorbol-13-acetate (TPA) in 7,12-dimethyl-benz[a]anthracene-initiated mouse skin. Pretreatment of JB6 cells with C3G inhibited UVB- and TPA-induced transactivation of NF-κB and AP-1 and expression of cyclooxygenase-2 and tumor necrosis factor-α. These inhibitory effects appear to be mediated through the inhibition of MAPK activity. C3G also blocked TPA-induced neoplastic transformation in JB6 cells. In addition, C3G inhibited proliferation of a human lung carcinoma cell line, A549. Animal studies showed that C3G reduced the size of A549 tumor xenograft growth and significantly inhibited metastasis in nude mice. Mechanistic studies indicated that C3G inhibited migration and invasion of A549 tumor cells. These finding demonstrate for the first time that a purified compound of anthocyanin inhibits tumor promoter-induced carcinogenesis and tumor metastasis in vivo.

Do, S. H., J. W. Lee, et al. (2008). "Bone-protecting effect of Rubus coreanus by dual regulation of osteoblasts and osteoclasts." Menopause **15**(4): 676-683.

Objective: Bone loss occurs with increasing age and/or as a secondary occurrence to chronic metabolic disease. Certain nutritional and pharmacological, as well as nonpharmacologic interventions such as weight-bearing exercise and muscle strengthening help prevent bone loss. We examined the effect of the methanol extract from the fruit of Rubus coreanus (RCM) on postmenopausal osteoporosis. Design: Ovariectomized rats were assigned to sham (negative control), vehicle control, positive control, safflower seed 200 mg/kg, RCM 100 mg/kg (RCM 100), RCM 200 mg/kg (RCM 200), and RCM 400 mg/kg (RCM 400) groups for 10 weeks after the operation. Serum biochemistry, histochemistry, immunohistochemistry, and other related biomarkers of bone metabolism were investigated. Results: We observed that RCM could prevent bone loss by increasing the femur trabecular bone area in a dose-dependent manner in ovariectomized rats. The mineral composition of RCM contains many more valuable elements, especially potassium, magnesium, and vitamins D and B2, than safflower seed. The effect of RCM increased not only osteoblast differentiation but also osteoclast apoptosis. In addition, the extract of RCM contained in quercetin suggests that the extract of RCM resulted in improved aging-related bone loss through an antioxidant effect. Conclusions: The present data provide the first direct in vivo evidence that RCM has a bone-protecting effect caused by estrogen deficiency, without undesirable side effects on the uterus and other solid organs. The beneficial effect of RCM may be mediated, at least in part, by dual regulation of the enhancement of osteoblast function and induction of osteoclast apoptosis.

Elisia, I., C. Hu, et al. (2006). "Antioxidant assessment of an anthocyanin-enriched blackberry extract." Food Chemistry **101**(3): 1052-1058.

Gel filtration of black berry (Rubus fruticosus sp) ethanolic extracts was employed to obtain an anthocyanin-enriched extract. The anthocyanin profile identified cyanidin-3-glucoside as the primary (e.g., 90% of total) anthocyanin present in blackberry. Gel filtration of crude extracts resulted in a 20-fold increase in total anthocyanin content, with no change in the proportion of cyanidin-3-glucoside. Antioxidant activities of both the crude and anthocyanin-enriched blackberry extracts were determined using cell-free (ORAC) and cell-based (INT-407 intracellular) antioxidant assays. Antioxidant activity, assessed by the ORAC assay, indicated a 7-fold increase in activity for the anthocyanin-enriched fraction. Similar results were obtained for the anthocyanin-enriched extract using the intracellular antioxidant assay with INT-407 cells. Our results indicate that the anthocyanin content, and more specifically the presence of cyanidin-3-glucoside, in blackberry, contributes a major part of the antioxidant ability to suppress both peroxyl radical-induced chemical and intracellular oxidation.

Elisia, I. and D. D. Kitts (2008). "Anthocyanins inhibit peroxyl radical-induced apoptosis in Caco-2 cells." Molecular and Cellular Biochemistry **312**(1-2): 139-145.

The antioxidant activity of anthocyanins has been well characterized in vitro; many cases has been postulated to provide an important exogenous mediator of oxidative stress in the gastrointestinal tract. The objective of this study was to evaluate the efficacy of anthocyanin protection against peroxyl radical (AAPH)-induced oxidative damage and associated cytotoxicity in Caco-2 colon cancer cells. Crude blackberry extracts were purified by gel filtration column to yield purified anthocyanin extracts that were composed of 371 mg/g total anthocyanin, 90.1% cyanidin-3-glucoside, and 4.9 mmol Trolox equivalent/g (ORAC) value. There were no other detectable phenolic compounds in the purified anthocyanin extract. The anthocyanin extract suppressed AAPH-initiated Caco-2 intracellular oxidation in a concentration-dependent manner, with an IC50 value of 6.5 ± 0.3 μg/ml. Anthocyanins were not toxic to Caco-2 cells, but provided significant (P < 0.05) protection against AAPH-induced cytotoxicity, when assessed using the CellTiter-Glo assay. AAPH-induced cytoxicity in Caco-2 cells was attributed to a significant (P < 0.05) reduction in the G1 phase and increased proportion of cells in the sub G1 phase, indicating apoptosis. Prior exposure of Caco-2 cells to anthocyanins suppressed (P < 0.05) the AAPH-induced apoptosis by decreasing the proportion of cells in the sub-G1 phase, normalized the proportion of cells in other cell cycle phases. Our results show that the antioxidant activity of anthocyanins principally attributed to cyanidin-3-O-glucoside and common to blackberry, are effective at inhibiting peroxyl radical induced apoptosis in cultured Caco-2 cells.

Feng, R., L. L. Bowman, et al. (2004). "Blackberry extracts inhibit activating protein 1 activation and cell transformation by perturbing the mitogenic signaling pathway." Nutrition and Cancer **50**(1): 80-89.

Blackberries are natural rich sources of bioflavonoids and phenolic compounds that are commonly known as potential chemopreventive agents. Here, we investigated the effects of fresh blackberry extracts on proliferation of cancer cells and neoplastic transformation induced by 12-O-tetradecanoylphorbol-13- acetate (TPA), as well as the underlying mechanisms of signal transduction pathways. Using electron spin resonance, we found that blackberry extract is an effective scavenger of free radicals, including hydroxyl and superoxide radicals. Blackberry extract inhibited the proliferation of a human lung cancer cell line, A549. Pretreatment of A549 cells with blackberry extract resulted in an inhibition of 8-hydroxy-2′-deoxyguanosine (8-OHdG) formation induced by ultraviolet B (UVB) irradiation. Blackberry extract decreased TPA-induced neoplastic transformation of JB6 P+ cells. Pretreatment of JB6 cells with blackberry extract resulted in the inhibition of both UVB- and TPA-induced AP-1 transactivation. Furthermore, blackberry extract also blocked UVB- or TPA-induced phosphorylation of ERKs and JNKs, but not p38 kinase. Overall, these results indicated that an extract from fresh blackberry may inhibit tumor promoter-induced carcinogenesis and associated cell signaling, and suggest that the chemopreventive effects of fresh blackberry may be through its antioxidant properties by blocking reactive oxygen species-mediated AP-1 and mitogen-activated protein kinase activation.

Fortalezas, S., L. Tavares, et al. (2010). "Antioxidant properties and neuroprotective capacity of strawberry tree fruit (Arbutus unedo)." Nutrients **2**(2): 214-229.

Berries contain significant amounts of phyto chemicals, including polyphenols, which are reported to reduce cancer risk, coronary heart disease and other degenerative diseases. These effects are mainly attributed to the antioxidant capacity of polyphenols found in berries. Strawberry tree (Arbutus unedo) berries are used in folk medicine but seldom eaten as fresh fruits. Their phenolic profile and antioxidant capacity reveal a high potential, but they are not well characterized as a "health promoting food". The aim of this study was to assess the antioxidant properties of the edible strawberry tree fruit in vitro and in a neuro degeneration cell model. Raspberry (Rubus idaeus), a well documented health promoting fruit, was used as a control for comparison purposes. A. unedo yielded a similar content in polyphenols and a slightly lower value of total antioxidant capacity in comparison to R. idaeus. Although the chemically-measured antioxidant activity was similar between both fruits, R. idaeus increased neuro blastoma survival in aneuro degeneration cell model by 36.6% whereas A. unedo extracts caused no effect on neuroblastoma viability. These results clearly demonstrate that a promising level of chemically-determined antioxidant activity of a plant extract is not necessarily correlated with biological significance, as assessed by the effect of A. unedo fruit in a neuro degeneration cell model.

Ghosh, D., T. K. McGhie, et al. (2007). "Cytoprotective effects of anthocyanins and other phenolic fractions of Boysenberry and blackcurrant on dopamine and amyloid beta-induced oxidative stress in transfected COS-7 cells." Journal of the Science of Food and Agriculture **87**(11): 2061-2067.

There is growing interest both from consumers and researchers in the role that berries play in human health. In the experiments reported here, we assessed the ability of anthocyanins and phenolic fractions of Boysenberry and blackcurrant to ameliorate the deleterious effect of the amyloid beta(25-35) (100 mu mol L-1, 24 h) and dopamine (1 mmol L-1, 4 h) on calcium buffering (recovery) of M1 muscarinic receptor-transfected COS-7 cells. Cell viability was also studied. Our results demonstrate that extracts of Boysenberry and blackcurrant showed significant protective effect and restored the calcium buffering ability of cells that had been subjected to oxidative stress induced by dopamine and the amyloid beta(25-35). Blackcurrant polyphenolics showed slightly higher protective effect against dopamine, whereas Boysenberry polyphenolics had a higher effect against the amyloid beta(25-35). In viability studies, all extracts showed significant protective effects against dopamine and amyloid beta(25-35)-induced cytotoxicity. Our results provide further evidence for the protective effects of berries against the neurotoxic effect of dopamine and amyloid beta(25-35) in brain cells.

Han, C., H. Ding, et al. (2005). "Inhibition of the growth of premalignant and malignant human oral cell lines by extracts and components of black raspberries." Nutrition and Cancer **51**(2): 207-217.

Black raspberries are a rich natural source of chemopreventive phytochemicals. Recent studies have shown that freeze-dried black raspberries inhibit the development of oral, esophageal, and colon cancer in rodents, and extracts of black raspberries inhibit benzo(a)pyrene-induced cell transformation of hamster embryo fibroblasts. However, the molecular mechanisms and the active components responsible for black raspberry chemoprevention are unclear. In this study, we

found that 2 major chemopreventive components of black raspberries, ferulic acid and β-sitosterol, and a fraction eluted with ethanol (RO-ET) during silica column chromatography of the organic extract of freeze-dried black raspberries inhibit the growth of premalignant and malignant but not normal human oral epithelial cell lines. Another fraction eluted with CH 2Cl2/ethanol (DM:ET) and ellagic acid inhibited the growth of normal as well as premalignant and malignant human oral cell lines. We investigated the molecular mechanisms by which ferulic acid and β-sitosterol and the RO-ET fraction selectively inhibited the growth of premalignant and malignant oral cells using flow cytometry and Western blotting of cell cycle regulatory proteins. There was no discernable change in the cell cycle distribution following treatment of cells with the RO-ET fraction. Premalignant and malignant cells redistributed to the G2/M phase of the cell cycle following incubation with ferulic acid, β-sitosterol treated premalignant and malignant cells accumulated in the G0/G1 and G2/M phases, respectively. The RO-ET fraction reduced the levels of cyclin A and cell division cycle gene 2 (cdc2) in premalignant cells and cyclin B1, cyclin D1, and cdc2 in the malignant cell lines. This fraction also elevated the levels of p21waf1/cip1 in the malignant cell line. Ferulic acid treatment led to increased levels of cyclin B1 and cdc2 in both cell lines, and p21waf1/cip1 was induced in the malignant cell line, β-sitosterol reduced the levels of cyclin B1 and cdc2 while increasing p21waf1/cip1 in both the premalignant and malignant cell lines. These results show for the first time that the growth inhibitory effects of black raspberries on premalignant and malignant human oral cells may reside in specific components that target aberrant signaling pathways regulating cell cycle progression. Copyright

Harada, N., K. Okajima, et al. (2008). "Effect of topical application of raspberry ketone on dermal production of insulin-like growth factor-I in mice and on hair growth and skin elasticity in humans." Growth Hormone and IGF Research **18**(4): 335-344.

Sensory neurons release calcitonin gene-related peptide (CGRP) on activation. We recently reported that topical application of capsaicin increases facial skin elasticity and promotes hair growth by increasing dermal insulin-like growth factor-I (IGF-I) production through activation of sensory neurons in mice and humans. Raspberry ketone (RK), a major aromatic compound contained in red raspberries (Rubus idaeus), has a structure similar to that of capsaicin. Thus, it is possible that RK activates sensory neurons, thereby increasing skin elasticity and promoting hair growth by increasing dermal IGF-I production. In the present study, we examined this possibility in mice and humans. RK, at concentrations higher than 1 μM, significantly increased CGRP release from dorsal root ganglion neurons (DRG) isolated from wild-type (WT) mice and this increase was completely reversed by capsazepine, an inhibitor of vanilloid receptor-1 activation. Topical application of 0.01% RK increased dermal IGF-I levels at 30 min after application in WT mice, but not in CGRP-knockout mice. Topical application of 0.01% RK increased immunohistochemical expression of IGF-I at dermal papillae in hair follicles and promoted hair re-growth in WT mice at 4 weeks after the application. When applied topically to the scalp and facial skin, 0.01% RK promoted hair growth in 50.0% of humans with alopecia ($n = 10$) at 5 months after application and increased cheek skin elasticity at 2 weeks after application in 5 females ($p < 0.04$). These observations strongly suggest that RK might increase dermal IGF-I production through sensory neuron activation, thereby promoting hair growth and increasing skin elasticity.

Harris, G. K., A. Gupta, et al. (2001). "Effects of lyophilized black raspberries on azoxymethane-induced colon cancer and 8-hydroxy-2′-deoxyguanosine levels in the fischer 344 rat." Nutrition and Cancer **40**(2): 125-133.

This study examined the effects of lyophilized black raspberries (BRB) on azoxymethane (AOM)-induced aberrant crypt foci (ACF), colon tumors, and urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels in male Fischer 344 rats. AOM was injected (15 mg/kg body wt ip) once per week for 2 wk. At 24 h after the final injection, AOM-treated rats began consuming diets containing 0%, 2.5%, 5%, or 10% (wt/wt) BRB. Vehicle controls received 5% BRB or diet only. Rats were sacrificed after 9 and 33 wk of BRB feeding for ACF enumeration and tumor analysis. ACF multiplicity decreased 36%, 24%, and 21% ($P < 0.01$ for all groups) in the 2.5%, 5%, and 10% BRB groups, respectively, relative to the AOM-only group. Total tumor multiplicity declined 42%, 45%, and 71% ($P < 0.05$ for all groups). Although not significant, a decrease in tumor burden (28%, 42%, and 75%) was observed in all BRB groups. Adenocarcinoma multiplicity decreased 28%, 35%, and 80% ($P < 0.01$) in the same treatment groups. Urinary 8-OHdG levels were reduced by 73%, 81%, and 83% ($P < 0.01$ for all groups). These results indicate that BRB inhibit several measures of AOM-induced colon carcinogenesis and modulate an important marker of oxidative stress in the Fischer 344 rat.

Hassan, H. A. and M. I. Yousef (2009). "Mitigating effects of antioxidant properties of black berry juice on sodium fluoride induced hepatotoxicity and oxidative stress in rats." Food and Chemical Toxicology **47**(9): 2332-2337.

Fluorosis is a serious public health problem in many parts of the world. As in the case of many chronic degenerative diseases, increased production of reactive oxygen species has been considered to play an important role, even in the pathogenesis of chronic fluoride toxicity. Black berry is closely linked to its protective properties against free radical attack. Therefore, the aim of this study was to demonstrate the role of black berry juice (BBJ) in decreasing the hepatotoxicity and oxidative stress of sodium fluoride (NaF). Results showed that NaF caused elevation in liver TBARS and nitric oxide (NO), and reduction in superoxide dismutase (SOD), catalase (CAT), total antioxidant capacity (TAC) and glutathione (GSH). Plasma transaminases (AST and ALT), creatine kinase (CK), lactate dehydrogenase (LDH), total lipids (TL), cholesterol, triglycerides (TG), and low density lipoprotein-cholesterol (LDL-c) were increased, while high density lipoprotein-cholesterol (HDL-c) was decreased. On the other hand, BBJ reduced NaF-induced TBARS, NO, TL,

cholesterol, TG, LDL-c, AST, ALT, CK and LD. Moreover, it ameliorated NaF-induced decrease in SOD, CAT, GSH, TAC and HDL-c. Therefore, the present results revealed that BBJ has a protective effect against NaF-induced hepatotoxicity by antagonizing the free radicals generation and enhancement of the antioxidant defence mechanisms.

Hogan, S., H. Chung, et al. (2010). "Antiproliferative and antioxidant properties of anthocyanin-rich extract from açai." Food Chemistry **118**(2): 208-214.

An anthocyanin-rich extract, generated from açai (AEA), was investigated for its antioxidant properties and antiproliferative activity against C-6 rat brain glioma cells and MDA-468 human breast cancer cells. AEA has an ORAC value of 2589 μmoles trolox equivalents (TE)/g dried powder and a DPPH radical-scavenging activity of 1208 μmoles TE/g, suggesting that AEA is an exceptional source of natural antioxidants. In addition, AEA remarkably suppresses proliferation of C-6 rat brain glioma cells, but has no effect on the growth of MDA-468 human breast cancer cells. Further experiments demonstrated that the AEA treatment dose-dependently inhibited the growth of C-6 rat glioma cells with an IC50 of 121 μg/ml. The DNA ladder fragmentation results indicated that AEA induced apoptosis of C-6 rat brain glioma cells. To compare açai with other anthocyanin-rich extracts, a number of berry extracts, including blueberry, strawberry, raspberry, blackberry and wolfberry, were assessed for potential antiproliferative activity against C-6 rat brain glioma cells. However, none of them showed suppressing effect. The results suggest that the active antiproliferative constituents in AEA are unlikely to be anthocyanins normally found in common berries.

Jeong, J. H., H. Jung, et al. (2010). "Anti-oxidant, anti-proliferative and anti-inflammatory activities of the extracts from black raspberry fruits and wine." Food Chemistry **123**(2): 338-344.

The purpose of the study was to determine polyphenols, anthocyanins and ascorbic acid in the extracts of black raspberry fruits and wine, along with their anti-oxidant, anti-proliferative and anti-inflammatory activities. Black raspberry fruits without or with seeds crushed were blended in 60% ethanol (FE and FES, respectively) or in water (FW and FWS, respectively). Black raspberry wine without or with seeds crushed (W and WS, respectively) were prepared. Polyphenol content was the highest in the FES (8.25 mg/g fruit). Generally the ethanol extracts with seeds crushed showed higher anti-oxidant activities with the lowest DPPH IC50 (130 μg/ml (freeze-dried extract/reaction solution)) for the FES and the lowest ABTS IC50 (198 μg/ml) for the WS. Cell viabilities were reduced by 13-70% when treated with 100 μg/ml (freeze-dried extract/medium) for HT-29 cells and 1000 μg/ml for LNCaP cells. The FES most actively suppressed nitric oxide production in LPS-stimulated RAW264.7 cells (p < 0.05). Superoxide dismutase and glutathione peroxidase activities treated with the extracts were higher than the control (p < 0.05).

Johnson, J. L., J. A. Bomser, et al. (2011). "Effect of black raspberry (Rubus occidentalis L.) extract variation conditioned by cultivar, production site, and fruit maturity stage on colon cancer cell proliferation." Journal of Agricultural and Food Chemistry **59**(5): 1638-1645.

Black raspberries have been shown to inhibit multiple stages of oral, esophageal, and colon cancer. The objective of this study was to evaluate how black raspberry extract variability conditioned by horticultural factors affected the antiproliferative activity of 75 black raspberry extracts using an in vitro colon cancer cell model. HT-29 cells grown in 96-well plates were treated with freeze-dried extracts at 0.6 and 1.2 mg of extract/mL of medium. Percent cell growth inhibition for each concentration of the extracts was determined using the sulforhodamine B assay. All extracts significantly inhibited the growth of HT-29 colon cancer cells in a dose-dependent manner. Cell proliferation was significantly influenced by cultivar, production site, and stage of maturity. The lack of correlation between growth inhibition and extract total phenolic and total monomeric anthocyanin assays suggested horticultural parameters influence bioactivity in a complex manner.

Jung, J., M. Y. Son, et al. (2009). "Antioxidant properties of Korean black raspberry wines and their apoptotic effects on cancer cells." Journal of the Science of Food and Agriculture **89**(6): 970-977.

BACKGROUND: The objective of the study was to determine the antioxidant activities of Korean black raspberry concentrate (KBR: Rubus coreanus Miq.) and Korean black raspberry wine concentrates (KBRW-1 and KBRW-2) using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The apoptotic effects of the KBRW concentrates on various cancer cells were also investigated. RESULTS: Both the KBR concentrate and KBRW concentrates showed dose-related antioxidant activities in the DPPH assay. At concentrations of 500 μg mL -1 and 1000 μg mL-1, the KBR concentrate antioxidant activities were 59% and 79%, respectively. KBRW-1 and KBRW-2 inhibited radical formation at 500 μgm L-1 by 19% and 48%, respectively. The antioxidant activity of KBRW-2 was comparable to various red wines (RW-1, RW-2, RW-3, and RW-4). However, at 1000 μg mL-1, the antioxidant activities of KBRW-1 and KBRW-2 were 53% and 83% that of α-tocopherol, respectively. Both RW-1 and KBRW-2 showed marked antiproliferative effects on all cancer cell types, with decreases in cell viability greater than 50%. This was co-related with apoptotic characteristics, including chromat in condensation and apoptotic bodyformation, asdetermined by cellmorphological assessment. Cell cycle analysis showed that treating AGS cells with RW-1 and KBRW-2 resulted in 25% and 30% apoptotic cell accumulation, respectively. KBRW-2 induced the cleavage of poly(ADP-ribose) polymerase (PARP) and the activation of caspase-3 within the AGS cells. Levels of PARP cleavage were increased five- and three-fold by RW-1 and KBRW-2, respectively, and the level of caspase-3 was significantly increased by the treatment with KBRW-2. CONCLUSION: Overall, the results of this study suggest that Korean black raspberry wine displays antioxidant activity comparable to that of imported red wines, and has anticancer effects that may be attributed to induction of the apoptotic pathway.

Juranic, Z., Z. Zizak, et al. (2005). "Antiproliferative action of water extracts of seeds or pulp of five different raspberry cultivars." Food Chemistry **93**(1): 39-45.

In many recent publications the importance of various constituents of berries, anthocyanins and polyphenols, is shown in vitro and in vivo to be beneficial in protecting cells from different forms of cancer. As red raspberries are very rich in the in content of ellagic acid, the goal of this work was to study the cancer suppressive action of water raspberry extracts obtained by extracting water soluble constituents from pulp and from seeds, of five different raspberry cultivars: K81-6, Latham, Meeker, Tulameen and Willamette. The further aim was to compare their antiproliferative action to malignant human colon carcinoma LS174 cells and to normal immune competent cells, with the action of ellagic acid alone. Results from this study show that water extracts of raspberries seeds or pulp possess the potential for antiproliferative action against human colon carcinoma cells in vitro. The antiproliferative action of seeds extract was correlated with its content of ellagic acid. The cytotoxic activity of seeds extracts was not pronounced on normal human PBMC.

Kim, Y. H., J. H. Choi, et al. (2011). "23-Hydroxytormentic acid and niga-ichgoside F1 isolated from Rubus coreanus attenuate cisplatin-induced cytotoxicity by reducing oxidative stress in renal epithelial LLC-PK1 cells." Biological and Pharmaceutical Bulletin **34**(6): 906-911.

The unripe fruits of Rubus coreanus (Rosaceae) are used in traditional Chinese medicine to relieve kidney dysfunction. In the present study, we evaluated the protective effects of the triterpenoid glycoside niga-ichigoside F1 (NIF1) and of its aglycone 23-hydroxytormentic acid (23-HTA) isolated from the unripe fruits of Rubus coreanus (Rosaceae) against cisplatin-induced cytotoxicity in renal epithelial LLC-PK1 cells. Pretreating LLC-PK1 cells with 23-HTA or NIF1 was found to prevent cisplatin-induced cytotoxicity and apoptosis. In addition, 23-HTA or NIF1 pretreatment significantly improved the changes associated with cisplatin toxicity by increasing levels of glutathione (GSH) and decreasing levels of malondialdehyde (MDA) and reactive oxygen species (ROS). The activity of antioxidant enzymes including catalase (CAT) and superoxide dismutase (SOD) was significantly lower in cisplatin-treated LL-PK1 cells, and 23-HTA or NIF1 treatment notably increased the these enzyme activity and protein and mRNA levels of CAT and manganese SOD (MnSOD). Moreover, cisplatin caused a significant decrease in nuclear levels of nuclear factor erythroid 2-related factor 2 (Nrf2) and pretreatment with 23-HTA or NIF1 significantly suppressed the cisplatin-induced translocation of Nrf2 in LLC-PK1 cells. Taken together, these results suggest that 23-HTA ameliorates cisplatin-induced toxicity via modulation of antioxidant enzymes through activation of Nrf2 in LLC-PK1 cells.

Kreander, K., A. Galkin, et al. (2006). "In-vitro mutagenic potential and effect on permeability of co-administered drugs across Caco-2 cell monolayers of Rubus idaeus and its fortified fractions." Journal of Pharmacy and Pharmacology **58**(11): 1545-1552.

This study investigated the mutagenic, anti-mutagenic and cytotoxic effects of acetone extract of raspberry, Rubus idaeus L. (v. Ottawa) Rosaceae, and the isolated and characterized ellagitannin and anthocyanin fractions thereof, suitable for food applications. The studied raspberry extract and fractions did not show any mutagenic effects determined in the miniaturized Ames test and were not cytotoxic to Caco-2 cells at the used concentrations. However, the anti-mutagenic properties were changed (i.e. decreased mutagenicity of 2-nitrofluorene in strain TA98, and slightly increased mutagenicity of 2-aminoanthracene in strain TA100) with metabolic activation. Further, their influence on the permeability of co-administered common drugs (ketoprofen, paracetamol, metoprolol and verapamil) across Caco-2 monolayers was evaluated. The apical-to-basolateral permeability of highly permeable verapamil was mostly affected (decreased) during co-administration of the raspberry extract or the ellagitannin fraction. Ketoprofen permeability was decreased by the ellagitannin fraction. Consumption of food rich in phytochemicals, as demonstrated here with chemically characterized raspberry extract and fractions, with well-absorbing drugs would seem to affect the permeability of some of these drugs depending on the components. Thus their effects on the absorption of drugs in-vivo cannot be excluded.

Lee, Y. I., K. E. Whang, et al. (2009). "Rubus coreanus extract attenuates acetaminophen induced hepatotoxicity; involvement of cytochrome P450 3A4." Biomolecules and Therapeutics **17**(4): 455-460.

Foods of plant origin, especially fruits and vegetables, have attracted attention because of their potential benefits to human health. In this report, Rubi Fructus (RF), the dried unripe fruit of Rubus coreanus Miq (Rosaceae) and ellagic acid (EA) purified from RF were used to test their potential hepatoprotective effect against acetaminophen (AAP)-induced hepatotoxicity in rats. RF extract (RFext) and EA reduced the elevated levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) in serum and the content of lipid peroxide in liver by AAP administration, while the increment of the cellular glutathione (GSH) content and the induction of glutathione S-transferase (GST) and glutathione peroxidase (GSH-PX) which were decreased by AAP administration. RFext and EA from RFext did not affect the two major form of cytochrome P450s, cytochrome P450 2E1 (CYP2E1) and cytochrome P450 1A2 (CYP1A2), but down-regulated the cytochrome P450 3A4 (CYP3A4) related to the conversion of AAP to N-acetyl-P-benzoquinone imine (NAPQI). These results suggest that RFext and EA from RF exhibit a hepatoprotective effect not only by increasing antioxidant activities but also by down-regulating CYP3A4 in the AAP-intoxicated rat.

Liu, Y., M. Liu, et al. (2010). "Fresh raspberry phytochemical extract inhibits hepatic lesion in a Wistar rat model." Nutrition and Metabolism **7**.

Background. Red raspberry possesses potent antioxidant capacity and antiproliferative activity against cancer in vitro. Methods. The objective of this study was to determine the protective effects of raspberry 80% acetone extract in a rat hepatic lesions model induced by diethylnitrosamine (DEN). Rats were treated with the red raspberry extract (0.75, 1.5 or 3.0 g/kg of body weight) by gavage starting 2 h after DEN administration and continuing for 20 weeks. Results. A dose-dependent inhibition by red raspberry extract of DEN-induced hepatic nodule formation which stands for hepatic lesions was observed. Corresponding hepatic nodule incidence rates were 45.0, 40.0, 25.0 and 5.0% in positive control, low, middle and high groups, respectively ($P < 0.01$ or 0.05). Gross findings, histopathological and ultrastructural evaluations of hepatic lesion were performed on 9, 8, 5 and 1 hepatic nodule in positive control, low, middle and high doses of groups, respectively, identified in rats from the respective groups of 20. A decreasing trend of proportions of hepatocellular carcinoma masses accompanied the increasing doses of red raspberry extract. Conclusions. These findings demonstrate that the potent capacity of red raspberry diet could not only suppress DEN-induced hepatic lesions in rats, but also reduce the definite diagnostic features of neoplasm.

Liu, Z., J. Schwimer, et al. (2005). "Black raspberry extract and fractions contain angiogenesis inhibitors." Journal of Agricultural and Food Chemistry **53**(10): 3909-3915.

Targeted therapies, such as agents that inhibit angiogenesis, offer hope as complementary agents in cancer therapy. Angiogenesis-inhibiting agents have the potential for inhibiting tumor growth and limiting the dissemination of metastasis, thus keeping cancers in a static growth state for prolonged periods. Black raspberry (Rubus occidentalis) extract was discovered to be antiangiogenic (0.1% w/v) in a novel human tissue-based in vitro fibrin clot angiogenesis assay. Assay-guided fractionation of a crude black raspberry extract resulted in a highly potent antiangiogenic fraction that accounted for only 1% of the fresh weight of whole black raspberries. At 0.075% (w/v), the active fraction completely inhibited angiogenic initiation and angiogenic vessel growth. Further subfractionation of this active fraction revealed the coexistence of multiple antiangiogenic compounds, one of which has been identified as gallic acid. However, the individual subfractions did not outperform the active whole fraction. These findings suggest that an active black raspberry fraction may be a promising complementary cancer therapy. It is natural and potent enough for manageable dosing regimens. These extracts contain multiple active ingredients that may be additive or synergistic in their antiangiogenic effects. These observations warrant further investigations in animals and human trials.

Madhusoodhanan, R., M. Natarajan, et al. (2010). "Effect of black raspberry extract in inhibiting NF B dependent radioprotection in human breast cancer cells." Nutrition and Cancer **62**(1): 93-104.

Black raspberry extracts (RSE) have been shown to inhibit cancer cell growth and stimulate apoptosis. Also, studies have demonstrated that RSE inhibits transcriptional regulators including NF B. Accordingly, we investigated the effect of RSE in inhibiting radiation (IR) induced NF B mediated radioprotection in breast adenocarcinoma cells. MCF-7 cells were exposed to IR (2Gy), treated with RSE (0.5, 1.0, 2.0 g/ml) or treated with RSE (1.0 g/ml) followed by IR exposure, and harvested after 1, 3, 6, 24, 48, and 72 h. NF B DNA-binding activity was measured by EMSA and phosphorylated I B by immunoblotting. Expression of IAP1, IAP2, XIAP and survivin were measured by QPCR and immunoblotting. Cell survival was measured using MTT assay and cell death using Caspase-3/7 activity. Effect of RSE on IR induced MnSOD, TNF, IL-1 and MnSOD activity was also determined. RSE inhibited NF B activity in a dose-dependent manner. Also, RSE inhibited IR-induced sustained activation of NF B, and NF B regulated IAP1, IAP2, XIAP, and survivin. In addition, RSE inhibited IR-induced TNF, IL-1, and MnSOD levels and MnSOD activity. RSE suppressed cell survival and enhanced cell death. These results suggest that RSE may act as a potent radiosensitizer by overcoming the effects of NF B mediated radioprotection in human breast cancer cells.

McDougall, G. J., H. A. Ross, et al. (2008). "Berry extracts exert different antiproliferative effects against cervical and colon cancer cells grown in vitro." Journal of Agricultural and Food Chemistry **56**(9): 3016-3023.

Polyphenol-rich berry extracts were screened for their antiproliferative effectiveness using human cervical cancer (HeLa) cells grown in microtiter plates. Rowan berry, raspberry, lingonberry, cloudberry, arctic bramble, and strawberry extracts were effective but blueberry, sea buckthorn, and pomegranate extracts were considerably less effective. The most effective extracts (strawberry > arctic bramble > cloudberry > lingonberry) gave EC 50 values in the range of 25-40 μg/(mL of phenols). These extracts were also effective against human colon cancer (CaCo-2) cells, which were generally more sensitive at low concentrations but conversely less sensitive at higher concentrations. The strawberry, cloudberry, arctic bramble, and the raspberry extracts share common polyphenol constituents, especially the ellagitannins, which have been shown to be effective antiproliferative agents. However, the

components underlying the effectiveness of the lingonberry extracts are not known. The lingonberry extracts were fractionated into anthocyanin-rich and tannin-rich fractions by chromatography on Sephadex LH-20. The anthocyanin-rich fraction was considerably less effective than the original extract, whereas the antiproliferative activity was retained in the tannin-rich fraction. The polyphenols composition of the lingonberry extract was assessed by liquid chromatography-mass spectrometry and was similar to previous reports. The tannin-rich fraction was almost entirely composed of procyanidins of linkage type A and B. Therefore, the antiproliferative activity of lingonberry was caused predominantly by procyanidins.

Ogawa, Y., M. Akamatsu, et al. (2010). "Effect of essential oils, such as raspberry ketone and its derivatives, on antiandrogenic activity based on in vitro reporter gene assay." Bioorganic and Medicinal Chemistry Letters **20**(7): 2111-2114.

The effect of essential oils, such as raspberry ketone, on androgen (AR) receptor was investigated using a MDA-kb2 human breast cancer cell line for predicting potential AR activity. Among them, eugenol had the highest AR antagonistic activity with its IC50 value of 19 μM. Raspberry ketone, which has threefold higher anti-obese activity than that of capsaicin, also had AR antagonist activity with its IC50 value of 252 μM. Based on these findings, a more precise CoMFA model was proposed as follows: pIC50 [log (1/IC50)] = 3.77 + [CoMFA field terms] (n = 39, s = 0.249, r2 = 0.834, scv = 0.507, q2 = 0.311 (three components).

Park, J. H., S. m. Oh, et al. (2006). "Induction of heme oxygenase-1 mediates the anti-inflammatory effects of the ethanol extract of Rubus coreanus in murine macrophages." Biochemical and Biophysical Research Communications **351**(1): 146-152.

Foods of plant origin, especially fruits and vegetables, draw increased attention because of their potential benefits to human health. The aim of the present study was to determine in vitro anti-inflammatory activity of four different extracts obtained from the fruits of Rubus coreanus (aqueous and ethanol extracts of unripe and ripe fruits). Among the four extracts, the ethanol extract of unripe fruits of R. coreanus (URCE) suppressed nitric oxide (NO) and prostaglandin E2 (PGE2) production in lipopolysaccharide (LPS)-stimulated RAW264.7 murine macrophages. We also demonstrated that URCE by itself is a potent inducer of heme oxygenase-1 (HO-1). Inhibition of HO-1 activity by tin protoporphyrin, a specific HO-1 inhibitor, suppressed the URCE-induced reductions in the production of NO and PGE2 as well as the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2). Our data suggest that URCE exerts anti-inflammatory effects in macrophages via activation of the HO-1 pathway and helps to elucidate the mechanism underlying the potential therapeutic value of R. coreanus extracts.

Park, S. M., H. Y. Choi, et al. (2010). "Dietary supplementation of red ginseng, Chinese lizard's tail, and Korean raspberry extracts alleviate oxidative stresses in aged rats." Food Science and Biotechnology **19**(4): 967-972.

To investigate antioxidant efficacy of medicinal plants known to possess anti-aging properties, the herb preparations were fed to 10-month-old rats. The herb preparations included ethanol extracts of red ginseng (Panax ginseng C.A. Mayer), Chinese lizard's tail (Saururus chinensis Hort.ex Loud), and Korean raspberry (fruits of Rubus coreanus Miquel). Plasma protein carbonyls, malondialdehyde, 8-iso-prostaglandinF2α, lymphocyte DNA damage, α-tocopherol concentration, and ferric reducing ability were measured as oxidative stress markers. The aged rats showed higher levels of malondialdehyde, 8-isoprostaglandinF2α, and protein carbonyl and lower level of α-tocopherol compared to young rats. Malondialdehyde and protein carbonyl levels were decreased in the aged rats fed diets supplemented with any of the herbal preparations. 8-Iso-prostaglandinF2α was lower in rats fed the 0.5 or 1.0% Chinese lizard's tail- or 1% Korean raspberry extract-supplemented diet. The 1:1:1 mixture of herbal preparations decreased the level of 8-iso-prostaglandinF2α. Rats fed 1.0% Chinese lizard's tail or Korean raspberry diets showed increased α-tocopherol level. These results indicated that the level of oxidative stress is increased in the aged animals and herbal preparations are effective in the alleviation of age-related oxidation.

Prior, R. L., X. Wu, et al. (2009). "Purified berry anthocyanins but not whole berries normalize lipid parameters in mice fed an obesogenic high fat diet." Molecular Nutrition and Food Research **53**(11): 1406-1418.

Male C57BL/6 mice received diets with either 10% of kcal from fat, or a high fat diet [45% (HF45) or 60% (HF60) kcal from fat]. Diets were prepared with or without freeze-dried powders (10%) from whole blueberries (BB), strawberries (SB), Concord grape or black raspberry. In the 2nd study, purified anthocyanins (ACNs) from SB or BB were added to the drinking water of the treatments fed the HF60 diet. In Study 1, serum triglycerides were increased by feeding the HF45 diet but were elevated further when black raspberry or BB was included in the HF45 diet. Liver total lipids and triglycerides were increased in mice fed HF45 diet and inclusion of any of the berry powders in the HF45 diet did not alter concentrations compared to HF45 controls. In the 2nd study, mice fed the HF60 diet plus purified ACNs from BB in the water had lower body weight gains and body fat than the HF60 fed. Serum cholesterol and triglyceride levels were elevated with the HF60 diet and decreased to control levels when ACNs from either SB or BB were included in the drinking water. Serum leptin levels were consistently decreased to control low fat levels in those ACN treatments in which measures of body fat were decreased. Administering purified ACNs from BB and strawberry via drinking water prevented the development of dyslipidemia and obesity in mice, but feeding diets containing whole berries or purple corn (PC) ACNs did not alter the development of obesity.

Ross, H. A., G. J. McDougall, et al. (2007). "Antiproliferative activity is predominantly associated with ellagitannins in raspberry extracts." Phytochemistry **68**(2): 218-228.

Raspberry extracts enriched in polyphenols, but devoid of organic acids, sugars and vitamin C, were prepared by sorption to C18 solid phase extraction matrices and tested for their ability to inhibit the proliferation of human cervical cancer (HeLa) cells in vitro. The raspberry extract reduced proliferation in a dose-dependent manner whether this was judged by cell number or measurements of cell viability. However, measurements based on cell viability were more accurate and gave an EC50 value of 17.5 μg/ml gallic acid equivalents (GAE) at day 4 of culture. Raspberry extracts were fractionated by sorption to Sephadex LH-20 into an unbound fraction, which was obviously enriched in anthocyanins, and a bound fraction. The unbound anthocyanin-enriched fraction was much less effective in reducing proliferation then the original extract and gave an EC50 value estimated at 67 μg/ml. The LH-20 bound fraction was more effective than the original raspberry extract (EC50 = 13 μg/ml) suggesting that the main anti-proliferative agents were retained in the bound fraction. Analysis of the original extract, the unbound and the LH20 bound fractions by LC-MS confirmed that the unbound fraction was enriched in anthocyanins and the bound fraction primarily contained ellagitannins. The ellagitannin-rich bound fraction had the highest antioxidant capacity as measured by the ferric reducing antioxidant potential (FRAP) assay. The mechanism by which the ellagitannins inhibit proliferation of cancer cells is discussed.

Rossi, A., I. Serraino, et al. (2003). "Protective effects of anthocyanins from blackberry in a rat model of acute lung inflammation." Free Radical Research **37**(8): 891-900.

Anthocyanins are a group of naturally occuring phenolic compounds related to the coloring of plants, flowers and fruits. These pigments are important as quality indicators, as chemotaxonomic markers and for their antioxidant activities. Here, we have investigated the therapeutic efficacy of anthocyanins contained in blackberry extract (cyanidin-3-O-glucoside represents about 80% of the total anthocyanin contents) in an experimental model of lung inflammation induced by carrageenan in rats. Injection of carrageenan into the pleural cavity elicited an acute inflammatory response characterized by fluid accumulation which contained a large number of neutrophils as well as an infiltration of polymorphonuclear leukocytes in lung tissues and subsequent lipid peroxidation, and increased production of nitrite/nitrate (NOx) and prostaglandin E2 (PGE2). All parameters of inflammation were attenuated in a dose-dependent manner by anthocyanins (10, 30 mg kg-1 30 min before carrageenan). Furthermore, carrageenan induced an upregulation of the adhesion molecule ICAM-1, nitrotyrosine and poly (ADP-ribose) synthetase (PARS) as determined by immunohistochemical analysis of lung tissues. The degree of staining was lowered by anthocyanins treatment. Thus, the anthocyanins contained in the blackberry extract exert multiple protective effects in carrageenan-induced pleurisy.

Rouanet, J. M., K. Décordé, et al. (2010). "Berry juices, teas, antioxidants and the prevention of atherosclerosis in hamsters." Food Chemistry **118**(2): 266-271.

The effects of raspberry, strawberry and bilberry juices and green and black tea on early atherosclerosis in hamsters were investigated. They received an atherogenic diet and at the same time either a juice or a tea at a daily dose corresponding to the consumption of 275 ml by a 70 kg human. After 12 weeks berry juices and teas inhibited aortic lipid deposition by 79-96% and triggered reduced activity of hepatic antioxidant enzymes, not accompanied by lowered plasma cholesterol. These findings suggest that moderate consumption of berry juices and teas can help prevent the development of early atherosclerosis. There were substantial differences between the five beverages in terms of composition and concentration of individual phenolic compounds that were present. This indicates that anti-atherosclerotic effects can be induced by a diversity of phenolic compounds rather than a few specific components. The possible mechanisms by which this is brought about are discussed.

Seeram, N. P., L. S. Adams, et al. (2006). "Blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry extracts inhibit growth and stimulate apoptosis of human cancer cells in vitro." Journal of Agricultural and Food Chemistry **54**(25): 9329-9339.

Berry fruits are widely consumed in our diet and have attracted much attention due to their potential human health benefits. Berries contain a diverse range of phytochemicals with biological properties such as antioxidant, anticancer, anti-neurodegerative, and anti-inflammatory activities. In the current study, extracts of six popularly consumed berries - blackberry, black raspberry, blueberry, cranberry, red raspberry and strawberry - were evaluated for their phenolic constituents using high performance liquid chromatography with ultraviolet (HPLC-UV) and electrospray ionization mass spectrometry (LC-ESI-MS) detection. The major classes of berry phenolics were anthocyanins, flavonols, flavanols, ellagitannins, gallotannins, proanthocyanidins, and phenolic acids. The berry extracts were evaluated for their ability to inhibit the growth of human oral (KB, CAL-27), breast (MCF-7), colon (HT-29, HCT116), and prostate (LNCaP) tumor cell lines at concentrations ranging from 25 to 200 μg/mL. With increasing concentration of berry extract, increasing inhibition of cell proliferation in all of the cell lines were observed, with different degrees of potency between cell lines. The berry extracts were also evaluated for their ability to stimulate apoptosis of the COX-2 expressing colon cancer cell line, HT-29. Black raspberry and strawberry extracts showed the most significant pro-apoptotic effects against this

cell line. The data provided by the current study and from other laboratories warrants further investigation into the chemopreventive and chemotherapeutic effects of berries using in vivo models.

Serraino, I., L. Dugo, et al. (2003). "Protective effects of cyanidin-3-O-glucoside from blackberry extract against peroxynitrite-induced endothelial dysfunction and vascular failure." Life Sciences **73**(9): 1097-1114.

Anthocyanins are a group of naturally occurring phenolic compounds as colorants in several plants, flowers and fruits. These pigments have a great importance as quality indicators, as chemotaxonomic markers and antioxidants. The content of blackberry (Rubus species) juice was investigated by HPLC/ESI/MS using narrow bore HPLC columns. Using this method we demonstrated that cyanidin-3-O-glucoside represents about 80% of the total anthocyanin contents in blackberry extract. Here we investigated antioxidant activity of the blackberry juice and cyanidin-3-O-glucoside on the endothelial dysfunction in cells and in vascular rings exposed to peroxynitrite. In human umbilical vein endothelial cells (HUVEC) in vitro, peroxynitrite caused a significant suppression of mitochondrial respiration (38 ± 2.1% of control cells), as measured by the mitochondrial-dependent conversion of the dye MTT to formazan. Peroxynitrite caused DNA strand breakage (63 ± 1.9% single strand vs 3 ± 0.9% single strand in control cells), as measured by the alkaline unwinding assay, and caused an activation of PARS, as measured by the incorporation of radiolabeled NAD+ to nuclear proteins. Blackberry juice (different dilutions that contained 80 ppm;40 ppm;14.5 ppm of cyanidin-3-O-glucoside) and cyanidin-3-O-glucoside (as chloride) (0.085 μM; 0.028 μM; 0.0085 μM) reduced the peroxynitrite-induced suppression of mitochondrial respiration, DNA damage and PARS activation in HUVECs. Vascular rings exposed to peroxynitrite exhibited reduced endothelium-dependent relaxant responses in response to acetylcholine as well as a vascular contractility dysfunction in response to norepinephrine. The development of this peroxynitrite-induced vascular dysfunction was ameliorated by the blackberry juice (different dilutions that contained 80 ppm;40 ppm;14.5 ppm of cyanidin-3-O-glucoside) and cyanidin-3-O-glucoside (as chloride) (0.085 μM;0.028 μM;0.0085 μM). In conclusion our findings clearly demonstrate that blackberry juice containing cyanidin-3-O-glucoside is a scavenger of peroxynitrite and that exert a protective effect against endothelial dysfunction and vascular failure induced by peroxynitrite.

Shin, T. Y., H. Y. Shin, et al. (2006). "Rubus coreanus unripe fruits inhibits immediate-type allergic reaction and inflammatory cytokine secretion." Natural Product Sciences **12**(3): 144-149.

The immediate-type allergic reaction (anaphylaxis) is involved in many allergic diseases such as asthma, allergic rhinitis, and sinusitis. The discovery of drugs for the treatment of immediate-type allergic diseases is a very important subject in human health. In this study, we investigated the effect of Rubus coreanus Miq. (Rosaceae) unripe fruits (RCF) on mast cell-mediated allergic reaction and inflammatory cytokine secretion. RCF inhibited compound 48/80-induced systemic reactions in mice. RCF attenuated immunoglobulin (Ig) E-mediated local allergic reactions. In addition, RCF dependently reduced histamine release from rat peritoneal mast cells activated by compound 48/80 or IgE. Furthermore, RCF decreased the phorbol 12-myristate 13-acetate plus calcium ionophore A23187-stimulated tumor necrosis factor (TNF)-α and interleukin (IL)-6 secretion in human mast cells. Our findings provide evidence that RCF inhibits mast cell-derived immediate-type allergic reactions.

Shukitt-Hale, B., V. Cheng, et al. (2009). "Effects of blackberries on motor and cognitive function in aged rats." Nutritional Neuroscience **12**(3): 135-140.

The polyphenolics in fruits and vegetables, when fed to rats from 19-21 months of age, have been shown to retard and even reverse age-related decrements in motor and cognitive performance. These effects may be the result of the polyphenols increasing antioxidant and/or anti-inflammatory levels, or by direct effects on signaling, in the brain. Increased dietary intake of berry fruit, in particular, has a positive and profound impact on human health, performance, and disease. Thus, the present study examined a 2% blackberry-supplemented diet for its effectiveness in reversing age-related deficits in behavioral and neuronal function when fed to aged (19-month-old) Fischer 344 rats for 8 weeks. The results showed that the blackberry diet improved motor performance on three tasks which rely on balance and co-ordination: the accelerating rotarod, wire suspension, and the small plank walk. Results for the Morris water maze showed that the blackberry-fed rats had significantly greater working, or short-term, memory performance than the control rats. These data support our previous investigations in which we have seen improved motor and cognitive performance in aged rats after supplementation with other berry fruits.

Smith, S. H., P. L. Tate, et al. (2004). "Antimutagenic activity of berry extracts." Journal of Medicinal Food **7**(4): 450-455.

Plants are proven sources of useful anti-tumor and chemopreventative compounds. Hence, identification of phytochemicals useful in dietary prevention and intervention of cancer is of paramount importance. The initial step in the formation of cancer is damage to the genome of a somatic cell producing a mutation in an oncogene or a tumor-suppressor gene. Fresh juices and organic solvent extracts from the fruits of strawberry, blueberry, and raspberry were evaluated for their ability to inhibit the production of mutations by the direct-acting mutagen methyl methanesulfonate and the metabolically activated carcinogen benzo[a]pyrene. Juice from strawberry, blueberry, and raspberry fruit significantly inhibited mutagenesis caused by both carcinogens. Ethanol extracts from freeze-dried fruits of strawberry cultivars (Sweet Charlie and Carlsbad) and blueberry cultivars (Tifblue and Premier) were also tested. Of these, the hydrolyzable tannin-containing fraction from Sweet Charlie strawberries was most effective at inhibiting mutations.

Tate, P., A. Kuzmar, et al. (2003). "Comparative effects of eight varieties of blackberry on mutagenesis." Nutrition Research **23**(7): 971-979.

Diets containing large amounts of fruits and vegetables are known to decrease the probability of developing cancer. The chemical composition of fruits can vary with their genetic characteristics and the environmental conditions under which they are cultivated. Because of this variability, different varieties of the same fruit could be expected to have different effects on processes leading to carcinogenesis. Blackberries have been shown to have anti-carcinogenic potential. Since somatic mutations play a major role in the initiation and progression of cancer, we have compared eight varieties of blackberry grown under the same conditions for their abilities to inhibit carcinogen-induced mutagenesis. Using the Ames assay, we have measured the effects of each of the eight varieties on: 1) mutation induction by 2-amino anthracene (2AA), 2) mutation induction by methyl methanesulfonate (MMS) and 3) cell survival. All varieties were found to strongly suppress 2AA mutagenesis, but have minimal effect on MMS mutagenesis. Experiments were performed with berry juice and with homogenized berries. In addition, berries extracts were acidified to simulate changes which might be caused by the digestive process.

Wu, Q. K., J. M. Koponen, et al. (2007). "Berry phenolic extracts modulate the expression of p21WAF1 and Bax but Not Bcl-2 in HT-29 colon cancer cells." Journal of Agricultural and Food Chemistry **55**(4): 1156-1163.

Previous studies have shown that anthocyanin-rich berry extracts inhibit the growth of cancer cells in vitro. The objective of this study was to compare the effects of berry extracts containing different phenolic profiles on cell viability and expression of markers of cell proliferation and apoptosis in human colon cancer HT-29 cells. Berry extracts were prepared with methanol extraction, and contents of the main phenolic compounds were analyzed using HPLC. Anthocyanins were the predominant phenolic compounds in bilberry, blackcurrant, and lingonberry extracts and ellagitannins in cloudberry extract, whereas both were present in raspberry and strawberry extracts. Cells were exposed to 0-60 mg/mL of extracts, and the cell growth inhibition was determined after 24 h. The degree of cell growth inhibition was as follows: bilberry>black currant>cloudberry>lingonberry>raspberry> strawberry. A 14-fold increase in the expression of p21WAF1, an inhibitor of cell proliferation and a member of the cyclin kinase inhibitors, was seen in cells exposed to cloudberry extract compared to other berry treatments (2.7-7-fold increase). The pro-apoptosis marker, Bax, was increased 1.3-fold only in cloudberry- and bilberry-treated cells, whereas the pro-survival marker, Bcl-2, was detected only in control cells. The results demonstrate that berry extracts inhibit cancer cell proliferation mainly via the p21WAF1 pathway. Cloudberry, despite its very low anthocyanin content, was a potent inhibitor of cell proliferation. Therefore, it is concluded that, in addition to anthocyanins, also other phenolic or nonphenolic phytochemicals are responsible for the antiproliferative activity of berries.

Xue, H., R. M. Aziz, et al. (2001). "Inhibition of cellular transformation by berry extracts." Carcinogenesis **22**(2): 351-356.

Recent studies have examined and demonstrated the potential cancer chemopreventive activity of freeze-dried berries including strawberries and black raspberries. Although ellagic acid, an abundant component in these berries, has been shown to inhibit carcinogenesis both in vivo and in vitro, several studies have reported that other compounds in the berries may also contribute to the observed inhibitory effect. In the present study, freeze-dried strawberries (Fragara ananassa, FA) or black raspberries (Rubus ursinus, RU) were extracted, partitioned and chromatographed into several fractions (FA-F001, FA-F003, FA-F004, FA-F005, FA-DM, FA-ME from strawberries and RU-F001, RU-F003, RU-F004, RU-F005, RU-DM, RU-ME from black raspberries). These extracts, along with ellagic acid, were analyzed for anti-transformation activity in the Syrian hamster embryo (SHE) cell transformation model. None of the extracts nor ellagic acid by themselves produced an increase in morphological transformation. For assessment of chemopreventive activity, SHE cells were treated with each agent and benzo[a]pyrene (B[a]P) for 7 days. Ellagic acid, FA-ME and RU-ME fractions produced a dose-dependent decrease in transformation compared with B[a]P treatment only, while other fractions failed to induce a significant decrease. Ellagic acid, FA-ME and RU-ME were further examined using a 24 h co-treatment with B[a]P or a 6 day treatment following 24 h with B[a]P. Ellagic acid showed inhibitory ability in both protocols. FA-ME and RU-ME significantly reduced B[a]P-induced transformation only when co-treated with B[a]P for 24 h. These results suggest that a methanol extract from strawberries and black raspberries may display chemopreventive activity. The possible mechanism by which these methanol fractions (FA-ME, RU-ME) inhibited cell transformation appear to involve interference of uptake, activation, detoxification of B[a]P and/or intervention of DNA binding and DNA repair.

Zhang, Y., Z. Zhang, et al. (2011). "Diuretic activity of Rubus idaeus L (Rosaceae) in rats." Tropical Journal of Pharmaceutical Research **10**(3): 243-248.

Purpose: To evaluate the diuretic activity of Rubus idaeus L in experimental rats. Methods: Hot-water and methanol extract of three kinds of Rubus idaeus L. fruits were administered to experimental rats orally at a dose of 2 and 5 mg/kg. Hydrochlorothiazide (10 mg/kg) was used as positive control in study. The diuretic effect of the extracts was evaluated by measuring urine volume, sodium and potassium excretion in the urine. Results: Compared with the

control group, significant increase in urine volume was observed from the experimental animal treated with wild raspberry methanol extract. In addition, we find that the methanol extract of wild raspberry fruits shows a potassium-conservation diuretic effect, which is a very interesting property in a phytodiuretic. Conclusion: Methanol extract of wild raspberry fruits have diuretic effect on experimental rats. This might be the first formal reports on diuretic effect of raspberry fruits, which can also, to some extent, explain the use of raspberry as a cure for renal diseases in Chinese traditional medical practice.

Zunino, S. J., Y. Zhang, et al. (2010). "Berry fruit extracts inhibit growth and induce apoptosis of high-risk acute lymphoblastic leukemia cells in vitro." Journal of Functional Foods **2**(3): 187-195.

Dark coloured fruits contain many phytochemicals that have anti-cancer activity. The ability of extracts of berry fruits to kill leukemia cell lines derived from patients with high-risk acute lymphoblastic leukemia (ALL) carrying the t(4;11)(q21;q23) chromosomal translocation was investigated. Extracts enriched in phenolic compounds were prepared from blackberries, blueberries, red, green, and black grapes, raspberries, and strawberry powder, and the leukemia-derived cell lines SEM, RS4;11, and REH were treated with 0, 25, 50, and 100 µg/ml of each berry extract daily for a total of 72. h. Mitochondrial membrane depolarization, cell death, cell cycle, and nitric oxide generation in response to treatment were measured by flow cytometry. Extracts from blackberries, blueberries, red and black grapes, and strawberry powder induced varying levels of apoptotic cell death in the leukemia cells. For most treatments, cell death was accompanied by an arrest in the G2/M phase of the cell cycle. An S phase arrest was also observed for SEM and REH cells treated with strawberry or blackberry extract. Our data suggest that phenolic phytochemicals found in berry fruits may have substantial potential for the prevention or treatment of high-risk ALL and further evaluations are warranted in vivo.