AMETIS, Closed Joint-Stock Company

Application of **DIHYDROQUERCETIN** in confectionery

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 Technical Regulations of the customs Union 029/2012 allows using Dihydroquercetin as an antioxidant for manufacturing cream, dry milk, processed cheese, chocolate. The content of Dihydroquercetin in these products is 200 mg/kg fat of the product.
- 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.
- GOST 53507-2009. Milk-containing sweetened condensed canned foods. General Specifications. Date of introduction: January 1, 2011;
- GOST R 54661-2011. Canned milk. Dry cream. Specifications. Date of introduction: January 1, 2011;
- Amendment No 1 №1 to Technical Requirements 9222-355-00419785-04. Cream
- Amendment No 6 to Technical Requirements 9225-146-04610209-2003. Processed cheese.

Table of contents

This presentation was made by managers of 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 fat-soluble 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, thereby enhances its detoxifying 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

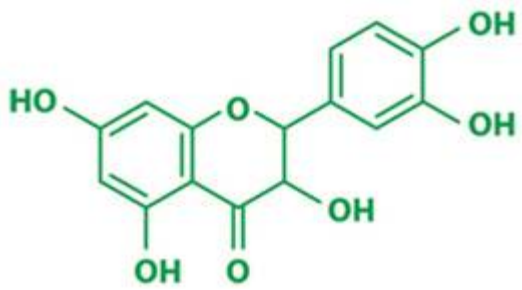
b) because of its capillary-strengthening properties, Dihydroquercetin can be used for functional products that are aimed at enhancing health.

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

Use of Dihydroquercetin (DHQ) in Confectionery

1) Improves oxidative stability of finished products

Hydrolysis and oxidation occurring in fats during their storage have resulted in the depreciation of their quality, shelf life reduction of fat-containing products and affected on their toxicological and microbiological safety.

Dihydroquercetin increases the shelf life of fat-containing products in 1.5 - 4 times, inhibiting oxidation reactions of food compounds. Dihydroquercetin suppresses the growth of microorganisms in foods, which have been already exposed to the oxidation process.

2) Preserves the initial organoleptic characteristics during storage

Lipid oxidation of food products leads to deterioration of organoleptic characteristics, loss in nutritional value, changes in appearance, etc. Dihydroquercetin preserves the initial organoleptic qualities of food products.

3) Enrichment of food products with antioxidants

Processing of food causing loss of many natural oxidants presented in raw materials, making the final product less resistant to the oxidation process.

Fortification of food products with dihydroquercetin promotes not only to supply with antioxidants, but also to slow down the oxidation process.

4) Supplies the product with parapharmaceutical properties

It is well known that end-products of lipid peroxidation may be mutagenic and cancerogenic, the most dangerous of them are free-radicals. Dihydroquercetin is the substance which “catches” and binds free radicals, preventing thereby the development of pathogenic processes and cell membranes lipid peroxidation.

5) Natural antioxidant

Dihydroquercetin is bioflavonoid extracted from natural plant raw material - Dahurian larch wood. Numerous studies have confirmed that Dihydroquercetin is non-toxic, physiologically harmless product for human.

APPLICATION OF DIHYDROQUERCETIN

The use of Dihydroquercetin in Confectionery

Chocolate

- A chocolate sample consisting of dry milk, grated cacao, cacao butter, and kernel nuts was used in the experiment. The addition of dihydroquercetin to the sample led to a decrease in the intensity of chemiluminiscence, especially at the concentration of 0.2% per lipid weight. At the concentrations of 1-2%, dihydroquercetin practically completely inhibited the oxidation process. The inhibition process had a dose-dependent correlation; thus at concentration 0.05% dihydroquercetin inhibited 20% oxidation, at 0.2%- 33%; at 0.5%- 47%; and at 1.0%- 62%. It was noted that dihydroquercetin was stable during 2 months of the experiment. From the 30th to 180th days of storage, there was an increase in the acid value in the control sample by 1.7 times. In the samples with dihydroquercetin, this value was much lower and remained stable and similar to dihydroquercetin added at concentrations of 0.2, 0.05 and 1.0% at 3 months of storage; these values increased by the end of the 6 month-period in 1.1-1.3 times. (*Tyukavkina, N.A., Rulenko, I.A., Kolesnik, Yu.A., et al., 1993 (1)*).

- When dihydroquercetin was added at 0.5% or 1.0% per lipid mass, there were no oxidation products found in the samples after 1 month of storage, while in the control samples there was an accumulation of peroxidation products in significant amounts. The addition of dihydroquercetin to the product at 0.05-1.0% per lipid mass resulted in the relatively unchanged acid number of the product within the first 3 months and, after 6 months of storage, the acid value started to increase. After 30 days of storage, the acid value in the control samples was 4.62, while in the samples with 0.05%, 0.2%, 0.5%, or 1% dihydroquercetin per lipid mass, the acid values were 4.34; 3.45; 3.55; and 3.53, respectively. At 6 months of storage, these values were 7.92 (in the control), 5.86 (in the 0.05% sample); 4.63 (in the 0.2% sample), 4.77 (in the 0.5% sample) and 3.98 (in the 1.0% sample). Dihydroquercetin lowered the level of oxidation products (such as saturated aldehydes and carbonyl acids) (*Tyukavkina, N.A., Rulenko, I.A., Kolesnik, Yu.A., et al., 1993 (2)*).

Grated cacao

- The study was conducted to examine the process of oxidation of grated cacao induced by Fe 2+ ions, and the antioxidant activity of dihydroquercetin added to this product. The addition of dihydroquercetin at 0.2% per the weight of the product's lipids resulted in chemoluminescence intensity equal to 7.5±7.8 as compared to 73.5±9.2 in the control sample (*Tyukavkina, N.A., Rulenko, I.A., Kolesnik, Yu.A., et al., 1993 (1)*).

Cacao butter

- The addition of dihydroquercetin at 0.05%, 0.2%, 0.5%, and 1.0% per the lipid mass resulted in the following chemoluminescence intensity of the lipids: 125±12.7; 94±4.2; 46±5.7; 21.5±5, respectively, versus 162±8.5 in the control. At the concentrations 0.05%, 0.2%, 0.5%, and 1.0% per lipid weight, dihydroquercetin inhibited peroxidation of lipids in cacao butter by 23%, 42%, 72%, and 37%, respectively. (*Tyukavkina, N.A., Rulenko, I.A., Kolesnik, Yu.A., et al., 1993 (1)*).

The Influence of Dihydroquercetin on Microbiological Indices

L. monolcytogenes	Dihydroquercetin inhibits the growth of L.monoclytogenes in sterilized sour cream. It kills on average **30%** of L.monocytogene
E.coli	Dihydroquercetin inhibits the growth of E.coli in sterilized sour cream. It kills on average **12%** of E.coli
S.aureus	Dihydroquercetin inhibits the growth of Staphylococcus aureus It killed on average **90%** of Staphylococcus aureus
Lipolytic microorganisms	Fortification with Dihydroquercetin inhibits the growth of lipolytic microorganisms in milk fat, inhibits 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 Dihydroquercetin is required for complete inhibition of 1 CFU of Rhodototorula
Lactic acid bacteria	**0.011 mg** of Dihydroquercetin is required for complete inhibition of 1 CFU of Lactic acid bacteria
Alicyclobacillus acidoterrestris	**2.5 mg** of Dihydroquercetin is required for complete inhibition of 1 CFU of Alicyclobacillus acidoterrestris

APPLICATION OF DIHYDROQUERCETIN

Ways of DHQ Introduction to fats and oils

Dihydroquercetin is weakly soluble in oils, including vegetable oils. It is recommended to introduce dihydroquercetin as alcohol solution allowed to use in food industry, or preliminarily dissolve Dihydroquercetin in hot water.

Dosage of introduction

Cocoa butter	0.02-0.5% by fat mass
Confectionery fat	1-2% by fat mass
Cacao powder	0.2% by fat mass
Grated kernels lipids	0.05-1% by fat mass
Hazelnut oil	0.05% by mass of raw material
Milk fat	0.01-0.1% by fat mass
Peanut butter	0.02% by fat mass
Palm oil	0.05% by fat mass
Palm fat	0.1% by fat mass
Chocolate	0.02% by fat mass
Confectioneries on fat basis	0.2 - 0.5% by lipid mass;

The use of Dihydroquercetin in Confectionery

Chocolate candies

- The addition of dihydroquercetin at 0.05%, 0.1%, 0.2%, 0.3%, 0.5%, 0.75%, 1.0% and 2.0% per lipid mass inhibited oxidation by 4%, 9%, 22%, 30%, 60%, 74%, 90%, and 91%, respectively (*Tyukavkina N.A., Rulenko I.A., Kolesnik Yu.A. et al., 1993 (1)*)
- Tests on oxidation products in confectioneries with dihydroquercetin showed that with an increase in the concentration of dihydroquercetin, there was a tendency toward the decrease of oxidation products in the samples. After 30 days of storage, the acid number in the control samples was 1.19; while in the samples with 0.2%, 0.5% and 1% dihydroquercetin per lipid mass, the acid number was 1.11; 1.06; 1.01; and 3.53, respectively. By the 6th month of storage, these values were 2.78 (in the control), 1.54 (in the 0.2% sample), 1.42 (in the 0.5% sample) and 1.15 (in the 1.0% sample); dihydroquercetin lowered the level of oxidation products (such as saturated aldehydes and carbonyl acids) in the confectioneries (*Tyukavkina N.A., Rulenko I.A., Kolesnik Yu.A. et al., 1993 (1)*).

Cacao powder

- The addition of dihydroquercetin at 0.2% resulted in the chemoluminscence intensity equal to 77.5±3.5 as compared to 158.5±4.9 in the control. At the concentration 0.2% per lipid mass, dihydroquercetin inhibited lipid peroxidation in cacao powder by 51% (*Tyukavkina N.A., Rulenko I.A., Kolesnik Yu.A. et al., 1993 (1)*).

Kernel nuts

- At the concentrations 0.05%, 0.2%, 0.5%, and 1.0% per lipid weight, dihydroquercetin inhibited peroxidation of lipids in kernel nuts by 17%, 55%, 76% and 93%, respectively (*Tyukavkina N.A., Rulenko I.A., Kolesnik Yu.A. et al., 1993 (1)*).

Confectionery fat

- The study was conducted to examine the oxidation of confectionery fat induced by Fe 2+ ions and the antioxidant activity of dihydroquercetin added to this product. The addition of dihydroquercetin at 0.05%, 0.2%, 1.0% and 2.0% per lipid weight resulted in the following chemoluminescence intensity of the lipids: 143.5±4.9; 125±5; 18±2.8; and 1.5±0.7, respectively, as compared to 143.9±2.1 in the control. At the concentrations 0.05%, 0.2%, 1.0% and 2.0% per lipid weight, dihydroquercetin inhibited peroxidation of lipids by 0.2%, 13%, 88%, and 99%, respectively (*Tyukavkina N.A., Rulenko I.A., Kolesnik Yu.A. et al., 1993 (1)*).

METHODS OF DHQ INTRODUCTION

Fillings for confectionery

The following technology of whipped cream fillings for confectioneries fortified with Dihydroquercetin could be used: Dihydroquercetin is introduced to warmed condensed milk, mixed with powdered sugar and softened butter, blend the ingredients until smooth (Patent RU 2345545C2, 2009).

Mass for preparing chocolate

The following technology of production of chocolate mass fortified with Dihydroquercetin could be used: mix all ingredients (powdered sugar, cocoa mass, part of cocoa butter. Dry milk products, kernels, etc could be also added); the received mixture is grounded; add the powdered mixture into a mixer, and add the remaining amount of cocoa butter; knead the mass Dihydroquercetin is introduced at the step of kneading the mass (Patent RU 2097977 C1, 1997).

Mass for chocolate

The following technology of production could be used: Mix Dihydroquercetin with other ingredients: powdered sugar, cocoa powder, dry milk products, grated walnut kernels and the part of the fat; grind the received mass; ground mixture fed into the mixer, where the remaining amount of fat is introduced; knead the mass (Patent RU 2097977 C1, 1997).

Mass for candies

The following technology for production of praline candies could be used: mix Dihydroquercetin with other ingredients: powdered sugar, cocoa powder, dry milk products, grated walnut kernels and the part of the fat; mixture is fed to the grinding mill; ground mixture fed into the mixer, where the remaining amount of fat is introduced; knead the mass (Patent RU 2097977 C1, 1997).

Practical Application (Russian Market)

Chocolate "Mishka"	Shelf life: 6 months. Nutritional facts: sugar, cacao grated, cacao butter, grated kernel nuts, emulsifier (lecithin), vanillin, **dihydroquercetin**	
Chocolate "Zdraviya Zhelayu"	Shelf life: 12 months. Nutritional facts: sugar, grated cacao, cacao butter, emulsifier (lecithin), vanillin, **dihydroquercetin**, dry cow foremilk;	
Chocolate "Sibirskiy"	No Data available	
Glazed chocolate bar "Vitagen"	Vitamin C – 60 mg, Anthocyanis – 30 mg, **dihydroquercetin** – 25 mg, sugar, molasses, dried milk, fried almond, cocoa oil, extract of bilberry, ascorbic acid, drinking water Shelf life: 8 months	
Dry biscuit "Madzheric Ultra"	Vitamin E – 0.27 g., **dihydroquercetin – 0.02 g.,** dietary fiber – 4g. Flour, salt, sugar, vegetable fat Shelf life: 12 months	
Crispbread "Samarskiy Zdorovyak"	Nutritional value (100 g): Proteins – 9.0 g, Fats – 1.0 g, Carbohydrates – 65.0 g, fiber – 37.0 g. Ingredients: whole wheat grains, natural honey, milk thistle and flax seed cake, jerusalem artichoke, fructose, **dihydroquercetin**	

Chocolates containing dihydroquercetin are intended for prevention of cardio-vascular diseases. Dihydroquercetin decreases the toxic products of oxidation in chocolates and extends the shelf life. Chocolates with dihydroquercetin are recommended for heightened physical and stress activity.