AMETIS, Joint-Stock Company

DIHYDROQUECETIN in production of Soft Drinks & Alcohol beverages

The application of Dihydroquercetin in the food industry is regulated by the following normative documentations in the Russian Federation:

• According to the Decision of the State Chief Medical Officer dated November 14, 2001 No 36 "About the application of the Sanitary and Epidemiological Conclusion (SEC) 2.3.2.1078-01", dihydroquercetin is classified as an antioxidant;

• The Decision of the State Chief Medical Officer dated April 18, 2003 No 59 "About the application of SEC 2.3.2.1293-03" allows using dihydroquercetin for manufacturing of cream, chocolate, dry milk. The maximal content of Dihydroquercetin in these products is 200 mg/kg fat of the product;

• The Methodical Recommendations of the State Sanitary and Epidemiological Regulations No 2.3.1.1915-04 "Recommended norm of consumption of food and bioactive supplements" has determined the appropriate and the highest allowable level of Dihydroquercetin consumption: 25-100 mg per a day;

• GOST R 52791-2007. Canned milk. Dry milk. Specifications. Date of introduction: January 1, 2009;

• GOST R 53436-2009. Canned milk. Milk and cream sweetened condensed. Specifications. Date of introduction: January 1, 2011.



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Prepared by Ametis JSC

BRIEFLY ABOUT DIHYDROQUERCETIN

Main Properties of Dihydroquercetin

1) Antioxidant properties

Dihydroquercetin is an antioxidant of direct action which binds free radicals. Dihydroquercetin inhibits free radical oxidation of both water soluble (luminol, ABTS) and fatsoluble substrates. dihydroquercetin as antioxidant could function as (1) the "catcher" of active forms of oxygen, (2) chelator of metal with variable valency, (3) chainformative agent.

2) Capillary-protective properties

Dihydroquercetin decreases the pathological capillary fragility and increases the resistance of normal capillaries to trauma. Dihydroquercetin tends to maintain the normal tensile strength of capillary walls.

3) Anti-inflammatory properties

Dihydroquercetin reduces capillary permeability, inhibits action of many enzyme systems involved in the development of inflammation and allergy, reduces release of histamine and other mediators of inflammation from mast cells and basophils, limits action of kinins and anti-inflammatory prostaglandins to tissues.

4) Radioprotective properties

Dihydroquercetin slows the development of free radical oxidation, decreases lipid peroxidation activity induced by gamma irradiation. Some studies reveal the possible use of dihydroquercetin as pharmaceutical to defend the human organism from a lipid peroxidation effects which are activated under various pathologic conditions including general irradiation by gamma rays.

5) Detoxifying properties

Detoxifying properties of Dihydroquercetin are related to the direct interaction with toxins. Dihydroquercetin binds toxins into a stable form with the subsequent excretion from the organism.

6) Hepatoprotective properties

Dihydroquercetin has the positive effect on the liver function: normalizes the cell membrane and the structure of hepatocytes, has an antioxidant effect, accelerates the regeneration of damaged liver parenchyma, therebv enhances its detoxifving function. Dihydroquercetin is the natural antioxidant of plant origin, bioflavonoid. Dihydroquercetin as an ingredient of phenolic compounds is found in many kinds of herbs and shrubs, but only in several kinds of trees dihydroquercetin is found to a greater extend. Dihydroquercetin, produced by Ametis JSC under the trade mark **"Lavitol"**, is a flavonoid, derived from Dahurian Larch (Larix gmelinii) by a water-ethanol extraction method.

Dihydroquercetin extract is an active antioxidant that could slow down oxidative reactions. The level of antioxidative activity allows to put dihydroquercetin on the first positions among the substances with similar spectrum of action.

The use of Dihydroquercetin in food products is determined by its ability to reduce oxidative reactions and to strengthen capillaries. Utilization of these properties can be beneficial in **two directions:**

a) as an antioxidant, Dihydroquercetin can reduce lipid peroxidation, with the consequent prolongation of food products' shelf life; and

b) because of its capillary-strengthening properties, Dihydroquercetin can be used for functional products that are aimed at enhancing health.

In the food industry, Dihydroquercetin is used in dairy products, meat products, alcoholic and non-alcoholic beverages, confectionary products, and products of functional nutrition.

The application of dihydroquercetin in food industry is caused by its ability to reduce the lipid peroxidation, with the prolongation of food products' shelf life in 1.5 - 4 times.

The lipid oxidation of food products leads to a deterioration of organoleptic characteristics, loss in nutritional value, color changes, microbial contamination, etc. Dihydroquercetin can improve the biological value of food products and retain the original organoleptic properties for a long time.

Dihydroquercetin slows down the oxidation processes not only in products, fortified with Dihydroquercetin, but also in human organism. The presence of even small amounts of Dihydroquercetin in the parapharmaceutical food prevents a number of diseases associated with the so-called "oxidative stress" and also helps to protect the body against free radicals.



APPLICATION OF DIHYDROQUERCETIN

The Efficiency of Dihydroquercetin in production of beverages

Available literature suggests a potentially wide application of Dihydroquercetin in beverage industry. Dihydroquercetin could be utilized in juices, functional waters, juices, carbonated drinks, fermented beverages, etc.

Carbonated soft drinks

- Preliminary animal experiments suggest potential functional benefits of Dihydroquercetin in a variety of non-alcoholic beverages. Thus, a weakly carbonated soft drink with Dihydroquercetin administrated for 7 days was shown to improve both absolute and relative indicators of physical working capacity of animals exposed to single and repeated swimming loads (*Pomozova, V.A., Babiy, N.B., etc., 2008*).

- Fortified with Dihydroquercetin carbonated drinks increased energy and well-being of the consumers (*Reshetnik, E.I., 2008*).

- The energy-enhancing properties of Dihydroquercetin in combination with its ability to enhance blood oxygenation would allow for a wide application of Dihydroquercetin in sport nutritional beverages.

Potable and Mineral water

- "Cosmetological Clinic "Institute of Beauty" together with OAO Plant of ecological equipment and eco-nutrition "DIOD" conducted clinical tests of potable water Aqua Minerale Beauty. Water composition: purified potable water, carbon dioxide, dihydroquercetin -2.0 mg/100 ml, natural mineral complex, chlorine 21.3 mg/100 ml, calcium - 0.16 mg/100 ml, sulfate - 0.65 mg/100 ml, mineralization of no more than 0.95 g/dl), vitamins (niacin - 0,7 mg/100 ml, calcium pantotenat -1.3 mg/100 ml, B6-0.1 mg/100 ml, H-biotin 0.01 mg/100 ml). The test results were following: high organoleptic properties of this water; regulating influence upon a water balance of an organism; improvement of skin micro-circulation, which is indirectly confirmed by restoration of uniform echogenicity of skin and reduction of sub-epidermal hypoechogenic zone in a smoking woman; clinically revealed increase of skin humidity, restoration of skin micro-relief, shown both clinically and by ultra sound skin scanning, increase of epidermis thickness and reduction of its echogenicity (Dolzhnikova, E.M., 2006).

The Influence of Dihydroquercetin on Microbiological Indices

L. monolcyto- genes	Dihydroquercetin inhibits the growth of L.monoclyto- genes in sterilized sour cream. It kills on average 30% of L.monocytogene
E.coli	Dihydroquercetin inhibits the growth of E.coli in steri- lized sour cream. It kills on average 12% of E.coli
S.aureus	Dihydroquercetin inhibits the growth of Staphylococ- cus aureus It killed on av- erage 90% of Staphylococ- cus aureus
Lipolytic micro- organisms	Fortification with Dihydro- quercetin inhibits the growth of lipolytic micro- organisms in milk fat, inhi- bits significantly their growth in sterilized cream. It kills on average 44% of lipolytic microorganisms in sterilized cream and 88% - in tallow.
Rhodototorula yeasts	0.014 mg of Dihydroquer- cetin is required for com- plete inhibition of 1 CFU of Rhodototorula
Lactic acid bac- teria	0.011 mg of Dihydroquer- cetin is required for com- plete inhibition of 1 CFU of Lactic acid bacteria
Alicyclobacillus acidoterrestris	2.5 mg of Dihydroquerce- tin is required for complete inhibition of 1 CFU of Ali- cyclobacillus acidoterre- stris

APPLICATION OF DIHYDROQUERCETIN

Application of Dihydroquercetin in Alcoholic Beverages Industry

Artificial aging of wines, spirits and cognacs;

Improves taste and quality of alcoholic drinks;

Binds and removes from the organism compounds of blood plasma proteins and acetaldehyde that cause alcoholic intoxication;

Prevent hangover;

Protects the liver from destruction by toxicants, removes them from the organism;

Dihydroquercetin added to liqueurs, vodkas and cognacs makes their taste milder and more delicate.

Application of Dihydroquercetin in Soft Drinks Industry

Suppress the yeast reproduction and decrease its viability;

Decrease the oxygen concentration in the beverage during its storage;

Improve the taste and odor;

Extend shelf life.

The Efficiency of Dihydroquercetin in production of beverages

Non-alcoholic beverages

- The addition of Dihydroquercetin to non-alcohol beverages neutralizes and blocks free radicals (*Skryabin, V.I., Kobelev, K.B., Gernet, M.B., Lavrova, V.L., 2005*).

- The addition of Dihydroquercetin to non-alcohol soft drinks improves organoleptic characteristics of the product *(Reshetnik, E.I., 2008).*

- Dihydroquercetin can be used for functional soft-drinks that are aimed at enhancing health.

Juices

- Fortification of directly squeezed juices, concentrated juices, reconditioned nectars and juice containing beverages with Dihydroquercetin allows to extend storage life of soft drinks, due to prevention reactions of free-radical oxidation, fermentation and mould formation.

Kvass

- The added to kvass dihydroquercetin at 20 mg/100 cm³, suppressed the function of the yeast (*Saccharomyces cerevisae*, strain Mariobru Lager 497) reproduction and decreased its viability (the added dihydroquercetin decreased the total number of yeast cells by 57% and increased the number of died cells by 42% compared with the control sample) (*Skryabin, V.I., Kobelev, K.B., Gernet, M.B., Lavrova, V.L., 2005*).

- Dihydroquercetin decreased the oxygen concentration in the beverage. The added dihydroquercetin also decreased the oxygen concentration in the beverage during the storage period to a greater extent than did the ascorbic acid. At the amount of 10 mg/ cm³, dihydroquercetin decreased the oxygen content in the kvass by 6.67 mg/ cm3 during five days of storage, while at 20 mg/ cm3, it decreased the oxygen content by 7.01 mg/ cm³ as compared to the original value (*Skryabin, V.I., Kobelev, K.B., Gernet, M.B., Lavrova, V.L., 2005*).

APPLICATION OF DIHYDROQUERCETIN

The Efficiency of Dihydroquercetin in production of beverages

Vodka

- Dihydroquercetin added to vodka improves taste and odor of the alcoholic beverages. Vodka enriched with Dihydroquercetin is said to promote detoxification of acetylaldehydes, prevent hangover and protect the liver (*Burachevskiy*, *I.I., Berezhnoy, A.G., 2006*).

- Dihydroquercetin, when added to vodka, promotes the liver detoxification and prevents hangover.

- Incubation with 100 μ M Dihydroquercetin at 37 °C for 3 hr of serum obtained from 5 healthy volunteers after the consumption of white wine was shown to reduce cytotoxicity of the albumin (Wickramasinghe, S.N., 1996).

Beer

- Dihydroquercetin can be used in beer. Preliminary evidence showed the ability of Dihydroquercetin to suppress the yeast reproduction and viability and to decrease the oxygen concentration (Yefremov, A.A., 2001).

- The fortification of beer with Dihydroquercetin could stabilize redox potential, improve the products' shelf life, preserve organo-leptic properties.

Dosage of Introduction

Beverages – 10-20 mg per 0.5 liters;

Beverages on the basis of mineral water – 26-30 mg/ 1 liter;

Compote – 57g. by 100 liters;

Juice – 15-19 mg/L

Kvass – 10-20 mg/dm³;

Low-calorie compote with prophylactic effect – 10mg by 1g.;

Mineral water (Aqua Minerale Beauty) – 2.0 mg by 100 ml of water;

Mineral water – 15-20 mg by 1 liter of water;

Tea composition on the basis of green tea – 2-20 g/kg.;

Vodka – 4-10 mg/L

Practical Application (Russian Market)

- *Aqua Minerale Beauty*. Nutritional facts: purified potable water, carbon dioxide, dihydroquercetin -2.0 mg/100 ml, natural mineral complex, chlorine 21.3 mg/100 ml, calcium – 0.16 mg/100 ml, sulfate – 0.65 mg/100 ml, mineralization of no more than 0.95 g/dl), vitamins (niacin – 0,7 mg/100 ml, calcium pantotenat –1.3 mg/100 ml, B6-0.1 mg/100 ml, H-biotin 0.01 mg/100 ml. *Aqua Minerale Beauty* is the innovative product for the Russian Market. The purified mineral water with vitaminous-mineral complex and natural extract dihydroquercetin is intended for strengthening capillary walls, preventing the skin from harmful impact.

- *Low-calorie dietary compotes* made of cherries, pears, sea-buckthorn, ashberries, apples, etc. is intended as the dietary product for those who suffered from diabetes, excess weight, cardio-vascular diseases, avitaminosis. Nutritional facts: in 1 g of compote contains: ascorbic acid – 30 mg., dihydroquercetin – 10 mg., pectines – 0.5 mg., fresh or fast-frozen fruits and berries.

- Alcohol-free cocktail "*Normoprotein*". Nutritional facts: dihydroquercetin, vitamins: C, B1, B2, B6, B12, folic acid, pantothenic acid, PP (niacin), biotin, A, E, D3, mineral substances: iron, calcium. "*Normoprotein*" is recommended for people with unbalanced or monotonous ration, vegetarians who don't receive the essential proteins and vitamins, smokers.

- Soft drink "*Olymp*". Nutritional facts: sea-buckthorn, vitamins: A, E, K, F, C, B1, B2, B3, B9, P, betacarotine, pectin, organic acids, mineral substances and microelements, antioxidant (dihydroquercetin).

- Tea line "*Vtalitea Super AOX*" bamboo, orange, rosehip, verbena. A cup of tea (200 ml) contains from 3 to 6 mg. of dihydroquercetin. This line of teas is recommended for immune system activation, normalization of cardiovascular system.