Antioxidant Properties of Berries: Review of Human Studies and their Relevance in the Context of the European Food Safety Authority
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Abstract
Berries have been traditionally consumed worldwide to prevent and treat diverse ailments. In the last decennium some of the berry products, traditionally used in certain parts of the world, have appeared on market shelves of the other parts under the label "superfoods". The efficient marketing policies, making use of selected scientific research, have resulted in rapidly increasing sales numbers. A great number of health claims is ascribed to berries and their constituents. The marketers largely stress antioxidant properties of berries. There is an outstanding number of research available on antioxidant activity of different berries. However, most of the research is represented by in vitro experiments, followed by animal studies. Human research is still scarce and often operate with the markers which are not considered reliable enough to substantiate health claims.

The European Food Safety Authority (EFSA) is the body responsible for authorization of health claims within the European Union. The authorization of 222 out of 2758 health claims and their adoption by the European Commission on May 16 2012 has raised sound discussions within food industry. Discussed are both the EFSA methodology and the consequences of the regulation for food producers. None of the submitted berry claims have been authorized. This paper provides an overview of the research available on five popular berries: açai, blueberry, cherry, goji and pomegranate and links it to the EFSA evaluation criteria. So far, for none of the studied berries there is a satisfactory (from the EFSA perspective) body of research available for substantiation of health claims related to antioxidant activities. Research on pomegranate and its products (juice, polyphenolic extracts) is the most pertinent, compared to the other four berries, but still more human long-term well-designed studies should take place before any antioxidant-related claim can get the green light in the European Union.

Key words
EFSA, health claims, berries, polyphenols
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Introduction

Different types of berries have been consumed in many parts of the world for centuries in fresh, dried and preserved (jams, condiments) form and have been traditionally used as nutraceuticals to prevent and treat numerous diseases of all kinds. Since the beginning of the 21st century some of the berry products, traditionally used in certain parts of the world, migrated to other parts under the brand "superfoods". Examples include goji berries, pomegranate juice, freeze-dried açai berry. The efficient marketing strategies led to that they have reached broad audiences thanks to the appraisal in media and advertisements for their outstanding qualities. These products are sold as health food articles, and the marketers are willingly making use of the selected research, mostly in vitro (in laboratory conditions) experiments, that stress the antioxidative, anti-inflammatory and other beneficial effects of the berries. Meanwhile, more common to the US and European audiences berries such as blueberries and cherries are also being intensively promoted by institutions such as U.S. Highbush Blueberry Council and Cherry Marketing Institute correspondingly. Those institutions stimulate and collect the research on health benefits of the berries and make it available to the public. The available experimental data have led to a vast number of health claims ascribed to berries by the marketers, presenting them as "cure all" products.

A great amount of in-vitro research and considerably smaller amount of human trials are obtainable through academic databases. Nevertheless, both consumers and trained nutritionists lack the accessible overview of the data available on the health benefits of the berries/their constituents. The European Food Safety Authority (EFSA) is the body responsible for authorization of all health claims made on foods within the European Union. Recent authorization of 222 out of 2758 (8%) health claims by EFSA and their adoption by the European Commission on May 16 2012 has raised sound discussions within food industry. Discussed are both the methodology hunted by the EFSA and the consequences of the regulation for food producers. Approximately forty of those 2758 claims are related to different berries, half of which are related particularly to antioxidative effects. None of them have been authorized by EFSA.

The research question of this thesis could be divided in two parts:
-- What criteria does EFSA use in its evaluation of antioxidant activity related claims, and
-- What is the weight of the available research on the selected berry types on the scale of EFSA

The aim of this paper is to provide an accessible review of the (human) studies for the five popular berries: açai, blueberry, cherry, goji and pomegranate with the focus on their antioxidative properties. Another aim is to give an insight into the global European policy
towards the health claims, focusing, again, on the authorization of health claims linked to antioxidative properties of food/food constituents. Finally, this paper will provide the link between the available research and its relevance in the EFSA discourse.

The first chapter of the paper will introduce the essential concepts, such as free radicals, oxidation, antioxidant, polyphenols, anthocyanins and others. The second chapter will describe the methodology of the European Food Safety Authority in regard to health claims evaluation criteria. The third chapter will introduce the selected berries and summarize theresearch available. The relevance of the outlined research will be discussed in the context of the evaluation criteria hunted by the EFSA. Concluding remarks will be made.

The PubMed, the database of the National Center for Biotechnology Information (NCBI), is the priority information source used in this paper. In some cases other resources are used too, in such cases it is explicitly mentioned.
Chapter 1. Definitions and Essential Concepts

The human body uses oxygen for routine metabolic reactions. Free radicals are sometimes created in the body during normal metabolic processes, and they could be introduced from the outside, such as by exposure to radiation (Sun rays, X-Ray), toxins, pollution, smoke, alcohol and unsaturated fats consumption and other factors. **A free radical** is readily formed when a covalent bond between entities is broken: basically, it is by definition an atom or a group of atoms with at least one unpaired electron in the outermost shell. An electron without a pair is unstable and highly reactive. A free radical involving oxygen can be referred to as reactive oxygen species (**ROS**). A free radical steals an electron from a neighbouring molecule, and thus a new free radical is formed in its place. The newly formed radical again steals an electron from another molecule, and a chain reaction occurs, which, if not intercepted by the antioxidative network, leads to oxidative damage. ROS have the high potential to damage vital biological systems and are incriminated to contribute to the ageing process and to over a hundred of disease conditions. (16, 25)

**Oxidant** is a compound that oxidizes other compounds. (16)

**Oxidation** is a chemical reaction involving transportation of electrons from a substance to an oxidizing agent.

**Peroxyl radical** (ROO') is a product of lipid peroxidation. The figure 1 shows a common series of ROS reactions. A hydroxyl radical removes a hydrogen atom from one of the carbon atoms in the fatty acid chain forming a molecule of water and leaving the carbon atom with an unpaired electron (in red); thus now a radical. One of the most probable things to happen next is the reaction with a molecule of oxygen forming a peroxyl radical. This may then steal a hydrogen atom from a nearby side chain making it now a radical too.

![Figure 1. Formation of peroxyl radical](image-url)
Oxidative stress is a condition when the production of oxidants and free radicals exceeds the ability of the body to cope with them in order to prevent damage.

Halliwell & Gutteridge (30) defined an antioxidant as 'any substance that, when present at low concentrations compared with that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate'. Antioxidant is a generic name for all elements of so-called antioxidant defence system. These elements elsewhere can also be called free radical scavengers, chain terminators, or reductants. The antioxidant defence system is responsible for cellular protection against oxidative stress. (16, 26, 27)

The major biological process leading to oxygen-derived O2 generation is electron transport associated with the mitochondrial membranes. The attack of free radicals can damage the polyunsaturated fatty acids in lipoproteins and in cell membranes, thus disrupting the transportation of substances into and out of cells. Free radicals can also damage DNA, RNA and proteins, contributing to cell distraction and disease development. By donating one of their own electrons, antioxidants may neutralize free radicals and end the chain reaction. Antioxidants do not become free radicals themselves because they are stable in either form. Antioxidants combat free radicals in several ways: they may destroy free radicals or their precursors, limit the free radical formation, stimulate antioxidant enzyme activity, repair the oxidative damage or stimulate repair enzyme activity (such as DNA glycosylases). (16, 26)

Under normal conditions the antioxidant defence system within the human body easily copes with the free radicals produced. The body produces several antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase, that neutralize free radicals. The proper action of these enzymes depends on the minerals selenium, copper, manganese and zinc. In addition the body uses vitamins that can act as antioxidants in their own right: vitamin C, vitamin E and vitamin B2. All these elements are part of so-called primary antioxidant defence system. Secondary defence mechanisms involve lipolytic enzymes, phospholipases, proteolytic enzymes, proteases, peptidases, DNA repair enzymes, endonuclease, exonuclease, and ligase (28).

Polyphenols exhibit evident antioxidant properties as well. The exact mechanisms are being extensively studied and are not always clear yet.

Antioxidant scavenging enzymes

Superoxide dismutase (SOD)

Dismutation of O2’ to H2O2 by SOD is essential, because the enzyme prevents further generation of free radicals. SODs are categorized into three groups depending on the metal ion content: Cu/ZnSOD, Mn SOD, and Fe SOD. Although some of the SOD activity appears to be extracellular, most of the activities are localized intracellularly, divided between the mitochondrial (Mn SOD) and cytosolic compartments (Cu/Zn SOD). Catalase (CT) is another major antioxidant defence component whose primarily function is to catalyse the decomposition of H2O2 to H2. CT performs this activity together with another enzyme, Glutathione peroxidise (GSH-PX). Both enzymes detoxify oxygen reactive radicals by catalysing the formation of H2O, derived from oxidant superoxide. Most species exhibit GSH-PX intracellularly located in the cytosol and mitochondrial matrix. Next to canalization of the reduction of H2O, GSH-PX also catalyses the reduction of organic hydroperoxides. (27)
Gallic acid (3,4,5-Trihydroxybenzoic acid) is a type of phenolic acid found in many plants. It is used as a standard for determining the phenolic content of plant constituents by the Folin-Ciocalteau assay; results are reported in gallic acid equivalents (GAE).

Phytochemicals are bioactive non-nutrient plant compounds. More than 10,000 phytochemicals have been identified to date, but a large percentage still remains unknown. Phytochemicals are responsible for food’s colour, flavour, aroma, taste and other characteristics. In human body phytochemicals can mimic hormones, act as antioxidants and probably suppress development of diseases.

Polyphenols (previously called collectively Vitamin P) are plant secondary metabolites. They are physiologically essential for processes as plants’ growth, pigmentation, lignification, pollination, allelopathy to name a few. Several thousand polyphenols have been identified to date, and several hundred of them have been found in edible plants. The antioxidant characteristics of the polyphenols are due to the hydrogen of the phenoxyl groups that is prone to be donated to a radical, and by the ensuing structure that is chemically stabilized by resonance.

Flavonoids are a chemically defined class of polyphenols which have a basic structure as shown on the Figure 2, and several subclasses of flavonoids are characterized by a substitution pattern in the B- and C-rings. There have been identified approximately 8,000 individual flavonoids. Most of the flavonoids are present in plants with sugars attached (glycosides), although occasionally they are found as aglycones. Most of the research is concentrated on flavonoids with a common C6-C3-C6 structure consisting of 2 aromatic rings linked through an oxygenated heterocycle. The main subclasses include flavan-3-ols (catechin, epicatechin), flavanones (hesperetin), flavones (luteolin, apigenin), isoflavones (genistein), flavonols (quercetin, kaempferol, myricetin), and anthocyanidins. The latter group will be discussed in details in the following paragraph.

Figure 2. Basic structure of a flavonoid.
The word *anthocyanin* originates from Greek *anthos* (flower) and *kyanos* (blue). *Anthocyanin* is a conjugated anthocyanidin. It is the blue, red, blue-red, or purple water-soluble pigment in berries, fruits, vegetables and leaves. It prevails in the skin of fruit and in buds and young shoots, and is an underlying pigment of chlorophyll in leaves, which becomes apparent as a purplish hue during late autumn. 

Approximately 400 individual ACN have been identified to date. ACN contain a positively charged oxygen in the central group of the molecule. Chemically anthocyanins are subdivided into the sugar-free anthocyanidine aglycons and the anthocyanin glycosides. The most widespread anthocyanin is cyanidin 3-glucoside. (15, 18, 22)
The greater the amount of ACN, the more intense the colour of the plant is. Particularly dark purple berries (blueberry, black raspberry) are rich in anthocyanins. ACN content in berries varies from 200 to 400 mg/100 g, increasing with the ripening process. Average amount of ACN consumed throughout the world is reported from 12.5 mg to 225 mg of anthocyanins daily. The positively charged oxygen atom in the anthocyanin molecule makes it a more potent and distinct hydrogen-donating antioxidant compared to other flavonoids. (10, 11, 12, 17, 26)

**Bioavailability** presupposes “that a fraction of an ingested nutrient or compound that reaches the systemic circulation and the specific sites can exert its biological action.” (24) The potential health benefits of berry polyphenols depend largely on their bioavailability. From the metastudy of Manach et al. (26) reviewing 97 articles on the issue of bioavailability it can be concluded that the most well-absorbed polyphenols are gallic acid and isoflavones, followed by catechins, flavanones, and quercetin glucosides. The least well-absorbed polyphenols are proanthocyanidins, catechins, and anthocyanins. Most studies report low ACN urinary excretions, ranging from 0.004% to 0.1% of the intake, although Lapidot et al. (30) and Felgines et al. (31) measured higher levels of anthocyanin excretion (up to 5%) after red wine or strawberry consumption. Not all of the anthocyanins’ metabolites might have been identified yet, and therefore their bioavailability might be underestimated. (26)
Chapter 2. European Food Safety Authority and Health Claims Authorization

European Food Safety Authority (EFSA) is the body responsible for the verifying the scientific substantiation of health claims submitted for authorization in the EU. One of the key objectives of the EFSA activity is to ensure that any claim made on a food label in the EU is clear and substantiated by scientific evidence.

Regulations
There are four essential regulations with in the area of health claims authorization.

**EU Regulation No 1924/2006** is on the use of nutrition and health claims for foods. One of the most important aims of the Regulation is to harmonize the domestic rules of the member states in the area of nutrition and health claims on food products. Another key aim is to establish rules governing the authorization of health claims made on foods in the European Union. The Regulation provides background information, definitions, conditions of use and characterizes the relevant procedures (authorization, monitoring, evaluation) for nutrition and health claims. In the annex of the Regulation nutrition claims (i.e. low energy, fat free, very low sodium/salt, source of fiber) and conditions applying to them are defined. (25)

According to the Regulation, the use of health claims shall only be permitted if the food/constituent, for which the claim is made, has been shown to have a beneficial physiological effect. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), which deals with issues related to dietetic products, novel foods, nutrition and food, makes a scientific judgment on whether the claimed effect is considered to be beneficial in the context of the specific claim and taking into account the population group for whom the claim is intended. The Regulation establishes that the list of authorized as well as rejected claims has to be published in a register for transparency reasons.

Article 13.1 of the EC Regulation is of the major interest for this thesis. It describes “General function” claims as referring “to the role of a nutrient or substance in growth, development and body functions; psychological and behavioural functions; slimming and weight control, satiety or reduction of available energy from the diet. These claims do not include those related to child development or health or disease risk reduction.”(25)

The consolidated database of Article 13.1 health claims contains the 4,637 main health claim entries submitted to EFSA by the European Commission for evaluation. All of these claims entries are available through the Register on the official EFSA web-site.(29)

**EU Regulation No. 353/2008** establishes the rules for applications for authorization of health claims as provided for in Article 15 of Regulation No 1924/2006. Technical rules for the preparation and presentation of the application for health claims are described in this regulation as well as general principles for scientific substantiation.

The Regulation proposes the following hierarchy of pertinent data substantiating the claims:
(a) human intervention studies, randomized controlled studies, other randomized studies (non-controlled), controlled (non-randomized) studies, other intervention studies;

(b) human observational studies, cohort studies, case-control studies, cross-sectional studies, other observational studies, such as case reports;

(c) other human studies dealing with the mechanisms by which the food could be responsible for the claimed effect, including the studies on bioavailability. (26)

Non-human research is defined as of secondary pertinence, and the hierarchy is the following:
(a) animal data
(b) ex vivo or in vitro data (26)

The Regulation notes that a food for which the claim is submitted should be sufficiently characterized, which includes: a) the description of the food/food category, including characterisation of the food matrix and the nutrient content, and b) the source and specifications of the food/food category and, in particular, the content of the constituent(s) related to the health claim.

It is worth mentioning that a part of the submitted to EFSA claims failed already in the area of characterization and therefore the scientific substantiation of these claims could not be assessed by the EFSA.

**Regulation No. 1169/2009** amends Regulation No. 353/2008. It allows the withdrawals of applications up to the moment the EFSA adopts its opinion. (27)

In June 2011 EFSA finalized the evaluation of the "general function" health claims (Article 13.1 of the EC Regulation No 1924/2006 on nutrition and health claims) prioritized by the European Commission and published 341 opinions where it provided scientific advice on 2,758 health claims. These were drawn from a list of main 4,637 claims submitted to EFSA by the European Commission between July 2008 and March 2010. For reference, in total, EU-countries provided national lists of approximately 44,000 health claims to the European Commission. Of the 2,758 claims only 222 have been authorized by the EFSA in December 2011. On May 16 2012 the claims, after scrutiny by the European Parliament and the Council, were adopted by the European Commission. The corresponding regulation, EU Regulation No 432/2012, was published in the official journal of the European Union on May 25 2012. (30)

**EU Regulation No 432/2012** established a list of permitted health claims made on foods. A scientific assessment was the first requirement for authorization. In its assessments EFSA looked at three consecutive elements:
- whether the substance of the claimed effect can be defined sufficiently for a scientific assessment,
- whether the claimed effect is beneficial for health, and
- whether the studies considered as pertinent by EFSA could allow establishing of a cause and effect relationship between the food and the claimed effect.
As was already mentioned, only 222 health claims passed successfully through the authorization process. The process of authorisation is also completed for another 1600 entries from the list, but these could not be authorised. The remainder, about 2200 claims, is represented mainly by so-called "botanical substances" and is still awaiting completion of the authorisation process.

The regulation shall enter into force on the 20th day following the publication, June 14 2012. The regulation shall be binding and directly applicable in all member states. The transition period for the food producers to adjust to the new regulation is six months, which means that by December 14 2012 the products with non-authorized health claims must disappear from the supermarket shelves of all the EU member-states. Claims for which the authorization process is complete will be listed in the Union Register of nutrition and health claims made on foods, as required by Regulation (EC) No 1924/2006. This Union Register can be permanently found on the Commission's website.

**EFSA Guidance for antioxidant activity related claims**

*Guidance on the scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health* (Guidance) was adopted in November 2011 and published in December 2011 in the EFSA Journal. The Guidance is a useful tool for the future applicants, as it summarizes what EFSA considers pertinent scientific substantiation for the proposed claims. The Guidance was developed by the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). Before adapting the document the NDA Panel organized public consultations which lasted for four months. Comments from 57 interested parties including applicants for health claims, governmental and nongovernmental organisations, industry organisations and academia were received. The NDA Panel claimed to take into consideration all relevant comments submitted.

In the document the Panel states that with the exception of well established risk factors (e.g. LDL-cholesterol concentration, blood pressure), the benefit of the reduction of a risk factor in the context of a suggested health claim should be considered on a case-by-case basis. Further, it reminds, that pursuant to Regulation 1924/2006, reference to general, non-specific benefits of the nutrient or food for overall good health or health-related well-being may only be made if accompanied by a specific health claim.

Regarding the hierarchy of scientific data confirming beneficial health effects, the hierarchy suggested in the Regulation 353/2008 is kept (see above).

In considering whether the studies provided are pertinent, the NDA Panel addresses the following criteria:

- Whether the studies have been carried out with the food/constituent for which the claim is made;
- Whether the design and quality of the studies allow conclusions to be drawn for the scientific substantiation of the claim;
- Whether the studies have been carried out in a study group representative of the population group for which the claim is intended;
Whether the studies used (an) appropriate outcome measure(s) of the claimed effect.

In paragraphs 3 and 4 of the Guidance the Panel describes the biomarkers and types of assays which it considers un/reliable. The complete text of the Guidance can be found in the corresponding appendix. In the following paragraphs assays to which the Guidance refers are briefly described.

Antioxidant activity assays
With regard to claims on antioxidant status and antioxidant defense, as far as EFSA concerns, assays which measure the overall antioxidant capacity of plasma, are not a reliable indication of any health benefits for humans. ORAC, TEAC, TRAP, FRAP, and FOX assays which assess changes in the antioxidant capacity of plasma are often used for research purposes; these assays are explicitly mentioned in the Guidance with the remark that “It is not established that changes in the overall antioxidant capacity of plasma exert a beneficial physiological effect in humans as required by Regulation (EC) No 1924/2006.” Further, those changes “do not predict a role of the food/constituent in the protection of body cells and molecules such as DNA, proteins and lipids from oxidative damage in vivo, and therefore are not suitable outcome measures for the scientific substantiation of the claimed effect.” (28) Therefore claims supported by the data referring to any of the above mentioned assays are (going to be) assessed by the EFSA with unfavorable outcome. The mechanism of action of these assays, as well as often used CAP-e and Folin–Ciocalteu assays is briefly outlined beneath.

ORAC refers to Oxygen Radical Absorbance Capacity. The concept of ORAC assay is based on the measurement of how effectively a compound eradicates the peroxyl radical as compared to the ability of Trolox (a vitamin E analogue) to act as antioxidant. The ORAC assay depends on the free radical damage to a fluorescent probe, such as fluorescein, to result in a downward change of fluorescent intensity. The assumption is that the degree of change is correlated to the degree of radical damage. The outcomes of the ORAC assay are expressed in micromoles of Trolox equivalents (TE) per gram. Indicator above one TE unit suggests that the compound has a stronger radicals scavenging activity. Food industry agents operate with so-called ORAC scores (elsewhere ORAC units or ORAC values), which are basically the outcomes of the measurements of antioxidant capacity of foods/food constituents expressed in mmol TE/g or converted into TE/100 g. The higher ORAC values, the higher the potential antioxidative effect of the compound. ORAC is used not only for measuring the antioxidant capacity of human plasma, but also for measurement of antioxidant capacity of food/food constituents. (10, 12, 13) Recently the USDA’s Nutrient Data Laboratory (NDL) removed the USDA ORAC Database for Selected Foods from its website "due to increasing evidence that the scores indicating antioxidant capacity have no direct link to the effects of specific bioactive compounds, including polyphenols, on human health." (22)
TEAC refers to Trolox Equivalent Antioxidant Capacity. The TEAC assay is based on the measurement of an antioxidant ability to scavenge the relatively stable ABTS radical. The ABTS is intensely colored, and when it reacts with an antioxidant the decolorization takes place. The reference compound is, as in the case of ORAC, Trolox, which has a TEAC score of 1. (5, 11) FRAP refers to the ferric reducing ability of plasma assay of Benzie and Strain. This assay also uses Trolox as the standard. The assay measures the ability of a sample to reduce Fe3+-TPTZ complex to the ferrous form at low pH. No oxidants are applied in the assay. (4, 11) TRAP refers to total radical trapping antioxidant parameter. This assay is based on the ability of azo-initiators to decompose, producing a peroxyl radical flow at a constant temperature-dependent rate. This flow has enough energy to abstract hydrogen from a substrate, thus initiating a (lipid) peroxidation chain. In TRAP the consumption of dissolved oxygen is the marker of the rate of lipid peroxidation and thus an indirect measure of plasma’s ability to inhibit the oxidation. Trolox is used as the standard. (14, 15) FOX refers to ferrous oxidation of xylene orange (an organic reagent). This assay uses dye xylene orange to form a blue-purple complex with a maximum absorption at 560 nm. The method is based on the principle of the rapid peroxide-mediated oxidation of Fe2+ to Fe3+ under acidic conditions. The latter, in the presence of xylene orange, forms a Fe3+-xylene orange complex which can be measured spectrophotometrically. (17) CAP-e refers to cell-based antioxidant protection in erythrocytes. During the measurement procedure, the red blood cells are first incubated with a test sample at a range of concentrations. The erythrocytes are then combined with fluorescein, which is subjected to oxidation, and the antioxidant activity of the tested substance is measured based on the degree of fluorescein inhibition, which is an indirect and nonspecific measure of reactive oxygen species production.(23) Interestingly enough, The CAP-e assay was developed by Alexander Schauss and Gitte Jensen, both of whom are devoted to the study of the properties of açaí berry.

The Total Phenolic Assay by Folin−Ciocalteu, also called the Gallic Acid Equivalence method, is a colorimetric assay of measurement of phenolic content in plants. It works by measuring the amount of the tested substance needed to inhibit the oxidation of the Folin-Ciocalteu reagent.

Lipid peroxidation measurement
Primary end products of lipid peroxidation include conjugated dienes and lipid hydroperoxides, while secondary end products include thiobarbituric reactive substances (TBARS), gaseous alkanes and a group of prostaglandin (PG) F2-like products termed F2-isoprostanes (F2-IsoPs).
The isoprostanes are a unique series of prostaglandin-like compounds formed in vivo via a non-enzymatic mechanism involving the free radical-initiated peroxidation of arachidonic acid. Milne et al. (19) notes, that a vast body of evidence indicates that measurement of F2-IsoPs in body fluids provides the most reliable approach to date to assess lipid peroxidation and represents a major advance in the ability to assess oxidative stress status in vivo. According to the EFSA, assessment of lipid peroxidation in vivo by measuring changes in F2-isoprostanes in 24-h urine samples, which is a preferable above plasma, using gas-chromatography techniques with various detection modes, of which mass spectrometry is preferred, is a reliable marker of lipid peroxidation. Lipid peroxidation could also be obtained in vivo by measuring oxidised LDL particles in blood using immunological methods with appropriate specificity. Primary oxidation product of phosphatidylcholine (PC) in blood plasma and tissues, phosphatidylcholine hydroperoxides (PCOOH) measured in blood or tissue by high-performance liquid chromatography (HPLC) is also a reliable method of assessing lipid peroxidation. TBARS is a lipid peroxidation measurement assay, which is often used by research teams. According to the EFSA, it is not a sufficient measurement on its own, but can be used as a supportive reference. TBARS refers to thiobarbituric acid reactive substances. TBARS assay measures decomposition of the unstable peroxides derived from polyunsaturated fatty acids, which results in the formation of malondialdehyde (MDA) and can be quantified colorimetrically following its controlled reaction with thiobarbituric acid. (21)

**Summary: Nine steps to have your antioxidant claims authorized**

These summarized guidelines, with the exception of Step seven, which is of particular interest for antioxidant activity related claims, are relevant for all health claims applicants under article 13.1. *General health claims* of the EU Regulation No 1924/2006.

**Step one.** The product should be sufficiently characterized.

Information on the characteristics of the food/constituent for which a health claim is made should be provided. Where applicable, this information should contain aspects considered pertinent to the claim, such as the composition, physical and chemical characteristics, manufacturing process, stability, and bioavailability.

**Step two.** The wording of the health claim should be proposed.

Where appropriate, this includes specification of conditions of use, such as the target population and population who should avoid using the product; the quantity of the food/constituent and pattern of consumption required to obtain the claimed effect, and whether this quantity could reasonably be consumed as part of a balanced diet; restrictions and directions of use.

**Step three.** The beneficial effect of the product consumption should be specified.
Reference to general, non-specific benefits of the food/food constituent for overall good health or health-related well-being may only be made if accompanied by a specific health claim. Examples of general and non-specific claim are “premature aging” and “healthy aging”.

**Step four.** The specified health effect should be beneficial for the human health. The claimed effect should be considered beneficial in the context of the specific claim and taking into account the population group for whom the claim is intended.

**Step five.** The claim should be accompanied by (all available) pertinent scientific data. The application must contain all relevant scientific data (published and unpublished) in favour as well as not in favour of the claim. The data provided forms the basis for substantiation of the health claim.

**Step six.** Human studies addressing the relationship between the consumption of the food/constituent and the claimed effect should be provided. Because of the scientific uncertainties in extrapolating non-human data to humans, data from studies in animals or *in vitro* experiments may be included only as supporting evidence. Human studies are central for the substantiation of health claims. Randomized controlled studies are prioritized.

**Step seven.** The provided human studies should operate with reliable biomarkers. Studies measuring (changes in) antioxidant capacity of plasma will not be regarded as pertinent. EFSA suggests the following biomarkers for measurements of oxidative damage to proteins, lipids and DNA:

- **Oxidative damage to proteins:** direct measurements in vivo (e.g. measurement of oxidative changes of amino acids in proteins) by means of HPLC-MS and other methods, as long as identification and separation of such molecules in plasma from other substances is successfully achieved (e.g. from protein tyrosine nitrination products);
- **Oxidative damage to lipids:** measurements of changes in F2-isoprostanes in 24-h urine samples, using gas-chromatography techniques, of which mass spectrometry is preferred. Other acceptable markers are PCOOH;
- **Oxidative damage to DNA:** direct measurements of oxidative damage to DNA *in vivo* using modifications of the comet assay, which allow the detection of oxidised DNA bases (e.g. use of endonuclease III to detect oxidised pyrimidines).

**Step eight.** Design and quality of the provided studies should allow conclusions to be drawn for the scientific substantiation of the claim. This presupposes that the provided data should be consistent in establishing a cause and effect relationship between the consumption of the food/constituent and the claimed effect. Further, the specific study group(s) in which the evidence was obtained should be representative of the target population for which the claim is intended.

**Step nine.** The quantity of the product and pattern of consumption required to obtain the claimed effect should reasonably be achieved as part of a balanced diet.
This boils down to that the consumer should be able to introduce the proposed food/food constituent in their daily diet with ease. For instance, the claim "Walnuts contribute to the improvement of the elasticity of blood vessels" may be used only for a product which provides a daily intake of 30 g of walnuts. In order to bear the claim, information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 30 g of walnuts.

**Authorized antioxidant claims**

ESFA has authorized claims on copper, manganese, riboflavin (Vitamin B2), selenium, vitamin C, vitamin E, and zinc with the wording *contributes to the protection of cells from oxidative stress*. All of these claims were authorized, because the EFSA concluded that the role of the reference vitamins and minerals as (indirect) components of the antioxidant defence system had been well established. The EFSA therefore was engaged in a profound examination of available research confirming the antioxidant activity of these micronutrients.

The only authorized of all the proposed claims on antioxidant activity of food/food constituents so far is the claim on *polyphenols in olive oil*, with the wording *Contribute to the protection of blood lipids from oxidative stress*.

EFSA issued a number of opinions where it summarized the outcomes of the scientific assessment of the proposed antioxidant activity related claims in batches. Claim on the antioxidant activity of pomegranate gained a separate opinion. It is discussed in the corresponding sub-chapter of this thesis.

*The claim may be used only for food which is at least a source of the constituent as referred to in the claim SOURCE OF [NAME OF VITAMIN/S] AND/OR [NAME OF MINERAL/S] as listed in the Annex to Regulation (EC) No 1924/2006.

**The case study: Polyphenols in Olive Oil in nine steps**

The case of polyphenols in olive oil is an exceptional one, because to date it is the only of all the antioxidant-related claims submitted to EFSA with favourable outcome. The case is examined using the proposed 9-steps model. The complete text of the EFSA *Scientific Opinion on the substantiation of health claims related to polyphenols in olive* can be found on the EFSA website. (10)

**Step one.** The product should be sufficiently characterized.

The food constituent is polyphenols (e.g. hydroxytyrosol and oleuropein complex) in olive (olive fruit, olive mill waste waters or olive oil, Olea europaea L. extract and leaf) standardised by their content of hydroxytyrosol and its derivatives (e.g. oleuropein complex).

**Step two.** The wording of the health claim should be proposed.

The claimed effects are “reduces oxidative stress”, “antioxidant properties”, “lipid metabolism”, “antioxidant activity, they protect body cells and LDL from oxidative damages”, and “antioxidant properties”. The target population is assumed to be the general population.
The conditions of use specify 200 mg/day of polyphenols, 2-15 mg per day of hydroxytyrosyl or oleuropein complex, and 250-500 mg of an Olea europaea L. extract standardised to 4-23% oleuropein.

**Step three.** The beneficial effect of the product consumption should be specified. In the context of the proposed wordings, the NDA Panel assumes that the claimed effects refer to the protection of low-density lipoproteins (LDL) particles from oxidative damage.

**Step four.** The specified health effect should be beneficial for the human health. The NDA Panel considers that protection of LDL particles from oxidative damage may be a beneficial physiological effect for the general population.

**Step five.** The claim should be accompanied by (all available) pertinent scientific data. The NDA Panel notes that the vast majority of the references provided for the scientific substantiation of this claim included narrative reviews, human intervention studies, animal studies and *in vitro* experiments on food/food constituents other than olive polyphenols, and/or on effects other than protection of lipids, including LDL particles, against oxidative damage. The Panel considers that no conclusions can be drawn from these references for the scientific substantiation of the claimed effect. In weighing the evidence, the Panel took into account only relevant data including five human studies, which were supported by markers of LDL peroxidation (conjugated dienes, *ex vivo* resistance of LDL to oxidation) going in the same direction, and by provided evidence for a biologically plausible mechanism by which olive oil polyphenols could exert the claimed effect.

**Step six.** Human studies addressing the relationship between the consumption of the food/constituent and the claimed effect should be provided. In weighing the evidence, the NDA Panel took into account one well conducted and powered study, and two smaller-scale studies, which showed a dose-dependent and significant effect of olive oil polyphenol consumption (for three weeks) on appropriate markers of LDL peroxidation. These studies were supported by one short-term and one acute trial (details below).

**Step seven.** The provided human studies should operate with reliable biomarkers. Olive oil polyphenols were shown to significantly decrease the amount of circulating oxidised LDL particles *in vivo* in a dose-dependent manner in one large (n=200, Covas et al., 2006b) and three small scale interventions (12 subjects, (Weinbrenner et al., 2004), 30 subjects, (Marrugat et al., 2004), and 36 subjects, (de la Torre-Carbot et al., 2010), respectively. A dose-dependent decrease in the amount of circulating oxidised LDL particles *in vivo* was also found in one small scale (n=12) acute post-prandial study (Covas et al., 2006a). The lowest daily dose of hydroxytyrosol and its derivatives (measured by HPLC) in olive oil which showed a significant effect on *in vivo* LDL peroxidation was 5 mg (Covas et al., 2006b). Further, a decrease of serum LDL un-induced conjugated dienes in relation to consumption of olive oil polyphenols (Covas et al., 2006b; de la Torre-Carbot et al., 2010) and *ex vivo* resistance of LDL to oxidation (Marrugat et al., 2004) was reported, which can be considered as supportive markers to assess LDL
particles peroxidation. Finally, a significant decrease in plasma C18 hydroxy fatty acids (Covas et al., 2006b; de la Torre-Carbot et al., 2010) and urinary MDA (Weinbrenner et al., 2004) was observed after consumption of olive oil polyphenols.

**Step eight.** Design and quality of the provided studies should allow conclusions to be drawn for the scientific substantiation of the claim.

Most of the human intervention studies described had been conducted in males (aged 20-60 years) using a wide range of daily doses of polyphenols in olive oil. Only studies on polyphenols present in (and consumed with) olive oil have been provided for the substantiation of the claimed effect, and no data have been made available for other food matrices (e.g. leaf tea or tea extract). The NDA Panel concludes that a cause and effect relationship has been established between the consumption of olive oil polyphenols (standardised by their content of hydroxytyrosol and its derivatives) and protection of LDL particles from oxidative damage.

**Step nine.** The quantity of the product and pattern of consumption required to obtain the claimed effect should reasonably be achieved as part of a balanced diet.

5 mg of hydroxytyrosol and its derivatives (e.g. oleuropein complex and tyrosol) in olive oil should be consumed daily in order to obtain the claimed effect (protection of LDL particles from oxidative damage). These amounts can be provided by moderate amounts of olive oil and can be easily consumed in the context of a balanced diet.
Chapter 3. Berries

Berries have been consumed all over the world since ancient times. Humans ate seasonal berries fresh and used preservation techniques (drying, cooking with preserving agents such as sugar) to keep them available for consumption for the rest of the year. In different cultures berries have been believed to possess diverse healing and protective qualities. Since the end of the 20th century there is an exploding interest for health benefits of berries from scientific perspective.

Berries in general are known to be nutrient-dense foods: they contain large amounts of water-soluble vitamins, minerals (potassium, manganese, zinc) and fibre. Scientists hypothesize though that berry polyphenols are the major health-beneficial component in them. In the last two decennia an impressive number of studies have been implemented on the potential health benefits of berries. Most of the studies are in vitro experiments focusing on quantification of polyphenols, their metabolic pathways and effect on different biomarkers. The vast majority of this research hypothesizes that health benefits of the berries are due to their antioxidative capacities. Numerous animal studies are also available. There is still a lack of reliable human data. Randomized controlled clinical trials are difficult to organize and are expensive.

This paper analyses the research available on the antioxidative properties of five berries: açai, blueberry, cherry, goji and pomegranate. All the available human trials for the chosen berries are outlined in the appendixes. A separate overview of human studies in the form of a table is attached as an appendix as well. When relevant, information on selected supporting animal and in vitro research is also provided. The selection had to be made regarding the great number of research and also taking into consideration the fact that for the EFSA ex-vivo, animal and in vitro studies play only a secondary role.
3.1 The case of Açaí berry

Açaí (pronounced ah-sigh-ee), Latin name Euterpe Oleracea, is a berry that grows on a palm tree in the Amazon. The berries are small (10-25 mm in diameter) and have a dark purple, almost black skin and red-purple pulp.*

**Synonyms**: acaí, acaizerio, assai, jicara, jussara, palmitiero, manac, manaka, naidi, pinapalm, piria, pinot.

**Interesting facts**
Dwellers of Belem, major commercial centre in the Northern Brazil, consume 250,000 l of fresh açaí juice daily, both from vendors and own yards. (1)

Açaí represented a major botanical dietary supplement in the United States in 2007. (9)

The following **health claims** are most commonly ascribed to the açaí berry by different manufacturers and wholesalers:

- Balanced Weight Loss
- Increased energy & stamina
- Detoxifies
- Boosts immune system
- Radiant/beautiful skin complexion
- Slows ageing process
- Powerful antioxidant
- Lowers cholesterol
- Healthy sex drive

Alex Schauss is the world’s major expert on the açaí berry. His research on this Amazonian fruit started in 1995. It is partly thanks to him that these berries -- mostly in the form of powder made from freeze-dried pulp -- have entered the US market with grandeur a decade ago and are still enjoying enormous popularity: they are added in chocolate and energy bars, yogurts, juices, energy drinks, immune boosting syrups or sold in the form of pure powder made from freeze-dried pulp and skin. Dr Schauss determined that the freeze-dried pulp and skin of açaí preserve the highest level of antioxidants able to eliminate free radicals *in vitro*, compared to other preservation methods. (13)

*There is also a white variety of açaí but only the purple variety is discussed in this paper.
Antioxidant profile
In his book *Açaí: An Extraordinary Antioxidant-Rich Palm Fruit* Dr Schauss emphasizes the high ORAC (Oxygen Radical Absorbance Capacity) score of the Euterpe Oleracea, and açai distributors use the reference to the ORAC willingly. The ORAC score of 100 g freeze-dried açai pulp is 1027 mmol TE/g. (1)

Five anthocyanins (ACN) were identified in freeze-dried açai berry, cyanidin 3-glucoside and cyanidin 3-rutinoside being predominant. The total ACN content was determined as 3.2-5.0 mg/g dry weight, which is lower than in most other dark coloured berries such as blueberries, blackberries, or cranberries. The ACN are considered to be only for 10% responsible for the overall antioxidant capacity of açai. Twenty two other compounds, comprising other flavonoids, benzenoids, lignans, monoterpenoids, norisoprenoids, and a quinone derivative could be responsible for the remaining 90%. (3, 6, 18, 19)

AÇAI in PubMed
36 results are available for the search terms “açai” + “antioxidant”. Only one of them is qualified as a human randomized controlled trial (RCT) – the study of Jensen et al. on antioxidant and anti-inflammatory capacities of a juice blend (JB) containing açai among other ingredients. (10) Further search in Google Scholar yields three more human studies – a pilot on the influence of açai on the markers of metabolic syndrome, plus a study on pain reduction in people with osteoarthritis, and a study on bioavailability of açai anthocyanins. The four studies are outlined in the appendix Açai Research Review.

Relevance of the studies in the context of the EFSA
To date there are data available on four human studies on açai, only one of which is RCT (Jensen, 2008). Two of the four studies are pilot studies, and one is a study on bioavailability.

The study of Jensen et al. on antioxidant activity of açai JB measured antioxidant status of plasma using ORAC assay, Total Phenolic Assay by Folin–Ciocalteu and CAP-e assay. The antioxidant activity of plasma is not considered to be correlated with any health benefits, according to the EFSA. In the same study lipid peroxidation was measured with the help of Thiobarbituric acid reactive substances (TBARS) assay. TBARS assay, is used alone, is not considered to be a reliable measurement of lipid peroxidation, according to the EFSA. *

To conclude, the study operated with the markers not approved by the EFSA as reliable. Besides, the study was acute, and thus its results could not be interpreted in the scope of long-term effects.

The other studies on açai have failed to show any relevant findings for antioxidant activity related claims either.

Açai and EFSA
In August 2010 a claim was submitted on antioxidative properties of açai. Unlike all the other claims discussed in this paper, the açai claim was submitted not under Article 13.1 (General function claims), but under Article 13.5 (New function claims). Claims under article 13.5 of the EC Regulation No 1924/2006 on nutrition and health claims are those based on newly developed scientific evidence and/or for which protection of proprietary data is requested. For these health claims authorization is required on a case-by-case basis,
following the submission of a scientific dossier to EFSA for assessment. The wording of the açaí claim was proposed as follows: “Assai (Açai) fruit and derivatives present antioxidant activity”. The claim was withdrawn by the applicant in the same year. For confidentiality reasons summaries of Article 13.5 claims applications are not published, therefore it is not possible to trace the scientific substantiation provided by the applicant of the claim. The available to the public body of research does not provide sufficient substantiation so far for açai antioxidant activity related claims.

* Guidance on the scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health. EFSA Journal 2011;9(12):2474.
3.2. The Case of Blueberry

Blueberry is a shrub belonging to the Heath (Ericaceae) family, whose other members are bilberry, cranberry, azalea, mountain laurel, and rhododendron. Blueberry colour is purplish-blue. The berries' flavours can vary from mildly tart (wild berries) to sweet (cultivated species).

There are three varieties of blueberries: highbush (most commonly cultivated), lowbush (also referred to as "wild") and rabbiteye. Blueberries are found on the Euro-Asian, American and Australian continents. Highbush varieties are found exclusively in North America.

Blueberries do not contain outstanding amounts of antioxidant vitamins; their in vitro antioxidant capacity has been attributed to the high concentration of phenolic compounds, particularly anthocyanins. (3, 27)

**Latin names** for blueberry sorts:
- Vaccinium corymbosum L (Highbush)
- Vaccinium angustifolium (Lowbush)
- Vacciniumashei reade (Rabbiteye)
- Vaccinium myrtillus L (Bilberry)

**Interesting facts**
- The United States supplies more than half of the global consumption of blueberries followed by Canada.
- The state of Maine is the largest lowbush blueberry producer in the world.
- Generally, the small-berried genotypes have much higher levels of anthocyanins than the large-berried genotypes. (8).
- The total antioxidant capacity of both highbush and lowbush blueberries species is about 3-fold higher than of strawberries or raspberries (13).
- To date, blueberry is the most studied in clinical conditions among all berry types.
Health claims most commonly attributed to blueberry
Improves vision
lowers blood sugar
rich in antioxidants
beneficial for intestinal health

Antioxidant profile
The total oxygen radical absorbance capacity (ORAC) value of 11 anthocyanins (ACN) identified in blueberry is up to 12.83 mmol of TE/g fresh product and accounts for 56.3% of the total ORAC value. It basically means that the ACN in blueberries are the major contributor to its antioxidant capacity. (33)

Lowbush blueberry contains a high concentration of total anthocyanins, composed of 27 different anthocyanins with no particular anthocyanin being really predominating; delphinidin 3-galactoside and delphinidin 3-glucoside being the highest in concentration (S, 33). Although in different blueberry genotypes the relative anthocyanins distribution is similar, the amount of ACN varies dramatically. Generally, the small-berried genotypes have much higher levels of anthocyanins than the large-berried genotypes. Genetics plays a more important role than growing season in influencing radical absorbance capacity and total phenolic content (TPH) in blueberries. The lowbush blueberry has a higher in vitro antioxidant capacity than the cultivated highbush blueberry. (14, 16, 18) ORAC values of different genotypes also vary enormously, ranging from 20.5 mmol TE/g (Magnolia sort) to 139.4 mmol TE/g for (US-497). (8, 34) Blueberry antioxidant activity, as well as TPH tends to decrease during ripening. The TPH between genotypes differs significantly between harvest years. A linear relationship is found between ORAC and FRAP values and ACN or TPH in blueberries. (10, 12, 18). ACN from blueberry are sensitive to alkaline conditions (>7.0), high temperature (>80 °C) and direct strong light. Freezing does not influence significantly total anthocyanins content in blueberries, while dry berries, in contrast, have a lower ACN level than fresh ones. (15, 20)

In a study on cellular antioxidant activity of 25 common fruits wild blueberry was the champion in demonstrating antioxidant activity. Namely, wild blueberry had the highest phenolic content, the highest ORAC score and the highest cellular antioxidant activity. (21)

Blueberry in PubMed
The terms "Blueberry" plus "antioxidant" hit 196 results, six of which are categorized as randomized controlled trials. Five of them are outlined in the appendix Blueberry Research Review although they are not equally relevant for this paper. The study "Blueberry Supplementation Improves Memory in Older Adults" of Krikorian et al. was excluded from the review, as it did not contain any obvious links to antioxidant mechanisms. Three more relevant human studies were found through Google Scholar. They are also described in the same appendix. Several relevant most quoted animal studies are reviewed there as well.

Relevance of the studies in the context of EFSA
To date, there are eight human studies available on antioxidant properties of blueberries. One of them, the study of Conception et. al (38), is a study on eating pattern and therefore is irrelevant in the scientific discourse. Trials of Wu et al. (14) and Mazza et al. (25) are
studies on bioavailability of blueberry ACN and therefore are only on the tertiary level of the EFSA hierarchy of scientific data. Studies of Kay and Holub (3) en Prior et al. (6) measured changes in antioxidant status in plasma, which is not directly related to any beneficial physiological effect in humans. Three other studies -- McAnulty et al. (36), McAnulty et al. (37), Wilms et al. (31) (please refer for detailed description to the appendix Blueberry Research Review) -- operated with the markers which, if used alone, could not be considered sufficient for scientific substantiation from the EFSA’s point of view.

**Blueberry and EFSA**

In total eleven entries were indexed by the EFSA on blueberry/blueberry products. For the overview of the non-authorized claims please refer to the appendix *Overview non-authorized antioxidant activity related claims on berries/berry products*. In summary, three of these claims have not been assessed, because the product was not sufficiently characterised. For one another claim, the claimed effect was not sufficiently defined to be able to be assessed. For the remaining two claims, the claimed effect has not been substantiated, “because no human studies which investigated the effects of the food(s)/food constituent(s) on reliable markers of oxidative damage to body cells or to molecules such as DNA, proteins and lipids were provided.” (41, 42) The latter two claims were proposed for fresh blueberries and blueberry extracts and included the following wording, correspondingly: “Natural antioxidant, protect organism from oxidative damage, natural way to avoid risks caused by oxidation and peroxidation process” and “Natural berries contain plenty of natural antioxidants (polyphenolic compounds, Vitamin C and carotenoids) and fibre but only a small amount of energy and sodium. For this reason they are very suitable for a heart-friendly diet.”

The following blueberry-related claims have been withdrawn by the applicants:

- Claim nr. 3938: Vaccinium myrtillus (common name: blueberry, bilberry) – Astringent
- Claim nr. 2163: VitaBlue® Blueberry Extract 40% Total Phenolics – Excellent source of healthy fruit polyphenols known to help in the management of heart health
- Claim nr. 2162: VitaBlue® Wild Blueberry Extract – Excellent source of healthy fruit antioxidants (40)

Two more blueberry-related claims (entry nr 2347 and 2050), which were categorized as "botanical substances", are still awaiting completion of the authorisation process, together with other 2200 "botanical" claims.

It could be concluded that available human trials were low-scale and operated with the markers which, if used alone, could not be considered sufficient for scientific substantiation of health claims from the EFSA’s point of view.
3.3. The Case of Cherry

Cherry is a fruit belonging to the genus Prunus in the Rosaceae family and counts more than hundred spices, of which sweet (Prunus avium) and sour (tart) (Prunus cerasus) sorts are the most recognized. Cherries are small (2 cm in diameter) berries with a non-edible pit inside. The colour of cherries varies per species from yellowish to red and dark purple.

**Interesting facts**

Cherries are traditionally used to treat gout and arthritis.

Turkey is number one exporter of cherries in the world.

Cherries contain the highest amount of melatonin of all the foods.

**Health claims** most often attributed to cherries by suppliers

- Help reduce post-exercise muscle and joint pain
- anti-inflammatory benefits
- reduced pain from gout and arthritis
- heart health benefits (4)

**Antioxidant profile**

Analysis of phenolic content of sweet and tart cherries demonstrate inconsistent outcomes, which indicates that it varies significantly. The study of Chaovanalkit et al. involving 3 sweet and 1 sour cherry spices indicated that Bing (sweet) cherry contained a considerably higher level of anthocyanins, but Montmorency (sour) cherry demonstrated the highest antioxidant activity measured by ORAC and FRAP assays. (18)

Cherry contains various kinds of polyphenolics that include cyanidin derivatives (mostly cyanidin 3-rutinoside), peonidin3-glucoside, pelargonidin, kaempherol, quercetin, isorhamnetin and derivatives and alkaloid melatonin. Of these, kaempherol and quercetin prove to be the most active antioxidants. (1) The study of Kirakosyan et al. shows that the bioactive components in cherries act synergetically, meaning that the effect of the consumption of cherry fruits is greater than the sum of the isolated cherry components. (1) As cherries ripen, the amount of anthocyanins increase. The amount of ACN varies per year of harvest. The amount of ultraviolet radiation in the environment during cherry ripening has a significant effect on the resulting accumulation of anthocyanins in cherries. (5)
Cherries in PubMed

Term "cherry" hits 7365 results in PubMed. It has largely to do with the fact that cherry berry belongs to the genus Prunus, which also coins plums, peaches, apricots and almonds. The 7365 articles are therefore not articles on cherry only but also on the mentioned fruits. Search for the terms "cherry" + "antioxidant" yields 35 results, 18 of which are classified as randomized controlled trials. After sorting out trials on prunes and almonds and those with the researcher with the surname Cherry 8 articles are left for further examination. All of them are outlined in details in the appendix Cherry Research Review.

Relevance of the studies in the context of EFSA

The vast majority of research on cherries focuses on the effect of cherry phenolics on the physical performance/muscle damage/feeling of exhaustion after strenuous exercise. The presented in the appendix Cherry Research Review trials of Howatson et al. (8), Conolly et al. (11)and Keuhl et al. (12) study this effect. The general conclusion which can be drawn from the three trials is that ingesting tart cherry juice prior to and during a strenuous exercise can minimize post-exercise muscle pain. There could not be drawn a conclusion that there is a direct correlation between antioxidant capacity of cherries and any physiological effects, as the studies lack reliable markers.

The most profound study from the EFSA’s perspective would be the intervention of Traustadottir et al. (9) The study used reliable markers, but the results showed that the tart cherry juice intervention did not have significant effects on those markers (urinary levels of F2-isoprostanes and dityrosine, marker of protein damage). There was however a significant decrease in markers of DNA and RNA damage, 8-OHdG and 8-oxo-G, but these markers if used alone cannot signify a direct damage to nucleic acids, in accordance with the EFSA.

The studies of Kelly et al. and Martin et al. (14, 16, 17) measured biomarkers of inflammation and/or plasma triglycerides neither of which is directly linked to anti/oxidation.

Study of Jacob et al. measured changes in antioxidant status in plasma after cherry consumption with ORAC, TEAC and FRAP assays and could not be considered a sufficient substantiation for any antioxidant-related health claims, because plasma levels of antioxidant does not have a direct influence on health benefits.

Six health claims on cherries were proposed by the applicants. Three of them had to do with antioxidant properties. One of the antioxidant claims was not authorized, because the claimed effect for the product did not prove to be a beneficial physiological effect on the basis of the substantiation proposed. The two other claims were not authorized “because no human studies which investigated the effects of the food(s)/food constituent(s) on reliable markers of oxidative damage to body cells or to molecules such as DNA, proteins and lipids were provided” (20) and therefore a cause and effect relationship was not established between the food constituent and health benefits.

It could be concluded that the existing body of research does not provide sufficient substantiation so far for cherry antioxidant activity related claims.
3.4. The Case of Goji Berry

Goji berry (Latin name *Lycium barbarum*), also known as Chinese Wolfberry, is shrubbery of Solanaceae family. The fruits are traditionally used as a nutraceutical in Asian countries such as China, Mongolia and Tibet. There are also American varieties of goji berries known; they grow in Southern states of the US. Goji is a small (1 cm) ellipsoid orange or red berry with tiny edible yellow seeds inside. The berries are harvested in summer and autumn, normally they are first dried in the shade and then exposed to the sun for further drying until the skin is hard and dry but the pulp remains soft. The reddish-orange colour of the berry derives from a group of carotenoids, zeaxanthin being predominant. The most well-researched chemical constituents of *Lycium barbarum* fruit are water-soluble glycoconjugates, collectively termed Lycium Barbarum Polysaccharides (LBP), they comprise 5% to 8% of the dried fruit. (1)

**Synonyms**: Matrimony Vine, Boxthorn, Desert Thorn, Duke of Argyll’s tea tree, Murali, Gouqizi, Kei Tze.

**Interesting facts**
Goji berries are used in Traditional Chinese Medicine (TCM) for balancing of Yin and Yan, for stimulation of kidney and liver function, weak joints and improvement of eyesight.

Chinese herbalist Li Ching-Yuen whose life-span was claimed to be 197 to 256 years old, advocated for goji berries and used to eat a goji soup daily.

**Health claims** ascribed to the goji berry by different suppliers:

- Improves liver functions
- improves kidney functions
- good for the eyesight
- anti-age properties
- blood glucose control
- anti-fatigue properties

**Nutritional profile**
Several types of compounds including vitamins (B1, B2, B3, B6, C, E ), 21 trace minerals, 18 amino acids, essential oil and fatty acids, carotenoids (mainly zeaxanthin dipalmitate,
followed by beta-cryptoxanthin monopalmitate), flavonoids (myricetin, quercetin, kaempferol), polysaccharides, and betaine have been identified in *Lycium barbarum*. Among these, carotenoids, betaine, and polysaccharides have been reported to be the biologically active. *Lycium Barbarum Polysaccharides* (LBP) are a mixture of proteoglycans and polysaccharides, consisting mainly of arabinose, galactose, glucose, xylose, and little amounts of rhamnose, mannose, and galacturonic acid as its glycosidic part. (9, 18)

**Goji in PubMed**
The term 'goji' produces 100 results, 'goji' + 'antioxidant' – 13, only two of which are randomized controlled trials (RCT), both of them outlined in the appendix Goji Research Review, along with a pilot study and a single-blinded trial on bioavailability of zeaxanthin in goji berries. Available animal studies are reviewed in the same document too.

**Goji & EFSA**
Clinical studies have been claimed to be taking place in China. The outcomes are published in the local journals, which are not accessible. The obtainable RCTs (1, 3, 4) operated with markers which can only be used as secondary, supportive markers (in EFSA’s definition), while the primary are missing.

Claim referring to health benefits of gojiberries with proposed wording: Contains antioxidant/s; Is a source of antioxidant/s; With antioxidant/s; Contributes to the cell protection against free radicals; Can protect your cells and tissues from oxidation; Can contribute to the total antioxidant capacity of the body has been proposed. EFSA concluded that for this claim the claimed effect has not been substantiated, “because no human studies which investigated the effects of the food(s)/food constituent(s) on reliable markers of oxidative damage to body cells or to molecules such as DNA, proteins and lipids were provided.”(21)

Two other claims on effects of Water Soluble Wolfberry Concentrate (entry nr. 1366, health claim Aging Management, and entry nr. 1364, health claim Normal Immune Function) were withdrawn by the applicant. The studies of Bucheli et al. (3) and Cheng et al. (14) measured plasma zeaxanthin after goji consumption, which was increased after the interventions. Increase of an antioxidant level in plasma is not a beneficial physiological effect of its own. Besides, in its *Scientific Opinion on the substantiation of health claims related to zeaxanthin and maintenance of normal vision* (22) EFSA already concluded that there was insufficient evidence for conclusion that increase of plasma zeaxanthin has a positive effect on maintenance of vision.

It could be concluded that the existing body of research does not provide sufficient substantiation so far for goji antioxidant activity related claims.
3.5. The case of pomegranate

The pomegranate (Latin name *Punica granatum*) is a fruit of a deciduous shrub belonging to Punicaceae family that grows up to 7 meters height. Inside the fruit, which on average is of an apple size, there are arils with 300-400 of edible seeds. The taste of the seeds varies depending on the sort and ripeness from sour to sweet with a hint of astringent taste deriving from the tannins. Pomegranate juice is consumed world-wide, and is gaining more and more popularity. The highest antioxidant activity among pomegranate polyphenols was observed for punicalagin (the pomegranate ellagitannin).

**Synonyms**
Anardana, Dadim, Fruit of the Dead, Granada, Grenade, Roma, Shi Liu Gen Pi.

**Interesting facts**
The fruit is present in Persian and Greek mythologies, as well as in Hinduism and Chinese folklore, symbolizing life, marriage, fertility, prosperity and regeneration; in Christianity its seeds are a symbol of individual worshipers gathered in one community of faith, while In Islam, the Quran indicates that pomegranates grow in the gardens of paradise. (2)

Many scholars believe that the forbidden fruit that Eve seduced Adam with in the Garden of Eden was actually not an apple but a pomegranate.

The sales of pomegranate juice increased from $84,507 in 2001 to $66 million in 2005 in the United States. (14)

**Health claims** most often attributed to pomegranate by suppliers
Weight reduction
cholesterol control
free radicals scavenging
skin nourishment
antiwrinkle effects
protection against Alzheimer’sdisease
protection against rheumatoid arthritis
increased libido

**Antioxidant profile**
Antioxidant properties of pomegranate are attributed to its polyphenolic complex including fatty acids, punicalagins (pomegranate ellagitannins), hydrolysed tannins, ellagic acid and anthocyanins, delphinidin, cyanidin, and pelargonidin being predominant in the last group. (9, 11) Ellagitannins are the major polyphenols in the pomegranate fruit and juice (juice-pressed whole fruit, arils, and seeds) and account for >90% of the antioxidant activity of the juice. (10, 11). The research demonstrates low bioavailability of the pomegranate polyphenols. It is therefore speculated that the health benefits of pomegranate are due to its metabolites (e.g., urolithins) rather than the compounds of the intact fruit. (5,6)

![Figure 9. Alpha-punicalagin](image)

Commercial pomegranate juices have shown an antioxidant activity three times higher (18-20 TEAC) than those of red wine and green tea (6-8 TEAC). The activity was remarkably higher in juices extracted from whole pomegranates than in experimental juices obtained from the arils only. (11)

**Pomegranate in PubMed**
The term ‘pomegranate’ yielded 28 results, ‘pomegranate’ + ‘antioxidant’ – 11 results, 5 of which are defined as randomized controlled trials. All these trials are outlined in the appendix *Pomegranate Research Review*, although some of them are of obviously less relevance than the others. Five more clinical human studies found through Google Scholar are described there as well.

**Pomegranate and EFSA**
There have been 12 applications made on pomegranate related health claims. The overview of the entries can be found in appendix *Overview non-authorized antioxidant activity related claims on berries/berry products*. Four of these entries were related to antioxidative properties of pomegranate/pomegranate juice. The EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to provide a scientific opinion on the pomegranate related health claims. The NDA issued its opinion in 2010. (28)The active food constituents were defined as punicalagin/ellagic acid, and claimed effects were “antioxidative function”, “antioxidant properties”, and “antioxidants and immunity”. The NDA assumed that the claimed effects related to the protection of lipids from oxidative damage caused by free
radicals, which is a beneficial physiological effect. In its opinion the NDA Panel summarized the provided human data for the proposed claims as follows:

A single arm, uncontrolled intervention study in 13 healthy male volunteers which assessed the effects of pomegranate juice consumption (50 ml per day containing 1.5 mmol total polyphenols) for two weeks on changes in the ex vivo activity of serum paraoxonase (an HDL-associated esterase), in plasma lipid peroxides (AAPH induced spectrophotometric method), and in the oxidation lag time of low-density lipoproteins (LDL) ex vivo was provided (Aviram et al., 2000). A second single arm (Rosenblat et al., 2006), uncontrolled intervention study in 10 healthy subjects and 10 non-insulin dependent diabetes mellitus (NIDDM) patients under pharmacological treatment was provided with the consolidated list. All subjects consumed 50 ml per day of pomegranate juice containing 1979 mg/l of tannins (1561 mg/L of punicalagin and 417 mg/l of hydrolysable tannins), 384 mg/l of anthocyanins (delphinidin 3,5-diglucoside, cyanidin 3,5-diglucoside, delphinidin-3-glucoside, cyanidin 3-glucoside and pelargonidine 3-glucoside) and 121 mg/l of ellagic acid derivatives for three months. Serum concentrations of lipid peroxides, thiobarbituric acid reactive substances (TBARS), serum SH groups, serum paraoxonase 1 (PON1) activity, cellular peroxides and glutathione content in monocytes-derived macrophages (HMDM), and oxidised LDL uptake by HMDM were measured at the beginning and end of the intervention.

The NDA Panel considered that no conclusions could be drawn from these small and uncontrolled studies for the scientific substantiation of the claimed effect, and concluded that a cause and effect relationship was not established between the consumption of punicalagin/ellagic acid in pomegranate/pomegranate juice and the protection of lipids from oxidative damage.

The NDA Panel did not take into consideration a 3 year-long study of Aviram et al., 2004 (17), which investigated the effects of regular PJ supplementation on the thickness of carotid intima-media, blood pressure and LDL oxidation, and which yielded positive results. Small number of participants and the choice of markers (which, if used alone, cannot be used for substantiation of health effects) will not allow the study to be qualified as a reliable scientific substantiation by the EFSA. But this study can probably be used as supporting evidence next to research focusing on other biomarkers. At the moment the research on pomegranate is actively going on (for instance in Rappaport Institute Haifa, Israel). It is not excluded that in the years to come enough evidence is piled to substantiate a claim related to antioxidative properties of pomegranate.

The detailed description of the studies mentioned as well as of the other available human studies can be found in the appendix Pomegranate Research Review.
Concluding Remarks

Berries have been traditionally consumed everywhere in the world to prevent and cure diverse diseases. Centuries-long observations make the establishment of the connection between ingestion of certain berries and (improvement of) certain conditions possible: pomegranate as part of the Mediterranean diet and lower incidence of cardiovascular diseases in the region; improvement of gout condition with abundant cherry consumption. These observations often become the point of departure for scientific research. In the last two decennia there is an obvious exploding interest for health benefits of berries from scientific perspective.

Berry polyphenols are generally considered to be nontoxic and beneficial for health, although the precise mechanisms of their metabolism and action remains largely unclear. Absorption peculiarities and routes of biological activity probably depend on the individual structure of polyphenols, as well as on humans inter-individual variation in their metabolism. Most studies report low falvonoids excretions, being particularly poor for anthocyanins, which are considered to play the major role in antioxidative mechanisms of berries (for instance in blueberries they are supposedly responsible for >50% of antioxidant activity). It could be therefore suggested that not all of the flavonoids’ metabolites might have been identified yet, and that their bioavailability might be underestimated.

Most of the berries studies available to date are in vitro experiments. Laboratory trials are on the lowest scale of the EFSA hierarchy of scientific data which can be used as substantiation for health claims on foods/foods constituents. An outstanding amount (for some berry types, for instance blueberries) of animal studies is not of much relevance for EFSA either, as it also can play only a supportive role in substantiation of health claims. That what needs to be supported — human data — is scarce. Moreover, in most cases the researches operate with the markers which, to EFSA point of view, can only be supportive to primary markers of direct measurements (of oxidative damage), while those primary markers are missing in the research. The question whether the criteria chased by EFSA are too limiting, is beyond the scope of this paper. What needs to be noted is that research teams worldwide have no consensus as how to assess metabolism of (berry) flavonoids and their impact on health. Further (human) research is needed in order to design a reliable model for assessment of utilization of berries active compounds by the humans and their alleged benefits.

Of the five berries examined in this paper pomegranate shows to be the most promising in the sense, that the number of the human research available is the greatest for this fruit (although still not really impressive: 10 controlled clinical trials) and that some of the studies are long-term and/or operate with reliable markers (of oxidation). It is not excluded that in the near future enough evidence is piled together to substantiate a claim related to
antioxidative properties of pomegranate. For now, the consumers should be aware that sound claims of marketers on antioxidative properties of berries are not (sufficiently) substantiated from the scientific perspective.

As berries are a safe, tasty and nutrient-dense, their consumption should be encouraged, and the years to come probably will shed more light on their benefits for human health.
Abbreviations

ACE: angiotensin converting enzyme
AUC: area under the curve
CAP-e: cell-based antioxidant protection in erythrocytes
CAS: carotid artery stenosis
COPD: chronic obstructive pulmonary disease
DAS(28): Disease Activity Score
DNA: deoxyribonucleic acid
EFSA: European Food Safety Authority
FCR: Folin–Ciocalteu reagent
FOX: ferrous oxidation-xylenol orange
FRAP: ferric reducing antioxidant potential
GAEs: gallic acid equivalents
GSH-Px: glutathione peroxidase
HDL: high-density lipoprotein
HPLC: high-performance liquid chromatography
GSH-Px: glutathione peroxidase
LBP: Lycium Barbarum Polysaccharides
LDL: low-density lipoprotein
MDA: malondialdehyde
NIDDM: non-insulin dependent diabetes mellitus
HMDM: monocytes-derived macrophages
ORAC: oxygen radical absorbance capacity
PCOOH: phosphatidylcholine hydroperoxides
PON1: serum paraoxonase 1
RA: rheumatoid arthritis
SDS: stress-induced ischemia
SOD: superoxide dismutase
TAS: total antioxidant status
TBARS: thiobarbituric acid reactive substances
TEAC: trolox-equivalent antioxidant capacity
TG: plasma triglycerides
TPH: total phenolic content
TRAP: total reactive antioxidant potential
UV: ultraviolet
VLDL: very low-density lipoprotein
**Açai Research Review**

**Human Studies**
A randomized, double-blinded, placebo-controlled, crossover trial of Jensen et al. involving 12 healthy subjects examined the antioxidant activity of juice blend MonaVie Active (JB) with açai as the major ingredient. Other ingredients included pomegranate, wolfberry, camu camu, passion fruit, aronia, acerola, bilberry, apricot, purple grape, white grape, lychee, banana, kiwi, pear, cranberry, blueberry, and prune. Blood samples at baseline, 1 h, and 2 h following consumption of 120 ml of JB or placebo were tested for antioxidant capacity using several antioxidant assays: ORAC Assay, Total Phenolic Assay by Folin–Ciocalteu and CAP-e assay. Lipid peroxidation was measured with the help of TBARS assay. In 11 out of 12 persons an increase in serum antioxidants at 2 h as well as inhibition of lipid peroxidation in 10 persons at 2 h postconsumption was observed. (10)

An open-label clinical pilot study of the same team, this time on pain reduction, involving 14 people with osteoarthritis and pain, showed a significant pain reduction after a treatment with 120 ml juice blend MonaVie Active (JB) daily during 12 weeks. Serum antioxidant status, measured by CAP-e assay, was improved after 2 weeks of consumption of JB and kept improving throughout the 12 weeks of the study. One of the observations emerging from the analysis of the data was the correlation between elevated pain levels and decreased plasma antioxidants status. (11)

In an uncontrolled pilot study of Udani et al., incorporating 10 participants with BMI > 25 &< 30, consumption of açai fruit pulp 100 g x 2 daily during 1 month resulted in reduced levels of selected markers of metabolic syndrome, including fasting glucose and insulin, total cholesterol and showed borderline significance in reduction of LDL-cholesterol and ratio total to HDL cholesterol (4).

**Bioavailability**
In an acute four-way crossover clinical trial of Mertens-Talcott et al. involving 12 people, the bioavailability of açai anthocyanins was studied. Açai pulp and clarified açai juice were used as compared to applesauce and a non-antioxidant beverage as controls. Açai pulp caused a significant increase in the antioxidant capacity of plasma which was measured by ORAC assay. The applesauce administration also caused an increase in plasma antioxidant capacity. The antioxidant capacity in urine, generation of reactive oxygen species, and uric acid concentrations in plasma were not significantly altered by the treatments. (13)

**Supporting Animal Study**
In one rat study seven berry types, including açai, demonstrated about an equal capability of inhibiting tumour progression in the oesophagus area in spite of known differences in levels of anthocyanins. (5)

**In vitro Study**
Jensen et al. implemented in vitro research using the CAP-e assay to evaluate the activity of antioxidants of juice blend MonaVie Active (JB) in a non-inflammatory cell-based system. It was determined that a significant amount of the antioxidants found in the JB are available
to living cells, capable of penetrating the plasma membrane of the cells, and able to protect the cells from intracellular oxidative damage. The antioxidant protection effect was dose-dependent. (10)
Blueberry Research Review

**Human research**

*In vivo*

A randomized, double-blind, crossover study of McAnulty et al. examined whether blueberries (150 g/d) or vitamin C (1250 mg) or placebo consumed for 7 days would attenuate oxidative stress and cytokine changes in athletes (n=9) exercising in hot environments. The combination of exercise and heat stress significantly elevated plasma concentrations of F2-isoprostanes and ROOH. The increase in these lipid markers indicated that the subjects underwent oxidative stress from exercising in the heat. The pattern of change between treatments was significant for lipid hydroperoxides (in favour of BB group) but not for any other marker. The administration of blueberries significantly suppressed ROOH production.

Plasma F2-isoprostanes were determined using gas chromatography mass spectrometry according to the methodology of Morrow and Roberts. The pattern of change in F2-isoprostanes post-run did not differ significantly between treatments. Lipid hydroperoxides were the only marker to change significantly. (37) However concentrations of lipid peroxides in blood could be used only as as supportive evidence (i.e. in addition to measurements of F2-isoprostanes in urine), in accordance with the EFSA criteria. F2-isoprostanes were determined in this study, but they were not significantly changed. Besides, they were measured in plasma and not in urine. Therefore the findings of this study could not be considered a strong evidence of blueberry health benefits.

In a randomized controlled trial of McAnulty et al. the effect of consumption of 250 g/d blueberries (or alternatively 250 g/d of mixed fruit) during three weeks was studied in relationship to attenuation angiotensin converting enzyme (ACE) activity and oxidative stress in chronic cigarette smokers (n = 20). The antioxidant level in plasma was not altered significantly in either group as measured by the FRAP assay. Lipid hydroperoxides were the only marker to change significantly at the end of treatment. (27) However, concentrations of lipid peroxides in blood could be used only as a supportive evidence (i.e. in addition to measurements of F2-isoprostanes in urine), in accordance with the EFSA criteria. F2-isoprostanes were determined in this study, but they were not significantly changed. Besides, they were measured in plasma and not in urine. Therefore the findings of this study could not be considered a strong evidence of blueberry health benefits.

In a randomized crossover study of Prior et al. involving 6 healthy women (aged 43.8 ± 3.8 years) the subjects consumed 1 cup of blueberries daily as part of their regular meals for a period of 14 days. It resulted in increased hydrophilic antioxidant capacity of area under the curve (AOC AUC) while AOC in control group (non-antioxidant meal) was decreased. (6)

A single blinded crossover study of Kay and Holub was performed involving 8 middle-aged (38–54 years) male subjects who consumed a high-fat meal and a control supplement followed 1 week later by the same high-fat meal supplemented with 100 g freeze-dried wild blueberry powder. Significant increases in serum antioxidant status (measured by ORAC and TAS assays) above the controls were observed at 1 h (ORAC PCA 8.5% greater; TAS 4.5% greater), and 4 h (ORAC total 15% greater; ORAC acetone 16.0% greater) post-consumption. (3)
In a study of Conception et al. involving 2 college athletes it was speculated that antioxidants from a blueberry beverage may impact plasma vitamins. During 30 days blueberry/placebo supplementation was administrated. It was basically a study meant to investigate whether the supplementation will alter the normal eating pattern of the athletes. Blueberry supplementation did not affect plasma vitamin concentrations and did not alter the eating habits of the athletes. (38)

**Ex vivo**

A pilot study of Wilms et al. involving 168 healthy participants (18-45 years) was designed to assess antioxidative and possible anti-genotoxic properties of fruit-borne antioxidants. The participants consumed 1 l of blueberry/apple juice per day, leading to a dose of 16 mg ascorbic acid and 97 mg of quercetin bound to a sugar moiety to ensure bioavailability. An increase in quercetin and ascorbic acid plasma concentrations and in TEAC value (from 781 IM (±3.95) to 800 IM (±4.02) TE) and a decrease (20%) in H2O2-provoked DNA damage *ex vivo* was observed, measured by comet assay. On average, men showed a larger protective effect upon blueberry/apple juice intervention than women. (31)

**Bioavailability of blueberry**

In a single-blind crossover study of Mazza et al. five healthy male subjects (46.9 +/- 1.9 years) consumed 100 g/d freeze-dried blueberry powder (lowbush) which contained 25 individual anthocyanins (ACN); the total amount of ACN was 1.20 g; oxygen radical absorbance capacity (ORAC) value of the preparation was 147 mmol of TE. The absorption of ACN was observed after the consumption of a high-fat meal with a freeze-dried blueberry powder. Nineteen of the 25 ACN present in the blueberries were detected in the blood serum. The appearance of total ACN in the serum was directly correlated with an increase in serum antioxidant capacity. The interpretation of the results is that consumption of blueberries is associated with a diet-induced increase in *ex vivo* serum antioxidant status. (14)

A study of Wu et al. involved 6 elderly women who acutely consumed 189 g lowbush blueberry (690 mg total ACN). The study demonstrated low absorption and excretion of ACN compared with other flavonoids in 5 out of 6 women. The researches were unable to detect ACN in plasma; ACN, however, were detected in the urine, but the total amount excreted during the first 6 hours post-consumption was only 0.004% of the quantity consumed. (25).

**Supporting animal research**

A study of Joseph et al. on 40 Fischer rats who were divided in 4 groups: control and fed for 8 weeks with strawberry, blueberry or spinach extracts (equalized by their ORAC activity), showed that supplementation was effective in reversing age-related deficits in several neuronal and behavioral parameters, blueberry being the most effective. (11)

Rachel et al. speculated that dietary supplementation with antioxidant rich foods could decrease level of oxidative stress in brain regions and therefore improve age-related deficits in neuronal and behavioural functions. An examination whether short-term supplementation with blueberries might enhance the brain's ability to generate a heat shock protein 70 (HSP70) mediated neuroprotective response to stress was done on 30 Fischer rats. HSP70 mediated protection against a stressor such as lipopolysaccharides (LPS)
increased significantly. The outcomes of the research suggest that short-term BB supplementation restored the ability of the aged hippocampal cells to respond to an inflammatory challenge with a large production of inducible HSP70, and that this process could play a role in shifting the balance between pro- and anti-oxidant forces. (19)

Another study of Rachel et al. tested the hypothesis that blueberries would reduce DNA damage and lipid peroxidation and increase phase II enzyme activities *in vivo* in rats. 8 rats (per group) were fed control diets or diets supplemented with blueberries or blueberry extracts (0.2% and 1% flavonoid concentrations) for 3 weeks. There was a significant alteration of only one marker: a slight decrease in liver DNA damage for the 1% flavonoid group. The research team notes that that the study "shows that in vivo results are not always in agreement with in vitro findings and that very high intakes of blueberry may be required to elicit significant effects in some tissues." (22)

In a study of Carrie et al. on male spontaneously hypertensive rats who received a BB-enriched diet or an isocaloric control diet for 6 or 12 weeks or 2 days, it was concluded that long-term BB-enriched diet lowered blood pressure, preserved renal hemodynamics, and improved redox status in kidneys and concomitantly demonstrated the potential to delay or attenuate development of hypertension-induced renal injury. Further, the results of experiment demonstrated that total ROS, superoxide, and peroxynitrite production rates were significantly lower and antioxidant activities were significantly higher in BB-fed rats. (29)
Cherry Research Review

Cherry and exercise induced stress

Persistent or chronic pain is a common symptom accompanying nearly every disease. It is speculated that phytochemicals in cherries may affect pain through modulation of cytokine biology and oxidants production. Pain associated with acute muscle exploration is likely due to oxidative tissue damage which leads to an inflammatory response, resulting in further production of free radicals and in increase in secondary muscle soreness. The vast majority of research on cherries focuses on the effect of cherry phenolics on the physical performance/muscle damage/feeling of exhaustion after strenuous exercise. The three following trials study this effect.

The purpose of the study of Howatson et al. was to examine the effect of a tart cherry juice blend supplementation on markers of muscle damage, inflammation and oxidative stress during strenuous exercise. Twenty recreational Marathon runners volunteered to consume either cherry juice or placebo for 5 days before, the day of and for 48 hours following a Marathon run. The cherry juice group took two 237 ml bottles of a commercially produced tart cherry juice blend, containing the equivalent of 50–60 cherries and at least 600 mg phenolic compounds, the oxygen radical absorbance capacity (ORAC) of one bottle was 55 mmol/L TE. Among other markers, total antioxidant status (TAS) and thiobarbituric acid reactive species (TBARS, marker of lipids peroxidation) were examined before and following the race. TAS appeared to be 10% greater in the cherry juice group for all post-supplementation measures. TBARS was lower in the cherry juice group at 48 h. (8) Decrease in TBARS level cannot be considered an evidence of antioxidative effect, because TBARS, if used alone, is not a reliable marker, in accordance with the EFSA criteria.

In a randomised, placebo controlled, crossover trial of Conolly et al. 14 male college students consumed 355 ml of a cherry juice blend twice a day or a placebo for 8 days. Each bottle of the cherry juice provided at least 600 mg GAE phenolic compounds and at least 40 mg anthocyanins (calculated as cyanidin-3-glucoside equivalents) thus being the equivalent of 50–60 cherries. A series of eccentric elbow flexion contractions (2620 max) was performed on the fourth day of supplementation. Isometric elbow flexion strength, pain, muscle tenderness, and relaxed elbow angle were recorded before and for four days after the exercise. Strength loss was 22% with the placebo but only 4% with the cherry juice group; the pain was experienced to a significantly lesser degree in the cherry group. (11)

In a randomized, double blind, placebo controlled study of Keuhl et al. 54 healthy runners ran 26.3 ± 2.5 km over a 24 hour period. The runners ingested 355 ml x 2 daily of tart cherry juice or placebo for 7 days prior to and on the day of the race. One bottle of the juice provided 600 mg GAE phenolic compounds and at least 40 mg anthocyanins (calculated as cyanidin-3-glucoside equivalents). The experience of pain was accessed. Both groups reported increased pain after the race, but it was significantly less for the cherry juice group (12 ± 18 mm compared to 37 ± 20 mm for the placebo group on a standard 100 mm Visual Analog Scale. (12)

The conclusion, which appears also to be relevant for the two studies mentioned above, is that ingesting tart cherry juice prior to and during a strenuous exercise can minimize post-
exercise muscle pain. A conclusion could not be drawn though that there is a direct correlation between antioxidant capacity of cherries and any physiological effects, as the studies lack reliable markers.

**Other human data**

A study of Traustadottir et al. checked the hypotheses if consumption of tart cherry juice containing high levels of anthocyanins would improve the capacity of older adults to resist oxidative damage during acute oxidative stress. In a double-blind, placebo-controlled, crossover trial 6 women and 6 men consumed either cherry juice (240 ml x 2 daily for 14 days) or placebo. The juice was commercially produced and the daily portion contained 120 mg of anthocyanins and 1100 mg gallic acid equivalents of phenolic compounds (GAE). The capacity to resist oxidative damage was measured as the changes in plasma F2-isoprostane levels in response to forearm ischemia-reperfusion (I/R) before and after each treatment. The tart cherry juice intervention reduced the I/R-induced F2-isoprostane response. Basal urinary excretion of oxidized nucleic acids was also reduced. Urinary F2-isoprostanes did not differ between the intervention and placebo, and there was no significant change in dityrosine, a marker of protein damage. There were significant changes in long-lived markers of oxidative damage of DNA and RNA. 8-OHdG and 8-oxo-G, measured in a pooled sample from urine collected during the last 5 days of the treatment period, were both significantly lower during the tart cherry juice treatment as compared to the placebo. (9) The EFSA notes that urinary 8-OHdG does not directly reflect DNA oxidation within cells, but could be used in combination with direct measurements of oxidative damage to DNA if appropriate techniques are used for analysis (e.g. HPLC). The study used reliable markers, but the results showed that the tart cherry juice intervention did not have significant effects on those markers (urinary levels of F2-isoprostanes or dityrosine). There was however a significant decrease in 8-OHdG and 8-oxo-G, but these markers if used alone cannot signify a direct damage of nucleic acids, in accordance with the EFSA. (9)

In a randomized, placebo-controlled crossover study of Martin et al. 10 volunteers with BMI>25.0 took part. They consumed 237 ml daily of either 100% tart cherry juice or a placebo beverage for 4 weeks. The erythrocyte sedimentation rate (ESR), an indicator of chronic inflammation, was significantly lower with treatment than with the placebo beverage.

During the same experiment plasma triglycerides were measured. Total cholesterol was not different between treatments, but plasma triglycerides (TG), TG/HDL-C, and VLDL were reduced by 10%, 17% and 15%, respectively after the juice consumption. The authors therefore suggest that “100% tart cherry juice may reduce biomarkers of inflammation often noted in chronic disease and may reduce the risk of CVD by reducing plasma TG.” (16, 17). During this study biomarkers of inflammation and/or plasma triglycerides were measured, neither of which is directly linked to anti/oxidation.

In the recent (2011) master study of L. Diemert the influence of 3x112 g/d consumption of fresh sweet cherries during 4 weeks was investigated in connection with COX-2 inhibition in men at elevated risk of Prostate cancer (n=30). The bioavailability of cherry anthocyanins (ACN) was examined in the same study. There was a high inter-individual variation of ACN levels excreted noted. It was therefore suggested that this could signify the individual variation in the ACN metabolism. With regard to the anti-inflammatory marker, the COX-2
metabolite PGEM, there was a significant reduction noted in men with elevated baseline values. (13)

To assess physiological effects of cherry consumption, in the study of Jacob et al. plasma urate, antioxidant and inflammatory markers were measured in healthy women (n=10) who consumed 280 g Bing sweet cherries after overnight fast during 10 days. Plasma urate decreased significantly over the 5-h period after cherry consumption. With regard to antioxidant capacity measures, the ORAC and TEAC measures did not differ after cherry consumption, lipophilic ORAC increased and FRAP decreased at all postdose sampling times. Plasma ascorbic acid increased significantly at 1.5 and 3 h postdose. Plasma creatinine decreased significantly at 1.5 and 5 h postdose, and marginally at 3 h postdose. Plasma albumin was unchanged throughout. (15)
Goji Research Review

Human studies
In the first randomized, double-blind, placebo-controlled clinical trial outside China of Harunobu Amagase et al. that has examined the general effects of the oral consumption of the standardized goji berry juice GoChi™ (120 ml/d which is equivalent to 150 g of fresh fruit, the amount traditionally prescribed by TCM specialists) to healthy adults (N 34) for 14 days an increase in subjective feelings of general well-being and improvement of neurologic/psychological performance and gastrointestinal functions have been reported. Additionally, of the 9 female subjects in the GoChi group, 5 reported a decrease in nonspecific complaints and pain during their menstrual cycle. Two male subjects in the GoChi group noted improvement in skin conditions, and 1 female subject reported harder nails. None of the subjects in the placebo group shared any information regarding any noticeable changes during the treatment period. (4)

Following this research, another study under leadership of Dr. Harunobu Amagase investigated the effects of consumption of 60 mlx2/d of goji juice (GoChi) containing 1632 mg of LBP on antioxidant markers (serum levels of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and lipid peroxidation (indicated by decreased levels of malondialdehyde (MDA)) in 50 Chinese healthy adults aged 55 to 72 years. The study lasted for 30 days. At the postintervention time point, serum SOD activity was significantly higher in the GoChi group (by 8.10%) relative to the level observed in the placebo group. GSH-Px had increased by 9.04% in the GoChi group. The MDA level had decreased by 5.95% in the GoChi group. (1)

As far as EFSA is concerned, concentrations of MDA in blood could be used as a supportive factor next to measurements of F2-isoprostanes or \textit{in vivo} LDL oxidation, neither of which been assessed during this research. Induction of antioxidant enzymes SOD and GSH-Px could be considered a beneficial physiological effect "only if such changes provide (additional) protection of cells and molecules from oxidative damage, which should be demonstrated \textit{in vivo} in humans."* Concluding from the above mentioned, this research will not regarded by the EFSA as a pertinent scientific evidence, as it is based on the measurements of markers which can only be secondary, while the primary are missing.

During the same study immunological biomarkers have been observed too. These observations were published in a separate article. The consumption period, number of participants and other variables of the trial coincide with the ones mentioned above. The results showed that daily consumption of GoChi significantly increased several immunological responses (number of lymphocytes and levels of interleukin-2 and immunoglobulin G compared to pre-intervention and the placebo group) and subjective feelings of general well-being without any adverse reactions. (13)

The three human trials described above were conducted under the leadership of Harunobu Amagase, an employee of \textit{Freelife int.} (23) which produces, among other, the goji products. The GoChi juice used in all this research is the product of \textit{Freelife int.}

Bioavailability of zeaxanthin
In a double-masked, randomized, placebo-controlled trial of Bucheli et al. on macular degeneration in healthy elderly subjects (n 150) receiving 13.7 g/d of milk-based formulation of goji berry, Lacto-Wolfberry (LWB) or placebo for 90 days an increase in plasma zeaxanthin and antioxidant levels was marked in the individuals receiving LWB. The antioxidant status was measured by high-performance liquid chromatography. Further, 13 individuals from the placebo group demonstrated hypopigmentation and 11 individuals from the placebo group demonstrated soft drusen accumulation in the macula, whereas the LWB group remained stable. Antioxidant capacity levels were stable over time for the placebo group but increased with 57% with the LWB. However, the mechanism of action of LBP remains unclear, given the lack of relationship between change in plasma zeaxanthin and change in macular characteristics. (3)

In a single-blinded, placebo-controlled, human intervention trial of parallel design of Cheng et al. 14 persons were given 15 g/d of goji berry (estimated to contain almost 3 mg zeaxanthin) for 28 days. Fasting blood was collected prior to the trial and on day 29. Control group (n 13) took no goji berry. It was concluded that plasma zeaxanthin in the goji group increased 2.5-fold. This study shows that zeaxanthin in whole wolfberries is bioavailable and that intake of a modest daily amount markedly increases fasting plasma zeaxanthin levels. (14)

Supporting Animal studies
A study of Reeve et al. on mice describes the potential for orally consumed goji berry juice to alter the photo damage induced in the skin by acute solar simulated UV (SSUV) irradiation. Dilutions of goji berry juice between 1% and 10% dose-dependently protected against SSUV-induced immunosuppression, as well as against suppression induced by the mediator, cis-urocnic acid. Furthermore, mice drinking 5% goji berry juice had the normal level of lipid peroxidation which remained not significantly different following UVA irradiation. Therefore it could be suggested that goji berry juice consumption was a strong inhibitor of UVA-induced lipid peroxidation in the skin (of mice). (19)

The effects of LBP on blood glucose, oxidative stress and DNA damage in rats with non-insulin dependent diabetes mellitus (NIDDM) were observed in the study of Wu et al. The results showed that LBP treatment (10 mg/kg daily) for 4 weeks led to decreased levels of blood glucose, MDA and nitric oxide (NO) in serum of fasting rats; and to increased serum level of SOD. It is speculated that LBP could reduce cellular DNA damage in peripheral lymphocytes of NIDDM rats. (16)

In a study of Wu et al. effect of LBP was investigated in relation to oxidative stress in high-fat mice. Results showed that blood and liver antioxidant enzymes activities and GSH level in model mice significantly decreased, and MDA level significantly increased compared to control group. (20)

It was found that Lycium barbarum fruit extracts could significantly reduce blood glucose levels and serum total cholesterol (TC) and triglyceride (TG) concentrations and at the same time markedly increase high density lipoprotein cholesterol (HDL-c) levels after 10 days treatment in
tested rabbits. The results are evidence of that Lycium barbarum polysaccharides (LBP) are major bioactive components of the hypoglycemic effect. However, both LBP and other compounds with antioxidant properties from Lycium barbarum fruits are possible triggers of hypolipidemic effect. According to the authors, the detailed mechanism of action needs further investigation. (15)

Research on pomegranate

Studies of Michael Aviram

The protective qualities of pomegranate juice against atherogenesis have been extensively studied in the last decade by Aviram’s and co-workers in Haifa, Israel. He took active part in the four following in vivo trials and in one ex-vivo trial, outlined below in the corresponding paragraph.

In a three-year long study of Aviram et al. (17) the effects of pomegranate juice (PJ) consumption by atherosclerotic patients with carotid artery stenosis (CAS) on the progression of carotid lesions and changes in oxidative stress and blood pressure was investigated. Ten patients were supplemented with PJ for 1 year and five of them continued for two more years. After 1 year the PJ group participants’ serum paraoxonase 1 (PON 1) activity was increased and LDL-oxidation was decreased significantly, and serum total antioxidant status (TAS) was increased by 130%. An increase in the (carotid) lesion glutathione(GSH) content, by 2.5-fold, was observed after PJ consumption for 3 or 12 months. In support to these results, LDL oxidation by lesions was significantly decreased (43% after 3 months, 32% after 12 months) in comparison to the control group. The author states that increase of PON 1 activity and decrease in LDL-oxidation were correlated. Serum paraoxonase 1 (PON 1) is a HDL-associated paraoxonase, which, according to the EFSA Guidelines*, is not a reliable in vivo marker of lipid peroxidation. EFSA states that “decrease in glutathione is considered a beneficial physiological effect only if such changes provide (additional) protection of cells and molecules from oxidative damage, which should be demonstrated in vivo in humans. “

The amount of LDL-associated lipid peroxides in the study was measured by the method of El-Saadani et al., which is a spectrophotometric assay for lipid peroxides in serum lipoproteins and is not mentioned in the Guidelines as a reliable assay for measurement of lipid peroxidation.

Further, it should be taken into account that the use of a small number of patients in this study (10) could cause a statistical error.

Concluded from the summarized above could be that the biomarkers observed during the study and the method of measurement of (lipid) peroxidation cannot be considered a convincing evidence for antioxidant activity of PJ. But this study can probably be used as a supporting evidence next to research focusing on other biomarkers.

In a study of Rosenblat, Hayek and Aviram 20 subjects whereof 10 healthy (controls) and 10 non-insulin dependent diabetes mellitus (NIDDM) patients participated. Both groups consumed 50 ml PJ per day for 3 months. In the patients versus controls serum levels of lipid peroxides and thiobarbituric acid reactive substances (TBARS) were both increased, whereas serum SH groups content and paraoxonase 1 (PON1) activity, were both decreased (measured before the intervention, which indicated oxidative stress in diabetic patients). In the patients versus controls (HMDM), increased level of cellular peroxides and decreased glutathione content was observed (before the intervention). After PJ consumption the lipid peroxides and TBARS levels were decreased by 56% and 28%, respectively, as compared to the levels observed in the patients’ serum prior to consumption, whereas serum total sulfhydryl groups content and PON1 activity, significantly increased by 12% and 24%, respectively. PJ consumption also significantly reduced cellular peroxides (by 71%), and
increased glutathione levels (by 141%) in the patients’ HMDM. The patients’ versus control HMDM took up oxidized LDL (Ox-LDL) at enhanced rate, and PJ consumption significantly decreased the extent of Ox-LDL cellular uptake \textit{in vitro}. It was thus concluded that PJ consumption by diabetic patients resulted in anti-oxidative effects on serum and macrophages. (18)

In a recent study (2011) of Aviram and Dornfeld the effect of pomegranate juice consumption (50 ml daily, 1.5mmol of total polyphenols) for 2 weeks by hypertensive patients (n=10) on their blood pressure and on serum angiotensin converting enzyme (ACE) activity was tested. A 36% decrement in serum ACE activity and a 5% reduction in systolic blood pressure were noted. (16) The results of the study cannot receive direct interpretation in the antioxidant activity context.

In a recent (2011) pilot study of Balbir-Gurman et al. (26) 6 subjects with rheumatoid arthritis consumed POMx preparation (10 ml daily) for 12 weeks. The daily dose of preparation (10 ml) contained 1300 mg GAE and consisted of 95% polymolecular mixture ellagitannins, mainly punicalagin, and 5% ellagic acid. The intervention significantly reduced the composite Disease Activity Index (DAS28) by 17%, and the tender joint count (by 62%). These results were associated with a significant reduction in serum oxidative status and a moderate but significant increase in serum high density lipoprotein-associated PON1 activity. The addition of POMx to serum from the participants reduced free radical-induced lipid peroxidation by up to 25%.

**Other human studies**

A randomized controlled clinical trial of Hashemi et al. was conducted among 30 adolescents (12–15 y) with metabolic syndrome. The participants had to drink whether pomegranate or grape juice daily during 30 days. The biomarkers of endothelial function (basal brachial artery dimension and flow-mediated dilation) and endothelial-dependent dilation were measured. After receiving nitroglycerin spray, these markers were evaluated by high resolution mode (ultrasonography) after 4 hours post-consumption and after 1 month. Flow-mediated dilation improved significantly within 4 hours of drinking juice in both groups. There was also significant improvement after 1 month of regular consumption of the aforementioned juices in both groups, but basal brachial dimension only improved significantly after 1 month of regular consumption of grape juice. The authors suggested therefore that consumption of antioxidant-rich drinks may improve endothelial function in children with metabolic syndrome. (1) This study is rather correlated with cardiovascular health, for which EFSA established specific criteria and which are beyond the scope of this thesis.

In the study of Summer et al. the effects of daily consumption of pomegranate juice for 3 months were studied in a randomized, placebo-controlled, double-blind study. 45 patients with ischemic coronary heart disease (CHD) were involved, and the affect on myocardial perfusion was investigated. After 3 months, the extent of stress-induced ischemia decreased in the pomegranate group but increased in the control group. (6)

In the study of Abidov et Al the effects of Radical Fruits TM (RF) supplement were studied on plasma cholesterol and urinary 8-epi PGF2a and 11-dehydro-TXB2 concentrations in
hypercholesteremic men (n=44). The RF supplement was composed of the following concentrated ingredients: prune, pomegranate, apple, grape, raspberry, blueberry, white cherry and strawberry. The RF group took 900 mg of the supplement 3 times daily before meals during 4 weeks. The results of the intervention indicate a significant inverse correlation between consumption of RF supplement and total plasma cholesterol concentration, as well as reduction of plasma LDL and increase in plasma HDL concentrations. (24)

A pilot study of Heber et al. involving 22 overweight subjects was designed for antioxidant activity assessment by administration of a pomegranate ellagitannin-enriched polyphenol extract (POMx). Two POMx capsules were provided per day, containing 1000 mg of extracts (610 mg of gallic acid equivalents (GAEs). Measurement of antioxidant activity in plasma were done before and after POMx supplementation. There was evidence of antioxidant activity through a significant reduction in TBARS, a biomarker of oxidative stress, measuring products of lipid oxidation in the blood. (10)

In a 5-week randomized, double-blind, placebo-controlled trial of Cerda et al. the effect of pomegranate juice (PJ) supplementation on patients with stable chronic obstructive pulmonary disease (COPD) (n=30) was investigated. The daily dose of PJ was 400 ml and contained 2.66 g polyphenols and provided 4 mmol/l TEAC. None of the polyphenols present in PJ were detected in plasma or in urine of the subjects. The most abundant PJ polyphenols, ellagittannins, were metabolized by the colonic microflora of the patients to yield two major metabolites in both plasma and urine (dibenzopyranone derivatives) with no TEAC. There was no difference with the control group for any of the evaluated parameters. The results suggest that PJ supplementation adds no benefit to the standard therapy in patients with stable COPD, and that "the high TEAC of PJ cannot be extrapolated in vivo probably due to the metabolism of its polyphenols by the colonic microflora." (7)

Di Silvestro et al. researched effects of pomegranate rinsing in relevance with gingivitis risk (randomized, single-blinded controlled intervention; n=32). Among other findings, it was concluded that thrice daily pomegranate mouth rinsing during 4 weeks resulted in increase of activities of the antioxidant enzyme ceruloplasmin (which is believed to give protection to oral oxidant stress) and increased radical scavenging capacity (though this increase was significant only by nonparametric statistical analysis). (5)

Supporting ex-vivo and animal studies

In a comparative 4 weeks study of Guo et al. on the effects of apple and pomegranate juice on antioxidant status of plasma, involving 26 elderly subjects, increased plasma antioxidant capacity and decreased plasma carbonyl content were demonstrated after daily consumption of pomegranate juice (250 ml). (12)

In a study of Aviram et al. potent antioxidative effects of pomegranate juice against lipid peroxidation in whole plasma and in isolated lipoproteins (HDL and LDL) were assessed in humans and in E0 mice after pomegranate juice consumption. The human study consisted of two parts, first one involving 13 healthy subjects (consuming daily 50 mg PJ during 2 weeks) and the second involving 3 subjects (consuming daily 20-80 ml/d of PJ during 10 weeks). The human studies were conducted ex-vivo.
Human plasma obtained after 2 wk of PJ consumption showed 6 % decreased susceptibility to AAPH induced lipid peroxidation. Additionally, a 9% increase in plasma total antioxidant status was observed after 2 weeks of PJ consumption. Following supplementation with 20 ml PJ/d for 1 week (n=3) resulted in a 11% decrease in plasma lipid peroxide content. Supplementation with 50 ml PJ/d for 1 more week resulted in a further 21% decrease in plasma lipid peroxidation, while yet an additional increase in PJ supplementation to 80 ml PJ/d for an additional week did not inhibit plasma susceptibility to lipid peroxidation further. The inhibitory effect of PJ consumption on plasma lipid peroxidation was maintained for 2 weeks after PJ supplementation ended.

In E0 mice, LDL oxidation by peritoneal macrophages was reduced by up to 90% after PJ administration, and this effect was associated with reduced cellular lipid peroxidation and superoxide release. The uptake of oxidized LDL and native LDL by peritoneal macrophages obtained after pomegranate juice administration was reduced by 20%. Finally, pomegranate juices supplementation reduced the size of the mice’ atherosclerotic lesions by 44% and also the number of foam cells. (15)

It is therefore speculated by the research team that pomegranate juice “had potent antiatherogenic effects in healthy humans and in atherosclerotic mice that may be attributable to its antioxidative properties”.

Bioavailability of pomegranate polyphenols

Pomegranate ellagitannins (ETs) comprise on average 70% of the polyphenols in commercial PJ (3), but are assumed to be non-absorbable due to the large size of the ET molecules. (21)

The study of Mertens-Talcott et al. investigated the absorption and antioxidant effects of a standardized extract from pomegranate in healthy volunteers (n=11) after the acute consumption of 800 mg of extract. Results showed that ellagic acid (EA) from the extract was bioavailable. The antioxidant capacity of plasma using ORAC Assay was measured and it was maximally increased after 0.5 h (31.8%). The second peak in antioxidant capacity was measured after 6 h (31.7%). No change in the reactive oxygen species generation was observed. (19)

In a study of Seeram et al. one person consumed 180 ml of PJ containing 25 mg ellagic acid (EA) and 318 mg hydrolyzable ellagitannins (ETs), and the bioavailability of EA and ETs was accessed. (20) ETs were not detected in plasma, while EA was.

Following study of Seeram et al., this time involving 18 subjects, further investigated the bioavailability of ETs. After an acute single oral dose of PJ concentrate (180 ml containing 318 mg punicalagins and 12 mg of free EA), EA increased rapidly and was cleared from plasma samples of all volunteers by 5 hours. (21).

These findings correspond with the outcomes of the research of Cerda et al. who studied the bioavailability of the pomegranate ellagitannines in vitro and in humans. The volunteers (n=6) consumed 1 litre of pomegranate juice (5.6 g of polyphenols including ellagitannins, ellagic acid derivatives and anthocyanins). Neither punicalagin nor ellagic acid present in the PJ were detected in both plasma and urine, but at least 3 microbial ellagitannin-derived metabolites were detected. The metabolites did not show significant antioxidant activity compared to punicalagin from PJ. The research team concluded that
“The potential systemic biological effects of pomegranate juice ingestion should be attributed to the colonic microflora metabolites rather than to the polyphenols present in the juice.” (25)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Duration</th>
<th>Study subjects</th>
<th>Control</th>
<th>Intervention</th>
<th>Significant findings</th>
<th>Findings significant for EFSA criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Udani et al., 2011 (4); uncontrolled pilot study</td>
<td>30 days</td>
<td>10 participants with BMI &gt; 25 &amp;&lt; 30</td>
<td>None</td>
<td>Açai fruit pulp 100 g x 2/d</td>
<td>Reduction total cholesterol from 159 ± 37 mg/dl to 142 ± 28 mg/dl; Reduction of the post-prandial increase in glucose levels: AUC from 205.6 ± 18.6 to 189.7 ± 26.3</td>
<td></td>
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<tr>
<td>Jensen et al., 2008 (10); randomized, double-blinded, placebo-controlled, crossover study</td>
<td>Acute</td>
<td>12 healthy subjects 19 to 52 years</td>
<td>Encapsulated placebo product from potato flakes with a purplish food-coloring blend</td>
<td>120 ml of juice blend MonaVie Active(JB); acute. Blood samples drawn at 1 and 2 h after ingestion</td>
<td>Increase in the serum antioxidant capacity within 2 h of consumption in 11 of 12 study participants; decrease in serum lipid peroxidation within 2 h of consumption in 10 of the 12 study participants (TBARS)</td>
<td>TBARS if used alone is not a reliable marker</td>
</tr>
<tr>
<td>Jensen et al., 2011 (11); open-label pilot study</td>
<td>12 weeks</td>
<td>14 people 44–84 years with osteoarthritis</td>
<td>None</td>
<td>120ml daily of JB</td>
<td>Serum antioxidant status, as measured by the CAP-e assay correlation between elevated pain levels and decreased plasma antioxidants status</td>
<td></td>
</tr>
<tr>
<td>Mertens-Talcott et al., 2008 (13); acute consumption trial</td>
<td>Acute</td>
<td>12 healthy people</td>
<td>Applesauce and a non-antioxidant beverage</td>
<td>Juice 7 mL/kg of body weight of both pulp &amp; juice</td>
<td>Plasma antioxidant capacity açai pulp and applesauce</td>
<td></td>
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<tr>
<td>Reference</td>
<td>Duration</td>
<td>Study subjects</td>
<td>Control</td>
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<tr>
<td>Kay &amp; Holub, 2002 (3); single-blind crossover study</td>
<td>Postprandial phases, one week apart</td>
<td>8 male subjects 38–54 years</td>
<td>High-fat meal</td>
<td>High-fat meal followed by 100 g freeze-dried wild blueberry powder</td>
<td>Increase in serum antioxidant status</td>
<td>Bioavailability studies are of tertiary importance</td>
</tr>
<tr>
<td>Prior et al., 2007 (6); randomized crossover study</td>
<td>14 days</td>
<td>6 healthy women 43.8 ± 3.8 years</td>
<td>A non-antioxidant meal</td>
<td>1 cup of blueberries daily</td>
<td>Increased plasma AOC in the postprandial state</td>
<td></td>
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<tr>
<td>McAnulty et al., 2004 (37); randomized double-blind crossover study</td>
<td>1 week for each treatment with 1 week washout</td>
<td>9 athletes</td>
<td>Blueberry-flavored shake</td>
<td>150 g/d blueberries in a milk shake/ 1250 mg/d vitamin C in a blueberry-flavoured shake</td>
<td>ROOH decrease in BB group; lipid peroxides</td>
<td>lipid peroxides (but only as a secondary evidence next to F2-isoprostanes in urine measurement)</td>
</tr>
<tr>
<td>McAnulty et al., 2007 (36); randomized control trial</td>
<td>3 weeks or postprandial</td>
<td>20 smokers 26-30 years</td>
<td>Restriction of large amounts of fruits and vegetables and vitamin supplements</td>
<td>Acute or daily consumption of 250 g blueberries</td>
<td>Decrease in lipid hydroperoxides in blueberry group at 3 weeks</td>
<td>Decrease in lipid hydroperoxides (but only as a secondary evidence next to F2-isoprostanes in urine measurement)</td>
</tr>
<tr>
<td>Mazza et al., 2002 (14); single-blind crossover study</td>
<td>Postprandial phases, one week apart</td>
<td>5 males, 47 ± 2 years</td>
<td>High-fat meal</td>
<td>High-fat meal followed by100 g freeze-dried wild blueberry powder</td>
<td>Increase in serum antioxidant status</td>
<td>Bioavailability studies are of tertiary importance</td>
</tr>
<tr>
<td>Wu et al., 2002 (25); single-blind crossover study</td>
<td>Acute</td>
<td>6 elderly women (60-70 years)</td>
<td>189 g lowbush blueberry blended in 315 ml water after fasting overnight as compared to 12 g elderberry extract blended in 500 ml water</td>
<td>Lower ACN excretion in urine than in elderberry group; no ACN in BB group detected in plasma</td>
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<tr>
<td>Reference</td>
<td>Duration</td>
<td>Study subjects</td>
<td>Control</td>
<td>Intervention</td>
<td>Significant findings</td>
<td>Findings significant for EFSA criteria</td>
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<tr>
<td>Traustadottir et al., 2009 (9); double-blind, placebo-controlled, crossover trial</td>
<td>14 days</td>
<td>6 healthy women &amp; 6 healthy men 61-75 years</td>
<td>Unsweetened black-cherry Kool-Aid soft drink mixed with water</td>
<td>240 mL/2 x daily</td>
<td>Reduction in the I/R-induced F2-isoprostane response; reduction in basal urinary excretion of oxidized nucleic acids; reduction in 8-OHdG and 8-oxo-G</td>
<td>Urinary F2-isoprostanes did not differ between the intervention and placebo; no significant change in dityrosine, a marker of protein damage; urinary 8-OHdG can be used as a supportive evidence in combination with direct measurements of oxidative damage to DNA (e.g. HPLC)</td>
</tr>
<tr>
<td>Diemert, 2011 (13); master thesis; single-arm clinical intervention</td>
<td>4 weeks</td>
<td>30 men at elevated risk of Prostate cancer</td>
<td>None</td>
<td>3x112 g/d consumption of fresh sweet cherries</td>
<td>Reduction inCOX-2 metabolite PGEM</td>
<td></td>
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<tr>
<td>Martin et al., (16, 17) randomized, placebo-controlled crossover study</td>
<td>4 weeks</td>
<td>10 subjects with BMI&gt;25.0</td>
<td>Placebo</td>
<td>237 ml daily of 100% tart cherry juice</td>
<td>Reduction in erythrocyte sedimentation rate (ESR); reduction in plasma TG (10%), TG/HDL-C (17%), and VLDL (15%)</td>
<td>Triglycerides are markers of cardiovascular health and therefore are not relevant for antioxidant related claims</td>
</tr>
<tr>
<td>Jacob et al., 2003 (15); single-arm clinical intervention</td>
<td>10 days</td>
<td>10 healthy women 22–40 years</td>
<td>None</td>
<td>280 g Bing sweet cherries after overnight fast</td>
<td>Decrease in plasma urate</td>
<td></td>
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<tr>
<td>Blando et al., 2004 (8)</td>
<td>8 days</td>
<td>20 recreational Marathon runners</td>
<td>Fruit flavoured concentrate mixed with water</td>
<td>237 ml x 2/d of cherry juice (min. 600mg phenolic compounds) 5 days before, the day of and for 48 hours following a Marathon run</td>
<td>TAS increase by 10% greater in the cherry juice group for all post-supplementation measures; TBARS lower in the cherry juice group at 48 h</td>
<td>TBARS as measure of lipid peroxidation, but only as supportive to more reliable measurements, i.e. F2-isoprostanes</td>
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<tr>
<td>Study Authors</td>
<td>Duration</td>
<td>Participants</td>
<td>Intervention</td>
<td>Protocol Details</td>
<td>Results</td>
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<tr>
<td>Conolly et al., 2006 (11)</td>
<td>8 days</td>
<td>14 male college students</td>
<td>Unsweetened black cherry soft drink mixed with water</td>
<td>355 ml of a cherry juice blend (min. 600mg phenolic compounds) twice a day. A bout of eccentric elbow flexion contractions (2 x 20 maximum contractions) was performed on the 4th day of supplementation</td>
<td>Less strength loss and less pain with the cherry juice group</td>
<td></td>
</tr>
<tr>
<td>Kuehl et al., 2010 (12)</td>
<td>8 days</td>
<td>54 healthy runners</td>
<td>Fruit punch soft drink mixed with water</td>
<td>355 ml of a cherry juice blend (min. 600mg phenolic compounds) twice a day or placebo for 7 days prior to and on the day of the race</td>
<td>Less muscle pain with the cherry juice group</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>Duration</td>
<td>Study subjects</td>
<td>Control</td>
<td>Intervention</td>
<td>Significant findings</td>
<td>Findings significant for EFSA criteria</td>
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<tr>
<td>Amagase et al., 2008 (4);</td>
<td>2 weeks</td>
<td>34 healthy adults</td>
<td>Solution matching the color, flavor, and taste of GoChi</td>
<td>Standardized goji berry juice GoChi™, 120 ml/d</td>
<td>Increase in subjective feelings of general well-being and improvement of neurologic/psychological performance and gastrointestinal functions</td>
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<tr>
<td>randomized, double-blind,</td>
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<td>placebo-controlled clinical trial</td>
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<tr>
<td>Amagase et al., 2009 (1);</td>
<td>30 days</td>
<td>50 Chinese healthy adults aged 55 to 72 years</td>
<td>Sucralose solution matching the color, flavor, and taste of GoChi</td>
<td>Standardized goji berry juice GoChi™, 2x60 ml/d</td>
<td>Increase in serum SOD and GSH-Px; decrease in MDA level in the GoChi group</td>
<td>SOD, GSH-Px and MDA only as additional markers but not as the primary evidence</td>
</tr>
<tr>
<td>randomized, double-blind,</td>
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<td>placebo-controlled clinical trial</td>
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<tr>
<td>Bucheli et al., 2011 (3);</td>
<td>90 days</td>
<td>150 healthy elderly subjects</td>
<td>Milk based formulation</td>
<td>13.7 g/d of milk-based formulation of goji berry, Lacto-Wolfberry (LWB) in the form of freeze-dried powder mixed with 200 ml of soup or hot water</td>
<td>Increase in plasma zeaxanthin and in antioxidant capacity levels</td>
<td>Studies on bioavailability only of tertiary importance</td>
</tr>
<tr>
<td>double-masked, randomized,</td>
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<td>placebo-controlled trial of</td>
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<tr>
<td>Cheng et al., 2005 (14)</td>
<td>28 days</td>
<td>14 persons</td>
<td>Placebo</td>
<td>15 g/d of goji berry</td>
<td>Increase in plasma zeaxanthin</td>
<td>Studies on bioavailability only of tertiary importance</td>
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<tr>
<td>single-blinded, placebo-</td>
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<tr>
<td>controlled intervention</td>
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</table>
Table 5. Summary of pomegranate human studies

<table>
<thead>
<tr>
<th>Reference</th>
<th>Duration</th>
<th>Study subjects</th>
<th>Control</th>
<th>Intervention</th>
<th>Significant findings</th>
<th>Findings significant for EFSA criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hashemi et al., 2010 (1)</td>
<td>30 days</td>
<td>30 adolescents (12–15 y) with metabolic syndrome</td>
<td>None</td>
<td>Pomegranate juice 240 ml/d or grape juice 18 ml/kg/d</td>
<td>Flow-mediated dilation improved significantly within 4 hours and 30 days</td>
<td></td>
</tr>
<tr>
<td>Summer et al., 2005(6); randomized, placebo-controlled, double-blind study.</td>
<td>3 months</td>
<td>45 patients with ischemic coronary heart disease (CHD)</td>
<td>Modified sports beverage of similar caloric content, amount, flavor, and color</td>
<td>240 ml/day pomegranate juice</td>
<td>Extent of SDS decreased in the pomegranate group</td>
<td></td>
</tr>
<tr>
<td>Cerda et al., 2006 (7); randomized, double-blind, placebo-controlled trial</td>
<td>5 weeks</td>
<td>30 patients with stable (COPD)</td>
<td>Synthetic orange-flavoured drink)</td>
<td>400 ml PJ daily</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Heber et al, (10); pilot study</td>
<td>acute</td>
<td>22 overweight subjects with excess abdominal fat as evidenced by a waist circumference of g35 in. for women and g40 in. for men</td>
<td>None</td>
<td>Two POMx capsules per day, containing 1000 mg of extracts (610 mg of GAEs)</td>
<td>Reduction in TBARs</td>
<td>TBARs if used alone is not a reliable marker of lipid peroxidation</td>
</tr>
<tr>
<td>Aviram et al., 2000 (15); single-arm clinical trial</td>
<td>2 weeks</td>
<td>13 healthy male volunteers</td>
<td>None</td>
<td>50 ml per day containing 1.5 mmol total polyphenols)</td>
<td>Decrease in plasma lipid peroxidation; increase in plasma total antioxidant, both ex vivo</td>
<td></td>
</tr>
<tr>
<td>Study Authors and Year</td>
<td>Duration</td>
<td>Participants</td>
<td>Intervention Details</td>
<td>Outcome Results</td>
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<tr>
<td>Aviram and Dornfeld, 2011 (16); single-arm clinical intervention</td>
<td>2 weeks</td>
<td>10 hypertensive non-smoking patients 62-77 years</td>
<td>None</td>
<td>50 ml PJ daily, 1.5mmol of total polyphenols</td>
<td>A 36% decrement in serum ACE activity and a 5% reduction in systolic blood pressure</td>
<td></td>
</tr>
<tr>
<td>Aviram et al., 2004 (17); randomized, double-blind, placebo-controlled trial</td>
<td>1 year/3 years</td>
<td>10/5 patients with CAS</td>
<td>Placebo, not specified</td>
<td>50 ml PJ daily, 1.5mmol of total polyphenols</td>
<td>Increase in PON 1 activity; decrease in LDL-oxidation; increase in TAS; increase in the (carotid) lesion GSH content; decrease in LDL oxidation by lesions</td>
<td></td>
</tr>
<tr>
<td>Balbir-Gurman et al., 2011 (26); pilot 12 week open-labelled study</td>
<td>12 weeks</td>
<td>6 postmenopausal women with active RA</td>
<td>None</td>
<td>10 ml daily POMx (1300 mg GAE)</td>
<td>Decrease in DAS28 an, tender joint count and serum oxidative status; an increase in PON1 activity</td>
<td></td>
</tr>
<tr>
<td>Rosenblat et al., 2006 (18); single arm uncontrolled intervention</td>
<td>3 months</td>
<td>20 subjects whereof 10 healthy (controls) and 10 NIDDM</td>
<td>None</td>
<td>50 ml PJ per day</td>
<td>Decreased serum levels of lipid peroxides and TBARS, whereas increase in serum SH groups content and PON1 activity; reduction in cellular peroxides and increase in glutathione levels in the patients’ HMDM</td>
<td></td>
</tr>
<tr>
<td>Abidov et al, 2006 (24); randomized double-blinded, placebo controlled clinical trial</td>
<td>4 weeks</td>
<td>44 hypercholesteremic men</td>
<td>Placebo capsule, not specified</td>
<td>900 mg of the supplement (Radical Fruits TM ) 3 times daily before meals</td>
<td>Inverse correlation between consumption of RF supplement and total plasma cholesterol concentration; reduction of plasma LDL and increase in plasma HDL concentrations</td>
<td></td>
</tr>
<tr>
<td>Mertens-Talcott et al (19), 2006; single-arm acute intervention</td>
<td>acute</td>
<td>11 healthy volunteers</td>
<td>None</td>
<td>Acute consumption of 800 mg of standardized pomegranate extract</td>
<td>Increase in antioxidant capacity of plasma</td>
<td></td>
</tr>
</tbody>
</table>
Table 6. Nutritional value of berries (per 100 g)

<table>
<thead>
<tr>
<th></th>
<th>Açaí, freeze-dried</th>
<th>Blueberry, raw</th>
<th>Cherry, raw, sour</th>
<th>Cherry, raw, sweet</th>
<th>Goji, dried</th>
<th>Pomegranate, raw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kcal</td>
<td>533.9</td>
<td>57</td>
<td>50</td>
<td>63</td>
<td>391</td>
<td>87</td>
</tr>
<tr>
<td>Carbohydrates, g</td>
<td>52.2</td>
<td>14.5</td>
<td>12</td>
<td>16</td>
<td>78</td>
<td>19</td>
</tr>
<tr>
<td>Sugars, g</td>
<td>1.3</td>
<td>10</td>
<td>8.5</td>
<td>12.8</td>
<td>56</td>
<td>13.7</td>
</tr>
<tr>
<td>Dietary fiber, g</td>
<td>44.2</td>
<td>2.4</td>
<td>1.6</td>
<td>2.1</td>
<td>7.4</td>
<td>4</td>
</tr>
<tr>
<td>Protein, g</td>
<td>8.1</td>
<td>0.7</td>
<td>1</td>
<td>1</td>
<td>11</td>
<td>1.7</td>
</tr>
<tr>
<td>Total fat, g</td>
<td>32.5</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>3.6</td>
<td>1.2</td>
</tr>
<tr>
<td>Saturated fat, g</td>
<td>8.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.9</td>
<td>0</td>
</tr>
<tr>
<td>Cholesterol, mg</td>
<td>13.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin C, mg</td>
<td>&lt;0.1</td>
<td>10</td>
<td>7</td>
<td>10</td>
<td>43</td>
<td>10</td>
</tr>
<tr>
<td>Vitamin A, IU</td>
<td>1002</td>
<td>54</td>
<td>1283</td>
<td>64</td>
<td>30600</td>
<td>0</td>
</tr>
<tr>
<td>Calcium, mg</td>
<td>260</td>
<td>6</td>
<td>16</td>
<td>13</td>
<td>288</td>
<td>10</td>
</tr>
<tr>
<td>Iron, mg</td>
<td>4.4</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>7.7</td>
<td>0.3</td>
</tr>
</tbody>
</table>
Table 7. Phenolic profile of berries

<table>
<thead>
<tr>
<th>Berry name</th>
<th>Açai pulp, freeze-dried</th>
<th>Blueberry, raw</th>
<th>Cherry, raw</th>
<th>Goji, dried</th>
<th>Pomegranate, raw</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORAC score, µmolTE/100 g</td>
<td>102700</td>
<td>4669 (highbush) - 9621 (lowbush)</td>
<td>1145 - 3747</td>
<td>3290</td>
<td>4479</td>
</tr>
<tr>
<td>Total polyphenols, mgGAE/100 g</td>
<td>1390</td>
<td>311 (highbush) – 429 (lowbush)</td>
<td>259</td>
<td>Not known</td>
<td>338</td>
</tr>
<tr>
<td>Total anthocyanins, mg/g</td>
<td>3.2-5.0</td>
<td>5.6</td>
<td>0,05-0,30</td>
<td>Not known</td>
<td>2.1 – 4.3*</td>
</tr>
</tbody>
</table>

* USDA Database for the Oxygen Radical Absorbance Capacity (ORAC) of Selected Foods, Release 2. U.S. Department of Agriculture
Table 8. Overview non-authorized claims on berries/berry products

<table>
<thead>
<tr>
<th>Nutrient, substance, food or food category/EFSA opinion reference / Journal reference</th>
<th>Claim</th>
<th>Health relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sqeez Wild Blueberry Juice Drink 2011;9(6):2244</td>
<td>Blueberries have a wide range of health benefits including anti-ageing properties and the maintenance of urinary tract and vision health.</td>
<td>not validated</td>
</tr>
<tr>
<td>blueberry extracts 2011;9(6):2244</td>
<td>Blueberry can support maintaining of proper night vision.</td>
<td>not validated</td>
</tr>
<tr>
<td>Berries and fruit juices/flavonoids + ascorbic acid 2011;9(4):2082</td>
<td>Includes (natural) flavonoids and other antioxidants. Berry/fruit juice contains a number of constituents with an antioxidative effect that protect the body from damage caused by free radicals. Symbol included in the claim: MarliVital</td>
<td>not validated</td>
</tr>
</tbody>
</table>

Reasons for non-authorisation: non-compliance with the Regulation because on the basis of the scientific evidence assessed, this claimed effect for this food is not sufficiently defined to be able to be assessed and the claim could not therefore be substantiated.

<table>
<thead>
<tr>
<th>Nutrient, substance, food or food category/EFSA opinion reference / Journal reference</th>
<th>Claim</th>
<th>Health relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prunus cerasus - common name: Sour cherry 2011;9(6):2228</td>
<td>Traditionally used to facilitate the digestion / Used to facilitate the digestion/ Contributes to the digestive comfort/ &quot;Helps to support the digestion / &quot;Contributes to support the digestion.</td>
<td>“Digestive function”</td>
</tr>
<tr>
<td>Sqeez Wild Blueberry Juice Drink 2011;9(6):2228</td>
<td>Blueberries have a wide range of health benefits including anti-ageing properties and the maintenance of urinary tract and vision health.</td>
<td>“Anti-aging properties”</td>
</tr>
<tr>
<td>Berry seed oils (supercritical carbon dioxide extract 2011;9(6):2228</td>
<td>The essential fatty acids in berry seed oils balance fatty acid metabolism in the body. Berry seed oils support the health of the cardiovascular system.</td>
<td>“Cardiovascular health”</td>
</tr>
</tbody>
</table>

Reasons for non-authorisation: non-compliance with the Regulation because on the basis of the scientific evidence assessed, this claimed effect for this food is not a beneficial physiological effect as required by the Regulation.

<table>
<thead>
<tr>
<th>Nutrient, substance, food or food category/EFSA opinion reference / Journal reference</th>
<th>Claim</th>
<th>Health relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cherries (Prunus cerasus), including Montmorency, Balaton or other sour/tart cherry varieties</td>
<td>[Tart/sour] cherries provide a rich source of antioxidants.</td>
<td>Antioxidant, antioxidant content, and antioxidant properties</td>
</tr>
<tr>
<td>Nutrient, substance, food or food category / EFSA opinion reference / Journal reference</td>
<td>Claim</td>
<td>Health relationship</td>
</tr>
<tr>
<td>-------------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Cherries (Prunus cerasus), including Montmorency, Balaton or other sour/tart cherry varieties</td>
<td>[Tart/sour] cherries help support a healthy heart.</td>
<td>protection of DNA, proteins and lipids from oxidative damage</td>
</tr>
<tr>
<td>Cherries (Prunus cerasus), including Montmorency, Balaton or other sour/tart cherry varieties</td>
<td>[Tart/sour] cherries help support healthy joints.</td>
<td>Maintenance of joints</td>
</tr>
<tr>
<td>Cherries (Prunus cerasus, P. domestica), including Montmorency, Balaton or other sour/tart cherry varieties</td>
<td>[Tart/sour] cherries help support healthy brain / mental function.</td>
<td>contribution to normal cognitive function</td>
</tr>
<tr>
<td>Cherries (Prunus cerasus), including Montmorency, Balaton or other sour/tart cherry varieties</td>
<td>Contains antioxidant/s; Is a source of antioxidant/s. With antioxidant/s. Contributes to the cell protection against free radicals. Can protect your cells and tissues from oxidation. Can contribute to the total antioxidant capacity of the body.</td>
<td>protection of DNA, proteins and lipids from oxidative damage</td>
</tr>
<tr>
<td>Berries (lingonberry, cloudberry, blueberry, currants, raspberry and strawberry)</td>
<td>Natural berries contain plenty of natural antioxidants (polyphenolic compounds, Vitamin C and carotenoids) and fiber but only a small amount of energy and sodium. For this reason they are very suitable for a heart-friendly diet.</td>
<td>protection of DNA, proteins and lipids from oxidative damage</td>
</tr>
<tr>
<td>blueberry extracts</td>
<td>Natural antioxidant, protect organism from oxidative damage, natural way to avoid risks caused by oxidation and peroxidation process.</td>
<td>protection of DNA, proteins and lipids from oxidative damage</td>
</tr>
<tr>
<td>Lycium Barbarum (Common Name : Wolfberry)</td>
<td>Contains antioxidant/s; Is a source of antioxidant/s. With antioxidant/s. Contributes to the cell protection against free radicals Can protect your cells and tissues</td>
<td>Protection of DNA, proteins and lipids from oxidative damage</td>
</tr>
<tr>
<td>Publication</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>Antioxidants from pomegranate juice 2010;8(2):1489</td>
<td>(Antioxidants from) pomegranate - plays an important antioxidative function - protect cells against oxidative damages - strengthen the immune system - strengthen the body's defences.</td>
<td></td>
</tr>
<tr>
<td>Pomegranate 2010;8(10):1750</td>
<td>Contributes to a healthy cholesterol level and healthy blood vessels.</td>
<td></td>
</tr>
<tr>
<td>Pomegranate Juice - phenolic compounds (anthocyanins, tannines, ellagic acid) 2010;8(10):1750</td>
<td>Helps maintain the cholesterol and lipids levels.</td>
<td></td>
</tr>
<tr>
<td>Pomegranate Juice - phenolic compounds (anthocyanins, tannines, ellagic acid) 2010;8(10):1750</td>
<td>With powerful antioxidant properties.</td>
<td></td>
</tr>
<tr>
<td>Punica granatum (Common Name : Pomegranate) 2010;8(10):1750</td>
<td>Contributes to a healthy cholesterol level and healthy blood vessels / antioxidants of pomegranate can be helpful for a healthy heart and arteries / antioxidants of pomegranate can help cells and arteries in their physiological function.</td>
<td></td>
</tr>
<tr>
<td>Punica granatum-fruits-Punicaceae-Dadhima-Pomegranate 2010;8(10):1750</td>
<td>Helps to maintain a normal glucose level.</td>
<td></td>
</tr>
<tr>
<td>VitaGranate® Pomegranate Extract 40% Ellagic Acid 2010;8(10):1750</td>
<td>VitaGranate® Pomegranate Extract is an excellent source of pomegranate polyphenols, compounds that have been associated with the maintenance of cardiovascular health.</td>
<td></td>
</tr>
</tbody>
</table>
References

Chapter 1. Definitions and Essential Concepts


Chapter 2. European Food Safety Authority and Health Claims Authorization


10. Scientific Opinion on the substantiation of health claims related to polyphenols in olive and protection of LDL particles from oxidative damage (ID 1333, 1638, 1639, 1696, 2865), maintenance of normal blood HDL cholesterol concentrations (ID 1639), maintenance of normal blood pressure (ID 3781), “anti-inflammatory properties” (ID 1882), “contributes to the upper respiratory tract health” (ID 3468), “can help to maintain a normal function of gastrointestinal tract” (3779), and “contributes to body defences against external agents” (ID 3467) pursuant to Article 13(1) of Regulation (EC) No 1924/2006.


31. Commission Regulation (EU) No 432/2012 of 16 May 2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children’s development and health EU Regulation No 432/2012.
33. List of permitted health claims. D009312/05.
34. Scientific Opinion on the substantiation of health claims related to polyphenols in olive and protection of LDL particles from oxidative damage (ID 1333, 1638, 1639, 1696, 2865), maintenance of normal blood HDL cholesterol concentrations (ID 1639), maintenance of normal blood pressure (ID 3781), “anti-inflammatory properties” (ID 1882), “contributes to the upper respiratory tract health” (ID 3468), “can help to maintain a normal function of gastrointestinal tract” (3779), and “contributes to body defences against external agents” (ID 3467) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. http://www.efsa.europa.eu/en/efsajournal/pub/2033.htm/ accessed May 01 2012
Chapter 3. Berries

3.1. Acai


### 3.2. Blueberry


41. Scientific Opinion on the substantiation of health claims related to various food(s)/food constituent(s) and protection of cells from premature ageing (ID 1668, 1917, 2515, 2527, 2530, 2575, 2580, 2591, 2620, 3178, 3179, 3180, 3181, 4329, 4415), antioxidant activity, antioxidant content and antioxidant properties (ID 857, 1306, 2515, 2527, 2530, 2575, 2580, 2591, 2629, 2728, 4327, 4365, 4380, 4390, 4394, 4455, 4464, 4507, 4694, 4705), protection of DNA, proteins and lipids from oxidative damage (ID 1196, 1211, 1216, 1306, 1312, 1440, 1441, 1666, 1668, 1692, 1900, 1914, 1948, 2023, 2158, 2517, 2522, 2527, 2575, 2591, 2620, 2637, 2639, 2663, 2860, 3079, 3276, 3564, 3818, 4324, 4329, 4351, 4397, 4416, 4424, 4507, 4527, 4528, 4542, 4611, 4629, 4659) and bioavailability of anthocyanins in black currents (ID 4220) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 2010;8(10):1752

42. Scientific Opinion Part I on the substantiation of health claims related to various food(s)/food constituent(s) not supported by pertinent human data (ID 411, 559, 1174, 1184, 1197, 1380, 1409, 1656, 1667, 1670, 1763, 1767, 1806, 1884, 1908, 1997, 2141, 2159, 2243, 2244, 2325, 2331, 2333, 2336, 2652, 2717, 2727, 2752, 2788, 2861, 2870, 2885, 2894, 3077, 3101, 3516, 3595, 3726, 4252, 4288, 4290, 4406, 4509, 4709) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 2011;9(6):2246

### 3.3. Cherry


20. Scientific Opinion on the substantiation of health claims related to various food(s)/food constituent(s) and protection of cells from premature ageing (ID 1668, 1917, 2515, 2527, 2530, 2575, 2580, 2591, 2620, 3178, 3179, 3180, 3181, 4329, 4415), antioxidant activity, antioxidant content and antioxidant properties (ID 857, 1306, 2515, 2527, 2530, 2575, 2580, 2591, 2629, 2728, 4327, 4365, 4380, 4390, 4394, 4455, 4464, 4507, 4694, 4705), protection of DNA, proteins and lipids from oxidative damage (ID 1196, 1211, 1216, 1306, 1312, 1440, 1441, 1666, 1668, 1692, 1900, 1914, 1948, 2023, 2158, 2517, 2522, 2527, 2575, 2591, 2620, 2637, 2639, 2663, 2860, 3079, 3276, 3564, 3818, 4324, 4329, 4351, 4397, 4416, 4424, 4507, 4527, 4528, 4542, 4611, 4629, 4659) and bioavailability of anthocyanins in black currants (ID 4220) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 2010;8(10):1752.

3.4. Goji

3.5. Pomegranate


27. Scientific Opinion on the substantiation of health claims related to pomegranate/pomegranate juice and maintenance of normal blood cholesterol concentrations (ID 1162, 1320, 2107, 2167), maintenance of normal erectile function (ID 1163), protection of lipids from oxidative damage (ID 1201, 1319, 2123), “antioxidant and anti-aging properties” (ID 1901), increase in appetite after unintentional weight loss
SCIENTIFIC OPINION

Guidance on the scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)

European Food Safety Authority (EFSA), Parma, Italy

SUMMARY

The Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked by the European Food Safety Authority (EFSA) to draft guidance on scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health. This guidance has been drawn from scientific opinions of the NDA Panel on such health claims. Thus, this guidance document represents the views of the NDA Panel based on the experience gained to date with the evaluation of health claims in these areas. It is not intended that the document should include an exhaustive list of beneficial effects and studies/outcome measures which are acceptable. Rather, it presents examples drawn from evaluations already carried out in order to illustrate the approach of the NDA Panel, as well as some examples which are currently under consideration within ongoing evaluations. A draft of this guidance document, endorsed by the NDA Panel on 25 March 2011, was released for public consultation from 26 April 2011 to 31 August 2011.

KEY WORDS

Health claims, scientific requirements, antioxidants, oxidative damage, cardiovascular health.

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1 On request from EFSA, Question No EFSA-Q-2010-01182, adopted on 24 November 2011.
2 Panel members: Carlo Agostoni, Jean-Louis Bresson, Susan Fairweather-Tait, Albert Flynn, Ines Golly, Hannu Korhonen, Pagona Lagiou, Martinus Lovik, Rosangela Marchelli, Ambroise Martin, Bevan Moseley, Monika Neuhausser-Berthold, Hildegard Przyrembel, Seppo Salminen, Yolanda Sanz, Sean (J.J.) Strain, Stephan Strobel, Inge Tetens, Daniel Tomé, Hendrik van Loveren and Hans Verhagen. Correspondence: nda@efsa.europa.eu

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**BACKGROUND AS PROVIDED BY EFSA**

Regulation (EC) No 1924/2006\(^4\) harmonises the provisions that relate to nutrition and health claims, and establishes rules governing the Community authorisation of health claims made on foods. According to the Regulation, health claims should only be authorised for use in the Community after a scientific assessment of the highest possible standard has been carried out by EFSA.

EFSA and its NDA Panel have been engaging in consultation with stakeholders, and have published guidance on scientific substantiation of health claims, since 2007\(^5\). Most recently, a briefing document on scientific evaluation of health claims was published for consultation in April 2010, followed by a technical meeting with experts from the food industry, Member States and the European Commission in Parma, in June 2010\(^6\).

Based on experiences gained with the evaluation of health claims, and to further assist applicants in preparing and submitting their applications for the authorisation of health claims, the NDA Panel is asked to develop guidance documents on the scientific requirements for the substantiation of health claims in selected areas, in addition to the guidance for the scientific substantiation of health claims related to gut and immune function (EFSA-Q-2010-01139).

**TERMS OF REFERENCE AS PROVIDED BY EFSA**

The NDA Panel is requested by EFSA to develop guidance documents on the scientific requirements for health claims in the following areas:

- Post-prandial blood glucose responses/blood glucose control
- Weight management, energy intake and satiety
- Protection against oxidative damage
- Cardiovascular health
- Bone, joints, and oral health
- Neurological and psychological functions
- Physical performance

Specific issues to be addressed in these guidance documents include:

- which claimed effects are considered to be beneficial physiological effects?
- which studies/outcome measures are appropriate for the substantiation of function claims and disease risk reduction claims?

Each guidance document should be subject to public consultation, and may be followed up as appropriate by scientific meetings with experts in the field.

Before the adoption of each guidance document by the NDA Panel the draft guidance shall be revised, taking into account the comments received during the public consultation. A report on the outcome of the public consultation for each guidance document shall be published. All guidance documents should be finalised by July 2012.

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Guidance on the scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health

ASSESSMENT

1. Introduction

To assist applicants in preparing and submitting their applications for the authorisation of health claims, EFSA and in particular its Scientific Panel on Dietetic Products, Nutrition and Allergies (NDA) has ongoing consultations with stakeholders, and has published guidance on the scientific substantiation of health claims since 2007. In April 2010, a draft briefing document on the scientific evaluation of health claims was published for consultation and was followed by a technical meeting with experts from the food industry, Member States and the European Commission in Parma, in June 2010. The draft briefing document has been transformed into a Panel output, taking into account the questions/comments received. This document constitutes the general guidance for stakeholders on the evaluation of Article 13.1, 13.5 and 14 health claims, and outlines the approach of the NDA Panel to the evaluation of health claims in general. In response to requests from industry, EFSA is engaged in further consultation with stakeholders, and is developing additional guidance on specific types of claims.

The present guidance, prepared by the NDA Panel, on the scientific requirements for the substantiation of health claims related to antioxidants, oxidative damage and cardiovascular health was, prior to its finalisation, endorsed by the NDA Panel on 25 March 2011 for public consultation, which was open from 26 April to 31 August 2011. All the public comments received that related to the remit of EFSA were assessed, and the guidance has been revised taking into consideration relevant comments. The comments received and a report on the outcome of the public consultation are published on the EFSA website.

The guidance document focuses on two key issues regarding the substantiation of health claims related to antioxidants, oxidative damage and cardiovascular health:

- claimed effects which are considered to be beneficial physiological effects.
- studies/outcome measures which are considered to be appropriate for the substantiation of health claims.

Issues which are related to substantiation and which are common to health claims in general (e.g. characterisation of the food/constituent) are addressed in the general guidance for stakeholders on the evaluation of Article 13.1, 13.5 and 14 health claims.

This document has been drawn from scientific opinions of the NDA Panel on health claims related to antioxidants, oxidative damage and cardiovascular health. Thus, it represents the views of the NDA Panel based on the experience gained to date with the evaluation of health claims in these areas. The document should be read in conjunction with the general guidance for stakeholders on the evaluation of Article 13.1, 13.5 and 14 health claims.

It is not intended that the document should include an exhaustive list of beneficial effects and studies/outcome measures which are acceptable. Rather, it presents examples drawn from evaluations already carried out in order to illustrate the approach of the Panel, as well as some examples which are currently under consideration within ongoing evaluations. Given that health claims are often technically complex and unique, additional health relationships and outcome measures for claimed

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effects need to be considered in the context of a specific application. This guidance document may be updated in the future in light of additional experience gained with the evaluation of health claims.

2. General considerations

2.1. Beneficial physiological effects

According to Regulation (EC) No 1924/2006, the use of health claims shall only be permitted if the food/constituent, for which the claim is made, has been shown to have a beneficial physiological effect. In assessing each claim, the NDA Panel makes a scientific judgement on whether the claimed effect is considered to be a beneficial physiological effect in the context of the specific claim, as described in the information provided and taking into account the population group for whom the claim is intended. For function claims, a beneficial effect may relate to the maintenance or improvement of a function.

For reduction of disease risk claims, ‘beneficial’ refers to whether the claimed effect relates to the reduction (or beneficial alteration) of a risk factor for the development of a human disease (and not to the reduction of the risk of disease). A risk factor is a factor associated with the risk of a disease that may serve as a predictor of development of that disease. Whether or not the alteration of a factor is considered to be beneficial in the context of a reduction of disease risk claim depends on the extent to which it is established that:

- The factor is an independent predictor of disease risk (such a predictor may be established from intervention and/or observational studies);
- The relationship of the factor to the development of the disease is biologically plausible.

Except for well established risk factors (e.g. LDL-cholesterol concentration, blood pressure), the extent to which the reduction of a factor is beneficial in the context of a reduction of disease risk claim needs to be considered on a case-by-case basis.

The NDA Panel considers that the population group for which health claims are intended is the general (healthy) population or specific subgroups thereof, for example, elderly people, physically active subjects, or pregnant women. In its evaluation, the NDA Panel considers that where a health claim relates to a function/effect which may be associated with a disease, subjects with the disease are not the target population for the claim, for example, patients with myocardial infarction. Applications for claims which specify target groups other than the general (healthy) population are the subject of ongoing discussions with the Commission and Member States with regard to their admissibility.

The NDA Panel also considers whether the claimed effect is sufficiently defined to establish that the studies identified for substantiation of the claim were performed with (an) appropriate outcome measure(s) of that claimed effect. Reference to general, non-specific benefits of the nutrient or food for overall good health or health-related well-being may only be made if accompanied by a specific health claim.

2.2. Studies/outcome measures appropriate for substantiation of claims

As human studies are central for the substantiation of health claims, this document focuses in particular on such studies. In considering whether the studies provided are pertinent (i.e. studies from which conclusions can be drawn for the scientific substantiation of the claim), the NDA Panel addresses a number of questions, including:
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- Whether the studies have been carried out with the food/constituent for which the claim is made. This requirement means that there should be sufficient definition of the food/constituent for which the claim is made, and of the food/constituent which has been investigated in the studies which have been provided for substantiation of the claim. The evaluation also considers how the conditions under which the human studies were performed relate to the conditions of use (e.g. quantity and pattern of consumption of the food/constituent) proposed for the claim.

- Whether the design and quality of the studies allow conclusions to be drawn for the scientific substantiation of the claim. The evaluation takes into account the hierarchy of evidence as described in the scientific and technical guidance of the NDA Panel\(^9\), for example, intervention studies generally provide stronger evidence than observational studies. Intervention studies should be appropriately conducted so as to minimise bias. In observational studies adequate control for factors other than the food/constituent known to have an impact on the claimed effect is important. Each health claim is assessed separately and there is no pre-established formula as to how many or what type of studies are needed to substantiate a claim. In this regard, the reproducibility of the effect of the food/constituent as indicated by consistency between studies is an important consideration.

- Whether the studies have been carried out in a study group representative of the population group for which the claim is intended. Can the results obtained in the studied population be extrapolated to the target population? For studies in groups (e.g. subjects with a disease) other than the target group for a claim (e.g. the general population), the NDA Panel considers on a case-by-case basis the extent to which it is established that extrapolation from the study group to the target group is biologically plausible.

- Whether the studies used (an) appropriate outcome measure(s) of the claimed effect. For this, the NDA Panel considers what is generally accepted in the relevant research fields (e.g. guidelines published by scientific societies based on rigorous methodological approaches), and consults experts from various disciplines, as appropriate.

3. **Antioxidant properties, antioxidant status, antioxidant defence**

3.1. **Claims on antioxidant properties of foods**

Claims on the antioxidant content/properties/activity of foods have been proposed. The references provided for the scientific substantiation of these claims included \textit{in vitro} studies on the capacity of foods/constituents to scavenge free radicals. Claims made on the antioxidant capacity/content or properties of foods/constituents based on their capability of scavenging free radicals \textit{in vitro} refer to a property of the foods/constituents measured in model systems, and it is not established that this capability exerts a beneficial physiological effect in humans as required by Regulation (EC) No 1924/2006.

3.2. **Claims on antioxidant status and antioxidant defence**

Claims referring to antioxidant status and antioxidant defence have been proposed. The references provided for the scientific substantiation of these claims included \textit{in vivo} human studies which assessed changes in the overall antioxidant capacity of plasma using methods such as the total reactive antioxidant potential (TRAP), the trolox-equivalent antioxidant capacity (TEAC), the ferric

reducing antioxidant potential (FRAP), the oxygen radical absorbance capacity (ORAC) or the ferrous oxidation-xylenol orange (FOX) assays. It is not established that changes in the overall antioxidant capacity of plasma exert a beneficial physiological effect in humans as required by Regulation (EC) No 1924/2006.

For claims related to the “antioxidant defence system”, references assessing the effects of foods/constituents on enzymes and endogenous compounds (e.g. glutathione) that are part of the body’s antioxidant network have been provided.

Some vitamins and essential minerals have a role in the function of enzymes which belong to the human antioxidant network that protects cells and molecules from oxidative damage. Their role in the human antioxidant network has been established based on a large body of scientific evidence. In the context of an adequate supply of these vitamins and essential minerals, a specific induction of antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px) and haemoxigenase, or limiting the decrease in glutathione, is considered a beneficial physiological effect only if such changes provide (additional) protection of cells and molecules from oxidative damage, which should be demonstrated in vivo in humans. Therefore, induction of antioxidant enzymes cannot be used alone as evidence for claims related to the “antioxidant defence system” for non-essential food constituents.

3.3. Claims on the protection of cells from premature ageing

Claims referring to the “protection of cells from premature ageing” or to “healthy aging” have been proposed in relation to antioxidant properties of foods/constituents. The references provided for the scientific substantiation of these claims included a variety of in vitro and in vivo animal and human studies assessing the effects of foods/constituents on a variety of outcomes, including the antioxidant capacity of foods; changes in antioxidant status; oxidative damage to proteins; lipids and DNA, non-oxidative DNA damage; neoplastic degeneration of cells, etc. For these claims, no definition has been provided of “premature aging” or of “healthy aging” in relation to the antioxidant properties of foods, and therefore the claimed effect is considered to be general and non-specific, and thus does not comply with the criteria laid down in Regulation (EC) No 1924/2006.

4. Oxidative damage, including photo-oxidative (UV-induced) damage

4.1. Claims on the protection of body cells and molecules (i.e. DNA, proteins and lipids) from oxidative damage, including photo-oxidative (UV-induced) damage

The protection of body cells and molecules such as DNA, proteins and lipids from oxidative damage, including photo-oxidative (UV-induced) damage, may be a beneficial physiological effect, assuming that any significant oxidative modification of the target molecule is potentially harmful. In this specific context, an appropriate method of assessment should be able to determine accurately and specifically the oxidative modification of the target molecule in vivo. The scientific substantiation of health claims on the protection of molecules from oxidative damage requires at least one appropriate marker of oxidative modification of the target molecule assessed in human studies, preferably in combination with other marker(s) as defined in sections 4.1.1 to 4.1.3. However, these other markers of oxidative damage to molecules cannot be used alone for substantiation as they have some limitations, either because they represent a result of two processes (oxidative damage and repair), or because they suffer from technical limitations (interferences from other unrelated processes or substances), or both. A marker cannot be accepted for substantiation when these limitations are considered to be severe. Different markers of oxidative damage to molecules should preferably be determined in the same study, but their determination in similar studies could be acceptable on a case-by-case basis.
The antioxidant properties of foods (measured in vitro), and changes in the overall antioxidant capacity of plasma (measured in vivo as, for example, TRAP, TEAC, FRAP, ORAC or FOX), do not predict a role of the food/constituent in the protection of body cells and molecules such as DNA, proteins and lipids from oxidative damage in vivo, and therefore are not suitable outcome measures for the scientific substantiation of the claimed effect.

4.1.1. Oxidative damage to proteins

Direct measurements of oxidative damage to proteins in vivo (e.g. measurement of oxidative changes of amino acids in proteins) could be obtained by means of HPLC-MS and other methods, as long as identification and separation of such molecules in plasma from other substances is successfully achieved (e.g. from protein tyrosine nitration products). Measures of protein oxidation by-products (e.g. protein carbonyls) using conventional assays (e.g. colorimetric procedure which involves dinitrophenylhydrazine (DNPH) derivatisation of carbonyl groups) or ELISA methods (either directly or after DNPH derivatisation) are usually susceptible to interferences by molecules other than proteins, and could only be used in combination with at least one direct marker of oxidative damage to proteins in vivo if assessed directly in blood or target tissue (e.g. skin).

4.1.2. Oxidative damage to lipids

Direct measurements of oxidative damage to lipids (i.e. lipid peroxidation) could be obtained in vivo by measuring changes in F₂α-isoprostanes in 24-h urine samples, which is a better matrix than plasma for this measurement, using gas chromatography techniques with various detection modes, of which mass spectrometry is preferred. F₂α-isoprostanes can also be measured using immunonassays. However, lack of specificity owing to possible cross reactions with other prostanoids needs to be taken into account.

Measurements of oxidative damage to lipids (i.e. lipid peroxidation) could also be obtained in vivo by measuring oxidised LDL particles in blood using immunological methods (i.e. antibodies) with appropriate specificity. Phosphatidylcholine hydroperoxides (PCOOH) measured in blood or tissue by high-performance liquid chromatography (HPLC) is also an acceptable marker of lipid peroxidation.

Other methods proposed are not reliable in vivo markers of lipid peroxidation (e.g. thiobarbituric acid reactive substances (TBARS), malondialdehyde (MDA), lipid peroxides, HDL-associated paraoxonases, conjugated dienes, breath hydrocarbons, auto-antibodies against LDL particles, and ex vivo LDL resistance to oxidation). However, concentrations of MDA or lipid peroxides in blood or tissue could be used as supportive evidence (i.e. in addition to measurements of F₂α-isoprostanes and in vivo LDL oxidation) if appropriate techniques are used for analysis (e.g. HPLC).

4.1.3. Oxidative damage to DNA

Direct measurements of oxidative damage to DNA may be obtained in vivo by using modifications of the comet assay, which allow the detection of oxidised DNA bases (e.g. use of endonuclease III to detect oxidised pyrimidines). Although the assay provides no absolute values, it allows quantitative comparison with an appropriate control. This assay directly reflects DNA oxidative damage within cells when assessed, for example, in circulating lymphocytes.

Measures of DNA damage using the traditional comet assay (single-cell microgel electrophoresis, SCGE) detect DNA strand breaks by the appearance of tailing, and are not specific for oxidative damage. Other variants of the comet assay determine resistance against oxidative modification using ex vivo pro-oxidant challenges. Neither of these measurements is appropriate for assessing in vivo oxidative damage to DNA.
Analyses of 8-hydroxy-2-deoxy-guanosin (8-OHdG) in blood (e.g. lymphocytes), tissue (e.g. skin) and urine have been used to assess oxidative damage to DNA. Free 8-OHdG results from oxidative damage and excision-repair; it may also result from oxidation of free bases or nucleotides, from oxidation of other nucleic acids, and from artefacts during sample work up. Urinary 8-OHdG does not directly reflect DNA oxidation within cells, but could be used in combination with direct measurements of oxidative damage to DNA if appropriate techniques are used for analysis (e.g. HPLC).

5. Cardiovascular health

Claims referring to cardiovascular health in general need to be accompanied by a specific claim (e.g. claims addressed in sections 5.1 to 5.5 of this guidance).

For a health claim on the normal function of the heart, evidence from human studies on specific outcomes (e.g. coronary events) can be used for substantiation.

5.1. Claims related to changes in the blood lipid profile

The scientific substantiation of health claims related to changes in the blood lipid profile requires identification of the particular markers which should be considered for the evaluation (e.g. LDL-cholesterol, HDL-cholesterol and triglycerides).

5.1.1. Claims related to blood LDL-cholesterol concentration

Maintenance of normal LDL-cholesterol concentration is a beneficial physiological effect. The scientific evidence for the substantiation of health claims on the maintenance of normal blood cholesterol concentration can be obtained from human intervention studies showing a short-term (e.g. three to four week) reduction in LDL-cholesterol concentration as compared to an appropriate food/constituent which is neutral with respect to the claimed effect, or exceptionally to no treatment (e.g. control group on usual diet). In this context, also a reduction in LDL-cholesterol concentration within the normal range is considered a beneficial physiological effect. Evidence for a sustained effect with continuous consumption of the food/constituent over longer periods of time (e.g. eight weeks) should also be provided.

Claims for a beneficial effect of the absence (or reduced content) of a food constituent in a food or category of food on LDL-cholesterol concentration have been proposed. Substantiation may be based on evidence for an independent role of the food constituent in increasing LDL-cholesterol concentration. For example, for claims on a reduced content of saturated fatty acids (SFAs) in relation to blood LDL-cholesterol concentration, SFAs in mixed diets have been shown to increase blood LDL-cholesterol concentration when compared to carbohydrates which have a neutral effect on LDL-cholesterol concentration, and therefore SFAs in mixed diets have an independent role in increasing LDL-cholesterol concentration.

Claims for a beneficial effect of a food constituent when used in replacement of a food constituent with an independent role in increasing LDL-cholesterol concentration have also been proposed. Substantiation may be based on evidence for an independent role of the replaced food constituent in increasing LDL-cholesterol concentration, together with evidence for the lack of an effect or a reduced effect of the food constituent which is used for replacement (e.g. claims for unsaturated fats and reduced LDL-cholesterol concentration when replacing saturated fats).

With respect to the study population, results from studies conducted in hypercholesterolaemic subjects treated with lifestyle measures only (e.g. diet) could be used for the scientific substantiation of these claims. However, the rationale for extrapolation of results obtained in hypercholesterolaemic
subjects under pharmacological treatment with cholesterol-lowering medications (e.g. statins) to the target population for the claim should be provided, and will be considered on a case-by-case basis (e.g. evidence for a lack of interaction between the food and the medications used on the claimed effect).

5.1.2. Claims related to blood HDL-cholesterol concentration

Maintenance of normal HDL-cholesterol concentration is a beneficial physiological effect as long as LDL-cholesterol concentration is not increased.

The scientific evidence for the substantiation of health claims on the maintenance of normal HDL-cholesterol concentration can be obtained from human intervention studies showing a short-term (e.g. three to four week) increase in fasting HDL-cholesterol concentration (without a concomitant increase in LDL-cholesterol concentration) as compared to an appropriate food/constituent which is neutral with respect to the claimed effect, or exceptionally to no treatment (e.g. control group on usual diet). In this context, also an increase in HDL-cholesterol concentration within the normal range is considered a beneficial physiological effect. Evidence for a sustained effect with continuous consumption of the food/constituent over longer periods of time (e.g. eight weeks) should also be provided.

5.1.3. Claims related to blood concentration of triglycerides

Maintenance of normal blood concentration of triglycerides may be a beneficial physiological effect. The scientific evidence for the substantiation of health claims on the maintenance of normal blood concentration of triglycerides can be obtained from human intervention studies showing a short-term (e.g. three to four week) reduction in fasting triglyceride concentration as compared to an appropriate food/constituent which is neutral with respect to the claimed effect, or exceptionally to no treatment (e.g. control group on usual diet). In this context, also a reduction in fasting triglyceride concentration within the normal range may be considered a beneficial physiological effect. Evidence for a sustained effect with continuous consumption of the food/constituent over longer periods of time (e.g. eight weeks) should also be provided.

With respect to the study population, results from studies conducted in hypertriglyceridaemic subjects treated with lifestyle measures only (e.g. diet) could be used for the scientific substantiation of these claims. However, the rationale for extrapolation of results obtained in hypertriglyceridaemic subjects under treatment with “triglyceride-lowering” medications (e.g. fibrates) to the target population for the claim should be provided, and will be considered on a case-by-case basis (e.g. evidence for a lack of interaction between the food and the medications used on the claimed effect).

5.2. Claims on the reduction of blood pressure

Maintenance of normal blood pressure is a beneficial physiological effect. The scientific evidence for the substantiation of health claims on the maintenance of normal blood pressure can be obtained from human intervention studies showing a short-term (e.g. three to four week) reduction in systolic blood pressure, or a reduction in diastolic blood pressure if accompanied by a reduction in systolic blood pressure as compared to a food/constituent which is neutral with respect to the claimed effect, or exceptionally to no treatment (e.g. control group on usual diet). In this context, also reductions in blood pressure within the normal range are considered beneficial physiological effects. Blood pressure should be measured using well-accepted protocols.

With respect to the study population, results from studies conducted in hypertensive subjects treated with lifestyle measures only (e.g. diet) could be used for the scientific substantiation of these claims.
However, the rationale for extrapolation of results obtained in hypertensive subjects under treatment with blood pressure-lowering medications (e.g. ACE-inhibitors, blockers of beta adrenergic receptors, calcium channel blockers and diuretics) to the target population for the claim should be provided, and will be considered on a case-by-case basis (e.g. evidence for a lack of interaction between the food and the medications used on the claimed effect).

5.3. **Claims on endothelial function**

Endothelial function *per se* is not sufficiently defined for a scientific evaluation, because endothelium-derived active factors play a role in the maintenance of several functions of the vascular system. These include vasomotion, smooth muscle proliferation, thrombosis, inflammation, coagulation, fibrinolysis and oxidation, which can be assessed by indirect methods. Some of the claims referred to the improvement of specific endothelial functions (e.g. endothelium-dependent vasodilation), which can be assessed *in vivo* using well established methods (e.g. the flow-mediated dilation technique). An improvement of specific endothelial functions (e.g. endothelium-dependent vasodilation) during sustained exposure to the food/constituent (e.g. four weeks) may be considered a beneficial physiological effect.

5.4. **Claims on reduced platelet aggregation**

Platelet hyperactivity and hypercoagulability states are more commonly observed in subjects with cardiovascular (CV) risk factors. Healthy subjects at very low risk of CV disease normally have non-activated circulating platelets. Decreasing platelet aggregation in subjects with platelet activation during sustained exposure to the food/constituent (e.g. four weeks) would be a beneficial physiological effect.

5.5. **Claims on homocysteine**

Maintenance of normal homocysteine metabolism is a beneficial physiological effect. It is well established that homocysteine metabolism is closely linked with both folate metabolism and one-carbon metabolism.

Evidence for the scientific substantiation of this claim may come from the well established role of a food in contributing to the remethylation or degradation of homocysteine (e.g. some vitamins), or can be obtained from human intervention studies showing a short-term reduction of homocysteine concentration as compared to an appropriate food/constituent which is neutral with respect to the claimed effect, or exceptionally to no treatment (e.g. control group on usual diet). Evidence for a sustained effect with continuous consumption of the food/constituent over longer periods of time (e.g. eight weeks) should also be provided.

5.6. **Disease risk reduction claims**

It is well established that elevated blood LDL-cholesterol concentration is independently associated with an increased risk of coronary heart disease (CHD), and that reducing blood LDL-cholesterol concentration (by dietary modification and drugs) would generally reduce the risk of development of CHD. It is also well established that elevated (systolic) blood pressure is independently associated with an increased risk of CHD and stroke, and that reducing (systolic) blood pressure (by dietary modification and drugs) would generally reduce the risk of development of CHD and stroke. Reduction in blood LDL-cholesterol concentration, therefore, is considered beneficial in the context of a reduction of disease risk claim for CHD, and reduction in (systolic) blood pressure is considered beneficial in the context of a reduction of disease risk claim for CHD and stroke.
For other proposed risk factors, the evidence may not be as strong. There is some evidence, for example, that low blood HDL-cholesterol concentration, elevated blood concentration of triglycerides, or elevated blood homocysteine concentration is associated with an increased risk of CHD. Reduction in blood concentration of triglycerides, reduction in blood homocysteine concentration, or an increase in blood HDL-cholesterol concentration, have been associated with a decreased incidence of CHD following certain dietary interventions in some human intervention studies. However, changes in any of these factors (by dietary modification or drugs) have not generally been shown to reduce the risk of CHD. Therefore, human studies on the risk of CHD are required for the substantiation of these claims in order to validate the association between these variables and the risk of disease in the context of a particular nutritional intervention.

CONCLUSIONS

The guidance document focuses on two key issues regarding the substantiation of health claims related to antioxidants, oxidative damage and cardiovascular health:

- claimed effects which are considered to be beneficial physiological effects.
- studies/outcome measures which are considered to be appropriate for the substantiation of health claims.

The document has been drawn from scientific opinions of the NDA Panel on health claims related to antioxidants, oxidative damage and cardiovascular health. Thus, it represents the views of the NDA Panel based on the experience gained to date with the evaluation of health claims in these areas.
Glossary and Abbreviations

8-OHdG  8-hydroxy-2-deoxy-Guanosin  
ACE  Angiotensin converting enzyme  
CHD  Coronary heart disease  
CV  Cardiovascular  
DNA  Deoxyribonucleic acid  
DNPH  Dinitrophenylhydrazine  
ELISA  Enzyme-linked immunosorbent assay  
FOX  Ferrous oxidation-xylenol orange  
FRAP  Ferric reducing antioxidant potential  
GSH-Px  Glutathione peroxidase  
HDL  High-density lipoprotein  
HPLC  High-performance liquid chromatography  
LDL  Low-density lipoprotein  
MDA  Malondialdehyde  
MS  Mass spectrometry  
ORAC  Oxygen radical absorbance capacity  
PCOOH  Phosphatidylcholine hydroperoxides  
SCGE  Single-cell microgel electrophoresis  
SFA  Saturated fatty acid  
SOD  Superoxide dismutase  
TBARS  Thiobarbituric acid reactive substances  
TEAC  Trolox-equivalent antioxidant capacity  
TRAP  Total reactive antioxidant potential  
UV  Ultraviolet