«AMETIS» JSC

“LAVITOL (DIHYDROQUERCETIN)”

IN FOOD INDUSTRY

Blagoveschensk, 2009
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References
1. GENERAL INFORMATION

Registration Number:
EINECS  207-543-4
CAS Number  480-18-2
BRN  0093548
BEILSTEIN HANDBOOK REFERENCE  5-18-05-00451
SYSTEM GENERATED NUMBER  000480182

Synonyms:
The formal chemical name: 3,5,7,3’4’-pentahydroxyflavanone
Other common names and synonyms: Taxifolin, Distylin, (2R,3R)-Dihydroquercetin, 2,3-Dihydroquercetin,
Molecular Formula: C_{15}H_{12}O_{7}
Molecular weight: 304.26

Identity:
Dihydroquercetin or Taxifolin relates to the large group of flavonoids.
The term “flavonoid” is generally used to describe a broad collection of natural products that include C_{6}-C_{3}-C_{6} carbon framework, or more specially a phenylbenzopyran functionality. Depending on the position of the linkage of the aromatic ring to the benzopyrano moiety, this group of natural products may be divided into 3 classes: the flavonoids, isoflavonoids, neoflavonoids [9].
Based on the degree of oxidation and saturation present in the heterocyclic C-ring, the flavonoids may be divided into the following groups and subgroups:

1) Flavones
   a) flavones: (ex.: Luteolin, Apigenin, Taugeritin);
   b) flavonols (ex.: Quercetin, Kaempferol, Myricetin, Fisetin);
   c) flavonones (ex.: Hesperetin, Naringenin, Eridictyol);
   d) flavononols (Dihydroquercetin (or Taxifolin), Dihydrokaempferol);
2) Isoflavones (ex.: Genistein, Daidzein, Glycitein);
3) Flavan-3-ols and Proanthocyanidins;
4) Anthocyanidins (ex.: Delphinidin, Cyanidin).
Flavononols or dihydroflavones are a much neglected group of which very little is known. About 40 flavonoids are known today. Dihydroquercetin (or Taxifolin) is the most common compound in this group.

**Sources of dihydroquercetin:**

Dihydroquercetin as an ingredient of phenolic compounds is found in many kinds of herbs and shrubs. Some of them are recognized to be drug plants. Among them are Polygonum nodosum Pers., Acacia catechu, Rhamnus lycoides L., Endelhardtia chrysdepis (Junglandaceae), Robinia pseudoacacia (Fabaceae) [8]. Dihydroquercetin is also occurs in the fruit of milk thistle (Silybum marianum) [40], in several citruses, grape seeds.

In several kinds of trees dihydroquercetin is found to a greater extend, especially in broad-leaved trees and conifers. In foliage trees dihydroquercetin presents in the bark of Salix caprea [8].

In conifers dihydroquercetin presents in Pinaceae family: Picea obovata L., Pinus sibirica, Picea ajanensis Fisch.. Bark of shore pine has high contents of the dihydroflavonols dihydromyricetin and dihydroquercetin. In Russia Larix sibirica Ledeb and Larix gmelini (Rupr.) wood (Pinaceae family) are the main sources of dihydroquercetin [26].

Lavitol (Dihydroquercetin) is a flavonoid derived from Larix gmelinii (syn. Larix dahurica) by a water-ethanol extraction method.

Dahurian Larch is a species of larch native to eastern Siberia, and adjacent northeastern Mongolia, northeastern China (Heilongjiang) and North Korea.

**2. PROPERTIES OF DIHYDROQUERCETIN**

**2.1. Physical and chemical properties**

Dihydroquercetin is a white to pale-yellow powder with wood bitterness.

The melting point of dihydroquercetin is 234-236°C.

Solubility of dihydroquercetin in water at different temperatures has an exponential character: at room temperature, the solubility is 0.1%; at 40°C- 0.3%; at 60°C- 1.0%; at 90°C- 3-5.3% [1; 30] and the solubility in boiling water is 9.3% [19] Dihydroquercetin is especially soluble in a 96% ethanol solution. Its solubility in a water-ethanol solution increases from 0.1% to 18% as the rate mass of alcohol increases from 30% to 90% [1; 30] Dihydroquercetin is soluble in ethyl acetate. The solubility is directly correlated to the temperature of the ethyl acetate solution. Thus, at a temperature of 20°C, the solubility...
of dihydroquercetin is 1.90%; at 40°C, it is close to 8 to 12%; and at 70°C, it is approximately 28% [1].
Dihydroquercetin is insoluble in chloroform, ether, and benzene [41].

2.1.1. **Structure of Lavitol (Dihydroquercetin)**

Dihydroquercetin is the dominant compound of Lavitol (Dihydroquercetin). Its content varies from 88% up to the 99%.
Depending on the purity of dihydroquercetin, Lavitol (Dihydroquercetin) could contain other flavonoids.

Table 1. Structure of Lavitol (Dihydroquercetin) of different purity

<table>
<thead>
<tr>
<th>Name</th>
<th>Lavitol (Dihydroquercetin) with purity 88-90%</th>
<th>Lavitol (Dihydroquercetin) with purity 93-94%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composition*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Diagram of Lavitol (Dihydroquercetin) with purity 88-90%](image)
![Diagram of Lavitol (Dihydroquercetin) with purity 93-94%](image)

<table>
<thead>
<tr>
<th>Name</th>
<th>Lavitol (Dihydroquercetin) with purity 95-96%</th>
<th>Lavitol (Dihydroquercetin) with purity 99%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composition*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Diagram of Lavitol (Dihydroquercetin) with purity 95-96%](image)
![Diagram of Lavitol (Dihydroquercetin) with purity 99%](image)

Notes: * - Arithmetic mean based on analyses results of 5 batches
Aromadendrin

- Synonyms: Dihydrokaempferol
- Molecular formula: C_{15}H_{10}O_{6}
- Aromadendrin is a flavanonol, a type of flavonoid.

Quercetin

- Molecular formula: C_{15}H_{10}O_{7}
- Quercetin is a plant-derived flavonoid, specifically a flavonol.
- Foods rich in quercetin include capers, lovage, apples, onion, red grapes, citrus fruit, tomato, etc. It has been shown to have anti-inflammatory and antioxidant properties and is being investigated for a wide range of potential health benefits.

Eriodictyol

- Synonyms: Eriodictiol
- Molecular formula: C_{15}H_{10}O_{6}
- Eriodictyol is a flavanone, a type of flavonoid. It is found in the Yerba Santa (Eriodictyon californicum), in the twigs of Millettia duchesnei, in rose hips.

Naringenin

- Molecular formula: C_{15}H_{10}O_{5}
- Naringenin is flavanone.
- Foods rich in naringenin include grapefruit, oranges, tomato skin. Naringenin has a bioactive effect on human health as antioxidant, free radical scavenger.

Kaempferol

- Synonyms: Kaempherol, Robigenin, Pelargidenolon, Rhamnolutin, Trifolitin
- Molecular formula: C_{15}H_{10}O_{6}
- Kaempferol has been isolated from tea, broccoli, grapefruit, brussel sprouts, apples, etc. Kaempferol is associated with reduced risk of heart disease. It has antidepressant properties.

Pinocembrin

- Synonyms: Dihydrochrysin, Galangin flavanone
- Molecular formula: C_{15}H_{12}O_{4}
- Pinocembrin is a flavanone. This antioxidant is found in honey and propolis.
2.2. Pharmacological properties of Dihydroquercetin

Dihydroquercetin has a wide range of pharmacological activity. Dihydroquercetin reveals an antioxidant, capillary protective, anti-inflammatory, hepatoprotective, irradiation protective, antiviral, immunemodulating effects. All these properties give the possibility to use dihydroquercetin in pharmacy for manufacturing different kinds of remedies, dietary and bioactive additives, in food industry as antioxidant, in cosmetology, in agriculture as the basis of feeds for animals and plant growth stimulant.

2.2.1. Antioxidant properties of Dihydroquercetin

Dihydroquercetin is an antioxidant of direct action which binds free radicals. The effect of dihydroquercetin exceeds essentially the number of wide famous vitamins A, C, E. It was demonstrated that Dihydroquercetin possesses antioxidant properties in a variety of model systems for oxidation. It slows the oxidation of ethylbenzene initiated by azo-bis-isobutyronitrile [45]. It also slows the free radical oxidation of liposomes from egg phospholipids, induced with the help of the Fe²⁺/ ascorbate system [39]. A number of studies concerns investigating antioxidant properties of dihydroquercetin using models of oxidative stress in vivo. Dihydroquercetin was shown to decelerate the development of free radical oxidation of lipids in the plasma and in the liver of mice after whole body irradiation [36] and in rats after tetrachloromethane poisoning [38]. After dihydroquercetin administration (130 mg/kg daily for 2 weeks) the resistance of the liver microsomes ex vivo to induction of lipid peroxidation was increased.

Teleskin and co-authors [36] investigated antioxidant effect in rats induced experimental hepatitis with the help of CCl₄ introduction. Authors discovered that the administration of dihydroquercetin in dose of 100 mg/kg, as aqueous suspension during 4 days before the addition of CCl₄ and next 14 days decreases the products of peroxide lipid oxidation in 1.5 times in comparison with control animals. The antioxidant activity of blood plasma of animals, introduced only CCl₄ was in 1.8 – 2.0 times lower than in experimental animals. Authors suppose that the received effect connects with antioxidant effect.

Antioxidant properties and cytoprotective activity of flavonoids (rutin, dihydroquercetin, quercetin, epigallocatechin gallate (EGCG), epicatechin gallate (ECG)) were studied. All these compounds inhibited both NADPH- and CCl₄-dependent microsomal lipid peroxidation. The I(50) values calculated for these compounds by regression analysis were close to the I(50) value of the standard synthetic antioxidant ionol (2,6-di-tert-butyl-4-methylphenol). The antiradical activity of flavonoids to O₂-* was studied in a model photochemical system. Rate constants of the second order reaction obtained by competitive kinetics suggested flavonoids to be more effective scavengers of

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Lavitol (Dihydroquercetin)
oxygen anion-radicals than ascorbic acid. By competitive replacement all flavonoids studied were shown to be chelating agents capable of producing stable complexes with transition metal ions (Fe2+, Fe3+, Cu2+). The flavonoids protected macrophages from asbestos-induced damage. The cytoprotective effect of flavonoids was in strong positive correlation with their antiradical activity to O2-• [27].

Moreover, it was reported, that dihydroquercetin (taxifolin) inhibited superoxide generation and lipid peroxidation. Taxifolin also inhibited sorbitol accumulation in human red blood cells and also been reported to protect human erythrocytes against oxidative stress [14].

It was established that dihydroquercetin inhibits free radical oxidation of both water soluble (luminol, ABTS) and fat-soluble substrates.

The antioxidant activity of dihydroquercetin was also approved in a study carried out by Japanese scientists [13]. They investigated that dihydroquercetin inhibited the production of lipid peroxides induced by microsomal NADPH oxidation. Dihydroquercetin was also effective in preventing microsomal lipid peroxidation, almost 80% inhibition being observed at 10 µg/ml. Moreover, dihydroquercetin at 30 µg/ml inhibited the lipid peroxidation completely, although, astilbin had no effect at 30 µg/ml. Many flavonoids are known to prevent lipid peroxidation. Dihydroquercetin has also been reported to inhibit brain mitochondrial lipid peroxidation that is induced by ascorbic acid or ferrous sulfate. Dihydroquercetin inhibited mitochondrial lipid peroxidation without affecting mitochondrial enzyme activities. This suggests that dihydroquercetin may be effective for preventing the functional depression of mitochondria. The received results showed that dihydroquercetin was effective for protecting tissues and cells against various types of oxidative stress. [13].

Wang Yea-Hwey and co-authors investigated that dihydroquercetin (taxifolin) inhibited leukocyte infiltration and COX-2 and iNOS expressions in CI/R-injured brain. Taxifolin also prevented Mac-1 and ICAM-1 expression. Production of both reactive oxygen species and nitric oxide by leukocytes and microglial cells was significantly antagonized by taxifolin. These data suggest that amelioration of cerebral ischemic reperfusion injury by taxifolin may be attributed to its antioxidative effect [44]. Thus, dihydroquercetin as antioxidant could function as (1) the “catcher” of active forms of oxygen, (2) chelator of metal with variable valency, (3) chaininformative agent.
2.2.1.1. **Antioxidant properties of Lavitol (Dihydroquercetin)**

Antioxidant activity reflects the ability of a product to destroy or neutralize oxygen free radicals. In general, the higher the product’s ability to destroy or neutralize reactive oxygen species, the higher the level of its antioxidative activity.

In 2008 and 2009, “Ametis” JSC entrusted the Advanced Botanical Consulting and Testing, Inc., Tustin, CA, USA and Brunswick Laboratories, Norton, MA, USA with testing the antioxidative activity of Lavitol (Dihydroquercetin). The method of the Oxygen Radical Absorption Capacity of the dissolved in water product (ORAC$_{\text{hydro}}$) was used in the testing. The test results showed that Lavitol (Dihydroquercetin) has very high ORAC value, which exceeds that of some known antioxidants.

Table 1. Comparative ORAC$_{\text{hydro}}$ value of some compounds considered to be powerful antioxidants

<table>
<thead>
<tr>
<th>No</th>
<th>Compound</th>
<th>ORAC$_{\text{hydro}}$ value (µmole TE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lavitol (Dihydroquercetin) 95% purity</td>
<td>32,744</td>
</tr>
<tr>
<td>2</td>
<td>Lavitol (Dihydroquercetin) 94% purity</td>
<td>21,940</td>
</tr>
<tr>
<td>3</td>
<td>Lavitol (Dihydroquercetin) 92-93% purity</td>
<td>19,925</td>
</tr>
<tr>
<td>4</td>
<td>Lavitol (Dihydroquercetin) 88 – 90% purity</td>
<td>15,155</td>
</tr>
<tr>
<td>5</td>
<td>Luteolin</td>
<td>12,500</td>
</tr>
<tr>
<td>6</td>
<td>Quercetin</td>
<td>10,900</td>
</tr>
<tr>
<td>7</td>
<td>Epicatechin (Green Tea)</td>
<td>8,100</td>
</tr>
<tr>
<td>8</td>
<td>Vitamin C</td>
<td>2,100</td>
</tr>
<tr>
<td>9</td>
<td>Vitamin E</td>
<td>1,300</td>
</tr>
</tbody>
</table>

Notes: Data provided by Brunswick Laboratories.

2.2.2. **Anti-inflammatory properties.**

Within wide spectrum of pharmacologic properties of bioflavonoids there are their anti-inflammatory properties. Mechanism of anti-inflammatory action is suggested to be governed by some bioflavonoids’ properties, such as:

- to reduce capillary permeability, inhibiting in such a way development of exudative inflammation;
- to inhibit action of many enzyme systems involved in the development of inflammation and allergy;

Lavitol (Dihydroquercetin)
• to reduce release of histamine and other mediators of inflammation from mast cells and basophils;

• to limit action of kinins and anti-inflammatory prostaglandins to tissues.

Anti-inflammatory properties of dihydroquercetin were studied in models for acute and chronic inflammation [26]. Acute inflammatory edema was modeled by administration of formalin or histamine under fascia of mice rear legs or by intraperitoneal administration of silver nitrate to rats. The test results showed that Dihydroquercetin inhibited development of acute inflammatory edema induced by formalin and histamine. In rats taken dihydroquercetin accumulation of exudates due to peritonitis decreased by 4 times compared with controls.

The anti-inflammatory effect was also described by the Gupta M.B. [10]. The effects of dihydroquercetin were studied on carrageenin induced oedema, formaldehyde induced arthritis and on granulation tissue formation by cotton pellet implantation in albino rats. Dihydroquercetin showed significant anti-inflammatory activities in all three tests. Dihydroquercetin was one-eight as active as hydrocortisone on carrageenin-induced oedema. In present studies dihydroquercetin prevented the increase in serum aspirate and alanine aminotransferase activities due to the inflammatory. ATP (adenosine triphosphate) phosphohydrolase activity in liver homogenate remained unaltered during inflammation but was significantly elevated by these agents [10].

The anti-inflammatory effect of dihydroquercetin was studied also by the Russian scientists Logvinov S.V, Plotnikov M.B and others. The study was carried out on 75 random-bred adult albino rats. Coagulation of the ovaries with a needle electrode was carried out in 4 points to a depth of 1.5 – 2.0 mm by the limiter. Intact rats served as control. “Ascovertin” (this drug contains dihydroquercetin and ascorbic acid in ratio 1:2.5) was administrated to animals of the main group in a dose of 70 mg/kg (20 mg/kg dihydroquercetin and 50 mg/kg ascorbic acid) in 1% starch orally for 5 days.

The test results showed that of growing follicles in the ovaries on days 3-5 after the operation had small specific volume compared to that in intact controls. By day 15 the volume ratio of follicles somewhat increased. “Ascovertin” treatment reduced inflammatory and destructive changes in the perifocal zone, specific volume of growing follicles on days 3 and 5 of experiment was higher than in the reference group. The ratio of arteric follicles and corpora significantly decreased on day 5 postoperation. Thus, the treatment by “Ascovertin” reduced the inflammatory reaction and intravascular changes, presumably, due to the anti-inflammatory effect of the main active component of the drug – dihydroquercetin [20].

Lavitol (Dihydroquercetin)
The effect of cromoglycate and of natural flavonoids on histamine release from peritoneal rat mast cells induced by compound 48/80 and ionophore A23187 was studied according to preincubation time of mast cells with drugs and to incubation time of cells with the triggering agent. Preincubation of cells with dihydroquercetin decreased the potency of drugs to inhibit the ionophore-induced release. Dihydroquercetin (taxifolin), previously considered as inactive, showed an interesting cromoglycate-like behaviour [4].

2.2.3. Capillary-protective properties

A new substance, which normalized permeability of blood vessels, was first designated as vitamin P (P stands for permeability). Years later it become clear that the substance was not vitamin, it was a flavonoid. According to present knowledge the mechanism by which flavonoids enhance the strength of capillary walls is involved in the mechanism of their anti-inflammatory action. This allows inhibition of exudate stage development. To make capillary protection more effective flavonoids are often used together with their synergist vitamin C.

The most studied effects of flavonoids as well as dihydroquercetin are related to the vascular system, particularly the decrease in pathological capillary fragility or the increase in resistance of normal capillaries to trauma. Dihydroquercetin tends to maintain the normal tensile strength of capillary walls. Dihydroquercetin may antagonize histamina’s effect in inducing capillary permeability. It appears to close capillary sphincters and to restrict blood entry by prolonging catecholamine action.

The angio-protective (capillary-protective) properties of dihydroquercetin were estimated by ability to decrease the exudative period of the aseptic inflammatory reaction i.e. by changing of vascular permeability. Effectiveness of normalizing capillary permeability by dihydroquercetin and quercetin was compared. Mice were given the flavonoids (100 and 300 mg/kg) an hour before intraperitoneal administration of 250 mcl of 1% solution of trypan blue. Permeability of vessels was estimated by the time taken for arrival of the dye at the inflammation focus. Inflammation was induced by xylene (50 mcl) overlaid on the dehaired skin. Dihydroquercetin in a dose of 100 mg/kg increased time interval between administration of the trypan blue and its arrival at the inflammation focus by 53% in comparison with control, quercetin increased the period by 15%. Dihydroquercetin at dose of 300 mg/kg increased the time period by 63% and quercetin – by 17%. [39].
It was also determined, that dihydroquercetin (as the main compound of the drug “Ascovertin”) increases the blood viscosity, erythrocyte aggregation, decreases the thickness of the blood and improve microcirculation. [20].

The results showed pronounced capillary protective effect of flavonoids in experiments with modeled inflammation induced by xylene. There was dose-dependent effect. Dihydroquercetin showed as more effective capillary protective affect as quercetin.

2.2.4. **Hepatoprotective properties**

Nowadays, compounds of bioflavonoid nature are the leaders among all antioxidants that are used in the therapy of liver diseases.

The hepatoprotective properties of one of the powerful bioflavonoid – dihydroquercetin were studied on rats with the hepatogenic pathologies.

The toxic injury of liver was reproduced by the hypodermic injection of tetracycline in a dose of 0,5 g/kg during 5 days and tetrachormethane in a dose 4 ml/kg during 4 days. Dihydroquercetin (30 and 100 mg/kg) was added daily during 3 days before the addition of toxicants and together with them during the experiment. The activity of ferments of hepatic origin: alanineaminotransferase, aspartaminotransferase, gamma-glutamate-transferase and alkaline phosphotase was determined in blood serum before the beginning and in 48 hours after the ending of tetracycline addition as well as on the 7th, 14th and 21st days after ending the addition of tetrachormethane. Dihydroquercetin slowed down the rate of rise of transaminases and alkaline phosphatase in blood serum in 16-24% in comparison with control. In this study dihydroquercetin decreased the accumulation rate of malonic dialdehyde in liver of rats on 36% in comparison with control.

The hepatoprotective properties of dihydroquercetin was studied in rats induced by CCl₄. It is well know that the metabolism of tetrachormethane in liver is accompanied by free radicals formation, which have damaging effect on the hepatic cells. It was observed that the value of TBA-reactive products (the products of oxidation able to react with the thiobarbituric acid) in liver and blood serum of poisoned animals of control group was increased and the antioxidant activity of blood serum was significantly decreased. In the 7th and 15th day of the experiment the antioxidant activity was in 1,5 and 2 time lower in comparison with intact animals. It was shown that the addition of dihydroquercetin into the experimental animals led to the decrease of TBA- reactive products in blood serum and liver. Thus, at
the end of the experiment there was no any significant difference between the intact group and the experimental animals received dihydroquercetin [26].

The antioxidant activity of “Diquertin” (the trade mark of dihydroquercetin) was investigated using of CCl₄-induced toxicity of male Wistar rats (155-220 g). The experimental animals were divided into three groups: the intact group (n=9); the control group (n=9), which was administered CCl₄ subcutaneously for 4 days at the dose of 4 ml/kg; and the experimental group (n=9), which was administrated “Diquertin” (100 mg/kg) for a period of 4 days prior to the administration of CCl₄ and until the end of the experiment. Fourteen days after the first CCl₄ injection, the blood plasma antioxidant activity and levels of lipid peroxidation products in the blood plasma and liver were measured. The content of products of lipid peroxidation, reacting with thiobarbituric acid in the serum and liver of the control animals, was increased by more than 1.5 fold compared with the intact and experimental animals (p < 0.01). The blood plasma antioxidant activity of the control animals was 1.8 to 2 times lower than that of experimental and intact animals (p < 0.01) It is suggested that the data obtained are dependent on the anti-oxidant properties of DHQ (P<0.01) [36].

Chang Liver cells were incubated with a cytokine mixture (CM) supplemented with the flavonols quercetin and kaempferol, the flavanone dihydroquercetin (taxifolin) and the flavone apigenin (5-50 microM). Concentrations of oxidised and reduced glutathione, generation of different ROS/RNS, and expression of antioxidant enzymes were measured. Oxidized glutathione concentration and the oxidised/reduced glutathione ratio were increased by the CM. These effects were significantly prevented by quercetin, kaempferol and dihydroquercetin at all tested concentrations. Dihydroquercetin 50 microM and apigenin 25-50 microM caused a significant increase in peroxides and nitric oxide generation [5].

It was determined that the content of thiobarbituric acid reactive substances (TBARS) of LP in the blood plasma and liver in the experimental animals was significantly lower compared to the control animals. The intensity of Fe²⁺-induced chemiluminescence of liver homogenates of experimental mice was 25-30% less compared with those of the control and intact animals (P<0.001). This can be possibly explained by accumulation of dihydroquercetin and the products of its metabolism in the liver [35].

2.2.5. Radioprotective properties

One of numerous studies was conducted on 155 female mice of the Balb strain. The animals were divided into three groups: the first untreated group, the second control irradiated group and the third group was irradiated and received dihydroquercetin. Dihydroquercetin was daily intubated in a dose of

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Lavitol (Dihydroquercetin)
100 mg/kg during the first 40 days after irradiation and 5 mg/kg during the remaining days of the experiment. A mice of the third experimental group that received dihydroquercetin didn’t have a different plasma content of 2-thiobarbituric acid (TBARP) from intact mice for the first 70 days of irradiation and only after that period was an increase observed. It was also shown the dihydroquercetin slows the development of free radical oxidation in the plasma and in the liver of mice, which were subjected to whole body irradiation [38].

Teselkin Y.O. and co-authors were examined the influence of dihydroquercetin on lipid peroxidation which is enhanced after exposure to ionizing radiation. This experiment was conducted with femail rats. The animals were divided into three groups: 1) intact, 2) control group with animals subjected to a single external gamma irradiation at 4 Gy, and 3) experimental animals which were irradiated, obtaining 100 mg/kg dihydroquercetin during the first 40 days after radiation exposure, followed by 5 mg/kg for the remaining 115 days. The test results demonstrated that dihydroquercetin decreases lipid peroxidation activity in serum and in the livers of mice which have been exposed to gamma radiation, revealing 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 [37].

The effect “Diquertin” (DQ) on the process of blood plasma and liver lipid peroxidation (LP) of female BALB/c mice (16.5-18 g) after a single 4 Gy dose of gamma –irradiation was examined. Animals were divided into three groups: the untreated group (n=45); the control irradiated group (n=50); and the experimental group (receiving irradiation plus DQ, n=60). DQ was daily inturbated per os, using a water crystalline suspension at the dose of 100 mg/kg during the first 40 days after irradiation and 5 mg/kg during the remaining of 115 days of the experiment. It was determined that the content of thiobarbituric acid reactive substances (TBARS) of LP in the blood plasma and liver in the experimental animals was significantly lower compared to the control animals. The intensity of Fe$^{2+}$-induced chemiluminescence of liver homogenates of experimental mice was 25-30% less compared with those of the control and intact animals (P<0.001). This can be possibly explained by accumulation of dihydroquercetin and the products of its metabolism in the liver [36].
3. MANUFACTURER

Ametis JSC is the 100% private company which was established on the 25th of June 1998 and is located in AMUR region (Far East) on the border with China Republic in Blagoveschensk city. In December 2003 the company begun to produce Dihydroquercetin. Ametis JSC is the largest dihydroquercetin manufacturer in the world with the annual capacity more than 11 tones.

“Ametis” JSC produces dihydroquercetin for different purposes:
- raw material “Raw Lavitol”. It is used for increasing the shelf life of oils, fuels, varnishes, paints and other technical production, for producing plant growth stimulant “Larixin”.
- raw material for manufacturing biologically active food supplements, medicine, foodstuffs - “Lavitol (Dihydroquercetin)”,
- raw material for manufacturing cosmetic products - “Lavitol cosmetics”.

Ametis JSC received the State registration for the manufacturing of all products that Ametis JSC produce, received the Sanitary and Epidemiological Conclusions. Ametis JSC is ISO 9001:2000 certified for the production of food and biologically active additives and substances for biologically active additives. Ametis JSC is the FDA registered facility.

4. QUALITY OF LAVITOL DIHYDROQUERCETIN

Ametis JSC has passed the State registration for the manufacturing of Lavitol (Dihydroquercetin), which is used as an antioxidant in the food industry in the Russian Federation. According to the State Institution of Research Institute of Nutrition (Moscow, the Russian Federation), the documentation-project of Technical Specifications for Lavitol (Dihydroquercetin) corresponds to the acting legislative act and normative regulations for the quality and safety of the population.

Lavitol (Dihydroquercetin) is being produced by “Ametis” JSC in accordance with the technical requirements TY 9325-001-70692152-07.

Lavitol (Dihydroquercetin) is regularly analyzed in the analytical laboratory of the enterprise as well as in the accredited testing analytical laboratory center based on the Public Health Federal State Establishment “Center for Hygiene and Epidemiology in Amur Region”.

In addition, Lavitol (Dihydroquercetin) is periodically tested by the independent laboratories the Advanced Botanical Consulting & Testing, Inc., Tustin, CA, USA and by the PhytoLab GmbH & Co,
KG, Vestenbergsgreuth, Germany. Each batch of the finished product is accompanied by a Certificate of Analysis and a chromatogram.

Lavitol (Dihydroquercetin) adheres to strict limits for levels of microorganisms, heavy metals, pesticides and radionucleotides to correspond to the requirements outlined in the Sanitary-Epidemiological Conclusion 2.3.2.1078-01 (index 1.10.5.).

The analysis of heavy metal contaminants in Lavitol (Dihydroquercetin) has shown that the levels of heavy metals are well within the stated specifications and accepted safety limits.

Table 1. Heavy Metal Profiles of Lavitol (Dihydroquercetin)

<table>
<thead>
<tr>
<th>Heavy Metals I&lt;sup&gt;†&lt;/sup&gt;</th>
<th>Heavy Metals II&lt;sup&gt;†&lt;/sup&gt;</th>
<th>Heavy Metals III&lt;sup&gt;†&lt;/sup&gt;</th>
<th>Arsenic (ppm)&lt;sup&gt;†&lt;/sup&gt;</th>
<th>Cadmium (ppm)&lt;sup&gt;†&lt;/sup&gt;</th>
<th>Mercury (ppm)&lt;sup&gt;†&lt;/sup&gt;</th>
<th>Lead (ppm)&lt;sup&gt;†&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND, &lt;10 ppm</td>
<td>ND, &lt;10 ppm</td>
<td>ND, &gt;10 ppm, &lt;20 ppm</td>
<td>Not more than 0.005</td>
<td>Not more than 0.049</td>
<td>Not more than 0.014</td>
<td>Not more than 0.076</td>
</tr>
</tbody>
</table>

Notes: 1 – USP Method I  
2 – USP Method II  
3 – USP Method III  
4 – ICP/MS, Prop. 65

Microbiological contaminants in Lavitol (Dihydroquercetin) are within the regulated safety limits.

Table 2. Microbiological Profiles of Lavitol (Dihydroquercetin)

<table>
<thead>
<tr>
<th>Total Plate Count&lt;sup&gt;†&lt;/sup&gt;</th>
<th>&lt;10 CFU/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast &amp; Mold&lt;sup&gt;†&lt;/sup&gt;</td>
<td>&lt;10 CFU/g</td>
</tr>
<tr>
<td>Escherichia coli&lt;sup&gt;†&lt;/sup&gt;</td>
<td>Negative/ 10g.</td>
</tr>
<tr>
<td>Salmonella&lt;sup&gt;†&lt;/sup&gt;</td>
<td>Negative/ 10g.</td>
</tr>
<tr>
<td>Staphylococcus aureus&lt;sup&gt;†&lt;/sup&gt;</td>
<td>Negative/ 10g.</td>
</tr>
<tr>
<td>Pseudomonas&lt;sup&gt;†&lt;/sup&gt;</td>
<td>Negative/ 10g.</td>
</tr>
</tbody>
</table>

Notes: 1 – USP & FDA – BAM Methods
STANDARD AMETIS CERTIFICATE OF ANALYSIS WITH A CHROMATOGRAM

Lavitol (Dihydroquercetin)
CERTIFICATE OF ANALYSIS

1. Dihydroquercetin [Lavitol (Dihydroquercetin)]3), Technical Requirements № 9325-001-70692152-07
2. Plant source: Dahurian Larch (Larix dahurica Turcz.)
4. Batch: 66a
5. Manufacturing date: March 23, 2009
6. Packaging: double polyethylene film
7. Quantity: 8 packs per 100 grams
8. Test results:

<table>
<thead>
<tr>
<th>№</th>
<th>Index name</th>
<th>Admissible Value</th>
<th>Result</th>
<th>Analysis method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Outward appearance</td>
<td>White or straw-colored powder</td>
<td>Straw-colored powder</td>
<td>Visual</td>
</tr>
<tr>
<td>2</td>
<td>Moisture</td>
<td>10</td>
<td>9.6</td>
<td>State Standard 16483.7-71</td>
</tr>
<tr>
<td>3</td>
<td>Mass of Dihydroquercetin</td>
<td>89</td>
<td>92.58</td>
<td>MV 72-08</td>
</tr>
<tr>
<td>4</td>
<td>Dichlorodiphenyltrichloroethane</td>
<td>Not more than 0.1</td>
<td>&lt;0.005</td>
<td>Method 2142-80</td>
</tr>
<tr>
<td>5</td>
<td>Mercury, mg/kg</td>
<td>Not more than 1.6</td>
<td>0.006</td>
<td>IPCMS, Prop. 65</td>
</tr>
<tr>
<td>6</td>
<td>Arsenic, mg/kg</td>
<td>Not more than 3.0</td>
<td>0.004</td>
<td>IPCMS, Prop. 65</td>
</tr>
<tr>
<td>7</td>
<td>Lead, mg/kg</td>
<td>Not more than 5.0</td>
<td>0.043</td>
<td>IPCMS, Prop. 65</td>
</tr>
<tr>
<td>8</td>
<td>Cadmium, mg/kg</td>
<td>Not more than 1.0</td>
<td>0.028</td>
<td>IPCMS, Prop. 65</td>
</tr>
<tr>
<td>9</td>
<td>Total Plate Count, CFU/g</td>
<td>5x10^5</td>
<td>&lt;10</td>
<td>USP &amp; FDA-BAM</td>
</tr>
<tr>
<td>10</td>
<td>Escherichia coli, per 10 g</td>
<td>Not allowed</td>
<td>Negative</td>
<td>USP &amp; FDA-BAM</td>
</tr>
<tr>
<td>11</td>
<td>Yeast &amp; Mold, CFU/g</td>
<td>100</td>
<td>&lt;10</td>
<td>USP &amp; FDA-BAM</td>
</tr>
<tr>
<td>12</td>
<td>Salmecella spp., per 10 g</td>
<td>Not allowed</td>
<td>Negative</td>
<td>USP &amp; FDA-BAM</td>
</tr>
<tr>
<td>13</td>
<td>Staphylococcus aureus, per 10 g</td>
<td>Not allowed</td>
<td>Negative</td>
<td>USP &amp; FDA-BAM</td>
</tr>
<tr>
<td>14</td>
<td>Pseudomonas spp., per 10 g</td>
<td>Not allowed</td>
<td>Negative</td>
<td>USP &amp; FDA-BAM</td>
</tr>
<tr>
<td>15</td>
<td>Solvent residues, Class 1</td>
<td>Not allowed</td>
<td>Non-detected</td>
<td>USPNF77 &lt;467-</td>
</tr>
<tr>
<td>16</td>
<td>Solvent residues, Class 2</td>
<td>Not allowed</td>
<td>Non-detected</td>
<td>USPNF77 &lt;467-</td>
</tr>
<tr>
<td>17</td>
<td>Solvent residues, Class 3</td>
<td>5,000 ppm Ethanol</td>
<td>1774 ppm</td>
<td>USPNF77 &lt;467-</td>
</tr>
</tbody>
</table>

* The admissible values are established by the regulatory organs of the Russian Federation.

9. Storage conditions: The product should be stored in dry, clean, well-ventilated places, without storage odors at temperature above 40°C and relative humidity ranges of 40% to 68%. It should be prevented from moisture and kept away from sunlight.

Chief of laboratory: Chernikha U.L.

Lavitol (Dihydroquercetin)
5. DIHYDROQUERCETIN IN FOOD INDUSTRY

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

1) According to the Decision of the State Chief Medical Officer dated 11/14/2001 #36 “About the application of the Sanitary and Epidemiological Conclusion (SEC) 2.3.2.1078-01”, Dihydroquercetin is classified as an antioxidant;

2) The Decision of the State Chief Medical Officer dated 04/18/2003 #59 “About the application of SEC 2.3.2.1293-03” allows using Dihydroquercetin for in manufacturing of cream, chocolate, and dry milk. The maximal content of Dihydroquercetin in these products is 200 mg/kg fat of the product;

3) The Methodical recommendations of the State Sanitary and Epidemiological Regulation #2.3.1.1915-04 “Recommended norm of consumption of food and biologically active supplements” has determined the appropriate and the highest allowable level of Dihydroquercetin consumption: 25–100 mg per day.

The use of Dihydroquercetin in food products is determined by its ability to reduce the oxidative reactions and to strength capillaries and by its pronounced P-vitamin activity. The 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 and P-vitamin activity, Dihydroquercetin can be used for functional products that are aimed at enhancing health.

Dihydroquercetin is used for manufacturing of the dairy products, meat products, alcoholic and non-alcoholic beverages, confectionary, and products of functional nutrition.

5.1. Application of Dihydroquercetin in meat products

Antioxidant activity of dihydroquercetin was studied in ground beef and pork meat [28]; ground lamb meat [3]; chicken rendered fat [18]; ground meat [11]; ground poultry meat [21]; cutlets [28]; dumplings [28]; and marinated semi-finished meat [23].

Ground meat

Samples of ground meat without dihydroquercetin (the controls) and with 0.001% dihydroquercetin, added in an alcohol solution, were packaged according to the Sanitary-Epidemiological regulations and stored for 7 days at a temperature of 4±2.C, and for 14 days at a temperature of -17.C. In the beginning of the experiment, the concentration of the primary peroxidation products in the ground meat stored at 4±2 .C was 0.018. By the 3rd day of storage, this number increased by 1.6 times, while at the end of the experiment, the number increased by 6.2 times. Addition
of dihydroquercetin resulted in a significant reduction in the oxidation process. Thus, on the 3rd, 5th and 7th days of storage, the peroxidation values in these samples were lower than in the corresponding controls by 13.8, 20.8, and 57.1%, respectively. The researchers suggested that the addition of dihydroquercetin to ground meat possibly results in a decreased formation of free radicals at the beginning of the storage period. Similar results were obtained in the samples with frozen ground meat. By the 14th day of storage, the number of primary oxidation products was 1.8 times less than that in the control [11].

**Ground poultry meat**

Lavitol (Dihydroquercetin) at 0.025 kg per 100 kg of ground chicken stored at -18°C lowered the peroxide value by 2.6-3.0 times as compared to the control. On the 10th day of storage, the peroxide value reached its minimum. The obtained results indicated that the fat oxidation process in the ground chicken with added dihydroquercetin had a 52% lower rate than that in the control. An analysis of the peroxide value and the acid number indicated that the shelf life of ground chicken meat increased by 30% and is equivalent to 6 months at a temperature of -18°C. In addition, there were no changes in the organoleptic parameters of the ground chicken meat, such as appearance (after defreezing), color, odor, and consistency, as compared to the fresh ground chicken meat [21].

Dihydroquercetin was added to ground poultry meat in the amounts of 0.006%, 0.02%, and 0.06% per fat mass. The products were packaged according to the Sanitary-Hygienic requirements and kept at temperature of -8°C for 45 days. The peroxide value in the control increased by 14 days of storage, after which it started to decline, indicating the formation of the secondary products of oxidation. In the experimental samples, the peroxide value was much lower than that in the controls. After one month of storage, the peroxide value of the control sample compared to the samples with 0.002%, 0.006% and 0.02% dihydroquercetin was 1.36, 2.03 and 2.96 higher, respectively. After 45 days of storage, there was no difference in the peroxide value in the control sample and in the sample with 0.002% dihydroquercetin, while compared to the samples with 0.006% and 0.02% dihydroquercetin, it was 1.25 and 1.29 times greater, respectively. The experiment indicated the time period during which the product remained fresh; thus, for the control and the sample with 0.002% dihydroquercetin, this time period was equal to 7 days, for the sample with 0.006% dihydroquercetin, it was equal to 1 month, and for the sample with 0.02% dihydroquercetin, it was equal to 45 days. The author concluded that, when added as an antioxidant to the ground chicken, the amount of dihydroquercetin should not be less than 0.006% per the product weight. Along with the peroxide values, an increase in the secondary products of oxidation was studied. In the samples with 0.006%, 0.002%, and 0.02% dihydroquercetin, the value of TBA increased after 1 month of storage by 6.4%, 6.4%, and 6.2%, respectively, and after 45
days of storage, by 11.0 times, 15.0 times, and 15.2 times, respectively. After 45 days of storage the TBA value in the samples with dihydroquercetin was 1.01 times, 1.10 times and 1.10 times less than that in the control samples, respectively. The author suggested that the addition of dihydroquercetin to the product can not only decrease the lipid peroxidation process, but also act as a bacteriocide toward the lipophilic microorganisms [28].

Lavitol Food Grade (Dihydroquercetin) was added to ground chicken meat (12.3-14% fat) to determine its effect on the meat’s storage time. The ground chicken was stored at a temperature of -18°C and the peroxide value and the acid numbers were calculated after 6 months of storage. The addition of Lavitol (Dihydroquercetin) decreased the peroxide value by 2.6-3.0 times, as compared to the controls. The peroxide value reached its minimum by the 60th day of storage, indicating the accumulation of the secondary products of oxidation. The analysis of the changes in the peroxide value indicated that the oxidation of the ground chicken fortified with dihydroquercetin was 52% lower than in the control sample. The addition of dihydroquercetin increased the storage time of the ground chicken by 50% (up to 6 months) at a storage temperature of -18.C without changing the appearance, color, smell, or consistency [11].

Lavitol (Dihydroquercetin) was added to the ground chicken in the amounts of 0.025%, 0.050% and 0.075% per weight of the meat. The samples were kept at a temperature of –18.C for 30 days, after which the organoleptic, physico-chemical and microbiological parameters of the experimental samples and the controls were studied. At the end of the experiment, the consistency, odor and color of the samples were similar to the freshly prepared ground meat. The microbiological analysis showed that during the storage time, there was no growth of pathological microorganisms in either the controls or experimental samples. The amount of toxic elements, pesticides, and radionuclides were within the regulated levels. In addition, it was shown that the highest rate of the primary oxidation products was seen in the control samples. The peroxide value in the sample with 0.025% dihydroquercetin per weight of the product was 0.0025, while in the control sample it was 0.015. On the 15th day of storage, the peroxide values were 0.007 and 0.028 for the dihydroquercetin sample and the control sample, respectively. On the 10th, 20th, and 30th days, the peroxide values in the dihydroquercetin samples were lower than in the controls by 57.0, 20.8 and 57.0%, respectively. When added at the concentrations of 0.05 and 0.075% per weight of the meat, dihydroquercetin has no lowering impact on the peroxide value [3].

Ground beef and pork meat

An experiment conducted on a product that contained ground beef and ground pork (at a 50:50 ratio) has shown that dihydroquercetin inhibited the formation of the primary oxidation products with

Lavitol (Dihydroquercetin)
the resulting decrease in the formation of the secondary oxidation products after 35 days of storage by 1.07 and 1.15 times at the concentration of 0.002% and 0.007%, respectively [5].

**Ground lamb meat**

Lavitol (Dihydroquercetin) was added to ground lamb meat in the amounts of 0.025%, 0.050% and 0.075%, according to the meat’s weight. The samples were kept at a temperature of -18°C for 30 days, after which the organoleptic, physico-chemical and microbiological parameters of the experimental samples and the controls were studied.

At the end of the experiment, the consistency, odor and color of the samples were similar to the freshly ground meat. The microbiological analysis has shown that during the storage time, there was no growth of pathological microorganisms in either the controls or experimental samples. The amount of toxic elements, pesticides, and radionuclides were within the regulated levels. In addition, during the experiment the highest rate of the primary oxidation products was seen in the control samples. The addition of Lavitol (Dihydroquercetin) at 0.050% and 0.075% per total weight of the meat resulted in a decreased formation of free radicals during the early stages of storage. The authors concluded that Lavitol (Dihydroquercetin) is capable of prolonging the storage time of ground meat [3].

**Chicken rendered fat**

The State University All-Russian Scientific Institute of Poultry Industry (GY VNII PP) conducted a study aimed at evaluating the antioxidant activity of dihydroquercetin in chicken rendered fat and developing technological methods that would determine the oxidative deterioration of lipids in the product. Dihydroquercetin (under the private label name Bianon) and ionol were diluted in 1 cm³ 96% ethyl alcohol and were added to 40 mg of chicken rendered fat at the initial stage of oxidation (peroxide value of 0.04% iodine, acid value 2.9 mg KOH). After the compounds were mixed, the dish with the mix was hermetically closed and heated for 15 minutes in a thermostat at 100°C. Then, the alcohol residues were removed with pure nitrogen within 5 minutes and the air was added to the mixture at a constant temperature of 97.0±0.1°C. The addition of dihydroquercetin to chicken rendered fat resulted in an increased induction period from 5.2 hrs at 0.01% dihydroquercetin to 25.6 hrs for 0.05% dihydroquercetin. At a concentration of 0.01%, dihydroquercetin increased stability of the chicken rendered fat to oxidation by 10-17 times [18].

**Cutlets**

Dihydroquercetin was added at different amounts depending on the amount of fat in the products varying from 0.002% to 0.006% per total weight of the product, with resulting concentrations of dihydroquercetin as 0.003% and 0.006%. The products were packaged according to the Sanitary-Hygienic requirements and kept at a temperature of -8°C. The product samples were kept for 35 days.
The addition of dihydroquercetin to cutlets resulted in the slower accumulation of the primary oxidation products. Thus, by the 35th day of storage, the peroxide value in the control sample was 4.4 times higher than at the beginning of the experiment, while in the samples with 0.003% and 0.006%, it was 4.2 and 3.3 times higher, respectively. A similar pattern was observed regarding the accumulation of the secondary products of oxidation [28]

Dumplings

Dihydroquercetin was added at different amounts depending on the amount of fat in the products, varying from 0.022% to 0.0034% per total weight of the product with resulting concentrations of 0.003% and 0.006%. The product samples were kept for 35 days. In both the control samples and in samples with dihydroquercetin, there was an increase in the peroxide values in 3.10 and 3.28 times on the 35th day of storage compared to the value at the beginning of the experiment. On the 35th day of storage, the TBA value in the sample with 0.003% dihydroquercetin was 66% lower than that of the control samples and in the sample with 0.006% dihydroquercetin it was 45.8% lower [28]

Marinated semi-finished meat

Fortified with dihydroquercetin, Shishkebab was shown by the Amur Center for Standardization and Meteorology along with the Far East State Agricultural University to have very low levels of heavy metals and pesticides as well as low levels of microorganisms. All tested values were below the standard values [23].

Thus, in meat industry Lavitol (Dihydroquercetin) can be added to:

<table>
<thead>
<tr>
<th>Name of meat product</th>
<th>Dose of Dihydroquercetin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground chicken</td>
<td>0.025% per total weight of the ground chicken(^1)</td>
</tr>
<tr>
<td>Ground lamb</td>
<td>0.05%-0.075% per product weight(^2)</td>
</tr>
<tr>
<td>Cutlets and dumplings</td>
<td>0.02% per net weight(^3)</td>
</tr>
<tr>
<td>Rendered beef fat</td>
<td>0.01% per fat mass(^4)</td>
</tr>
<tr>
<td>Ground beef</td>
<td>0.05%-0.075% per product weight(^5)</td>
</tr>
<tr>
<td>Ground roe deer</td>
<td>0.05%-0.075% per product weight(^5)</td>
</tr>
<tr>
<td>Ground poultry of mechanical boning with fat content 15±1%</td>
<td>0.02 – 0.04% per fat mass(^6)</td>
</tr>
<tr>
<td>Poultry fresh-jerked sausage</td>
<td>0.02% per mass of lipids(^7)</td>
</tr>
</tbody>
</table>

\(^1\) [21]


2 [6]
3 [28]
4 [32]
5 [43]
6 [25]
7. Patent RU 2303914 C2

5.2. Application of Dihydroquercetin in dairy products

Russian research has shown that, as an antioxidant, dihydroquercetin can be utilized in dry milk [15], dry whole milk [12; 34]; soymilk concentrates [21]; butter [16; 17; 21]; milk concentrates [21]; sour-milk products [2], sour cream [16]; yogurt [16]; cottage cheese [16; 17]; and dry milk and milk concentrates for children. Several patents were awarded for technologies incorporating dihydroquercetin in the dairy products.

**Dry milk**

The inhibiting effect of dihydroquercetin on the free radical oxidation of dry milk lipids was studied by the Russian State Medical University, Moscow Medical Academy, and the All-State Scientific Institute of the Dairy Industry. The addition of 0.02, 0.08, or 0.2% of dihydroquercetin per weight of lipids to samples of dry milk with Fe $^{2+}$-induced oxidation resulted in a dose-dependent decrease in both the intensity of chemiluminescence and the accumulation of malondialdehyde (MDA). An additional experiment was conducted on dry milk that was stored for 8 months at 20±2°C. The samples were analyzed at an intensity of Fe $^{2+}$-induced chemiluminescence in the presence of luminol at the beginning of the experiment, and at 3, 6, and 8 months. It was shown that the intensity of Fe $^{2+}$-induced luminescence in the control sample without dihydroquercetin increased during the first six months of storage, reaching 1.7 times higher values than at the beginning of the experiment. In the samples with dihydroquercetin, although the changes in the intensity of chemiluminescence were similar to that of the controls, the values were significantly lower. The greatest inhibition was observed in the samples with 0.08% and 0.2% dihydroquercetin. For example, after 6 months of storage, the intensity of chemiluminescence in the samples with 0.2% dihydroquercetin was four times lower than that of the control samples, and 2.6 time lower after 8 months of storage. In addition, at the end of the experiment, the amount of MDA in the sample with 0.2% dihydroquercetin was 90% lower than that of the control sample [15].

The antioxidant activity of dry milk fortified with Flukol D, a private label name used for dihydroquercetin, was studied with the application of such chemical methods as the degree of oxidation

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*Lavitol (Dihydroquercetin)*
of milk lipids by the chemiluminescence and stability of milk lipids by the Monin-Shetty method. In addition, the antioxidant activity was evaluated by several biological methods. In particular, the antioxidant activity of dry milk fortified with dihydroquercetin was evaluated by the examining lipid peroxidation processes in homogenates of rats' livers. The latest experiment comprised of two parts: a model of a pathological process in vitro, and in vivo on the tetrachloromethane-induced toxicity of the liver. The effect of dihydroquercetin was determined by changes in the concentration of MDA in the liver homogenate compared to the controls. In in vivo experiments, lipid peroxidation was induced by the Fe 2+-ascorbate system. The addition of dihydroquercetin-containing dry milk caused a significant decrease in the rate of lipid peroxidation as compared to the controls in approximately 2 times at 1-2 mg of dihydroquercetin per 1 gram of the liver. In the experiment with the tetrachloromethane-induced toxic damage of the liver, the rats were fed dihydroquercetin-fortified dry milk for seven days. At the end of the feeding period, the animals were injected with CCl4 with resulting hepatitis. Injection of CCl4 resulted in a three time-increase in the rate of lipid peroxidation in the controls, while in the experimental group no pathological changes were observed [29].

**Dry whole milk**

The effect of dihydroquercetin on the storage stability of vacuum foam-dried milk powder was studied organoleptically by the Dairy Products Laboratory, Eastern Utilization Research and Development Division, USDA, Washington, D.C. Statistical analysis of the data conducted on the samples that were stored at 80°F for six months in air packs showed that dihydroquercetin produced a significant improvement in the flavor scores of the powder packed in nitrogen containing 0.1 to 1.0% oxygen [34].

As a result of the joined work of the VNIIMI and VNII (the Russian Federation), a technological process of manufacturing of dry milk fortified with Flukol D (Dihydroquercetin) was developed. The dry milk fortified with dihydroquercetin has a storage time of 24 months as opposed to 8 months for dry milk without dihydroquercetin [12].

**Soymilk concentrate**

Soymilk concentrates of different ratios of vegetable and animal fat (30:70; 50:50; 40:60) were used in an experiment aimed at determining the effect of dihydroquercetin on the storage time of the products. Dihydroquercetin was added in a 40%-alcohol solution at 0.02%, 0.05%, 0.08%, 0.2%, and 0.5% by the product’s fat weight. The addition of dihydroquercetin to the soymilk concentrate had no effect on the taste, smell, color, and appearance of the product. There were no pathogenic microorganisms detected in the product at the end of the experiment. At the end of the 6-month experiment, there were no changes in the quantity of dihydroquercetin added to the product. The thermal

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*Lavitol (Dihydroquercetin)*
parameters had little impact on the quantity of dihydroquercetin in the product. Thus, in the refrigerator, heated (t = 20°C) and unheated chambers (t = 10°C), the amounts of dihydroquercetin decreased by 6.8%, 10.3%, and 3.2%, respectively. Stability of dihydroquercetin in the product stored in the non-heated chamber was the highest. As was shown by the chemiluminescence method, dihydroquercetin is capable of quenching free radicals during soymilk lipid oxidation. The intensity of Fe 2+-induced chemiluminescence in the concentrate’s lipids was much lower in the samples with dihydroquercetin than that in the controls. As a result of these experiments, it was concluded that the addition of dihydroquercetin to soymilk concentrate increased the storage time of the product by two times [21].

**Milk concentrate with sugar**

The addition of dihydroquercetin at 0.02%, 0.05%, 0.1%, 0.3%, and 0.5% by lipid mass of the product to the sugary milk concentrate improved the quality and storage time of the product. The samples were kept at a temperature range of 2-6°C, -3°C, and -18°C. There were no changes in the physico-chemical parameters of the milk concentrate after fortification with dihydroquercetin. The level of toxic elements, pesticides, radionuclides and microbiological data did not change [21].

**Butter**

The antioxidant activity of Flukol D (Dihydroquercetin) in butter (82.2% lipids by weight) was studied by the Moscow State University. The samples of the butter fortified with different concentrations of dihydroquercetin (0.1%, 0.05%, and 0.025% per 100 g of the product) and the controls were kept in a thermostat at 37±2°C and in a refrigerator at 4±2°C for one month. The efficacy of the antioxidant was determined by its ability to inhibit lipid peroxidation as was calculated by changes in the peroxide value and in the thiobarbituric acid number, which indicates the accumulation of the secondary products of lipid peroxidation, aldehydes, in both the samples and controls. It was shown that the peroxide value in the sample with dihydroquercetin was 2-2.5 times lower than that in the control. Similar results were obtained for the carbonyl compounds. The amount of carbonyl compounds in the samples fortified with 0.025% dihydroquercetin changed from 0 ppm within the first three days of storage to 42±4 ppm after 24 days of storage, while in the control samples these values changed from 8±1 ppm to 155±2 ppm, respectively. The oxidation coefficient was calculated to be equal to 0.626 [17].

The addition of Lavitol (Dihydroquercetin) to butter improved the quality and storage time of the product. Dihydroquercetin was added at the amounts of 0.02%, 0.05%, 0.1%, 0.3%, and 0.5% per lipid mass in the product. The samples were kept at a temperature range of 0°C, +10°C, and +20°C. At the storage temperature lower than 0°C, deterioration of the product took place much slower than at higher temperatures. When stored at the above 0°C temperature, microbiological processes and fermentation changed the plasma butter and deteriorated its qualities. The addition of dihydroquercetin to the butter...
resulted in a significant inhibition of peroxidation as was evident by the peroxide value within 240 days of the experiment. There were no changes in the physico-chemical parameters of the butter after its fortification with dihydroquercetin. The level of toxic elements, pesticides, radionuclides and microbiological data did not change [21].

The addition of Lavitol (Dihydroquercetin) to the animal butter at 0.1% per fat mass decreased the intensity of lipid oxidation. After eight months of storage, the quantity of malonaldehyde in the butter fortified with dihydroquercetin was 90% lower than in the control sample without dihydroquercetin [32].

**Sour-milk products**

The addition of 0.02% Flukol D (Dihydroquercetin) per the lipid mass of the sterilized milk (4% lipids by weight) prior to its fermentation with either *Lactobacillus delbruecki subsp. Bulgaricus* (sample 1), *Streptococcus thermophilus* (sample 2) or both (sample 3) showed positive results on the growth and development of the bacteria. The experimental samples (with dihydroquercetin) and the control samples (without dihydroquercetin) were fermented under similar conditions at 42±1°C. After cooling down to 4±2°C, the samples were packaged under aseptic conditions and kept at the above temperature for 108 days. During the experiment, there was an increase in acidity in both the control and experimental samples. Nevertheless, an increase in acidity took place at a 5-15% slower rate in the experimental samples than in the controls, with an exception of the sample containing both bacteria. The acidity in sample 1 was 12-13% lower at 20-74 days than in the corresponding control. After 86 days, the acidity increased to 270.T in the control samples and to 225.T in the experimental samples. By the 108th day, these numbers increased to 330. T and 270.T, respectively. The acidity levels in sample 2 and the corresponding control were similar during the first five days of the experiment, 80-81.T. From 2 to 108 days, the acidity in the experimental samples was lower by 12-15% compared to the controls. In the third sample, the acidity remained 5% lower than in the corresponding control sample during the whole period of experiment. By the 86th day of the experiment, the number of live bacteria in both sample 1 and its corresponding control remained at a relatively high level (2.5x10⁷), with a consequent decrease by the end of the experiment. In sample 2, the number of live bacteria was twice higher than in the control; after the 36th day, the number of live bacteria in the experimental sample started to decline and was lower than that in the control sample at the end of the experiment. In sample 3, the number of live bacteria was higher than in the control during the 86 days of the experiment. By the 108th day, the number of live bacteria was relatively similar and equal to 6x10⁵ [2].

**Sour cream**

*Lavitol (Dihydroquercetin)*
The active acidity of sour cream fortified with 0.025% Flukol D (Dihydroquercetin) decreased from 4.50±0.02 to 4.22±0.01 after 39 days of storage, while in the control sample, it reached 4.10±0.01 during that storage period. The value of the titratable acidity (.T) of sour cream fortified with 0.025% dihydroquercetin increased from 66±1 to 83±2 after 39 days of storage, while that of the control increased from 66±1 to 84±2.

Analysis of the fatty acid composition of the product, fortified with dihydroquercetin and stored for 40 days, showed that this composition remained unchanged during the storage period. In addition, there were no changes in the microbiological, heavy metal, pesticide, and radionuclide profile at the end of the experiment. The author concluded that fortification with 0.025% dihydroquercetin can increase storage time of sour cream to 40 days [16].

Yogurt

The experiment has shown that the active acidity of yogurt fortified with 0.025% Flukol D (Dihydroquercetin) decreased from 4.44±0.03 to 4.06±0.01 after 54 days of storage, while in the control sample, it reached 4.05±0.02. The value of the titratable acidity (.T) of yogurt fortified with 0.025% dihydroquercetin increased from 85±2 to 115±2 after 54 days of storage, while that of the control increased from 84±2 to 116±2. The analysis of the fatty acid composition of the product, fortified with dihydroquercetin and stored for 54 days, showed that this composition remained unchanged during the storage period. In addition, there were no changes in the microbiological, heavy metal, pesticide, and radionuclide profile at the end of the experiment. The author concluded that fortification with 0.025% dihydroquercetin can increase storage time of yogurt to 60 days [16].

Cottage cheese

The antioxidant effect of Flukol D (Dihydroquercetin) at 0.025% per 100 g cottage cheese (5.5% lipids) on the quality parameters of cottage cheese during 39 days of storage was studied by the Moscow State University. The addition of dihydroquercetin to cottage cheese resulted in low levels of aldehydes. The consistency, taste and odor of the product fortified with dihydroquercetin were close to those of the control [17]. It was also shown that the active acidity of cottage cheese fortified with 0.025% dihydroquercetin decreased from 4.56±0.02 to 4.54±0.01 after 54 days of storage, while in the control sample, it reached 4.30±0.01. The value of the titratable acidity (.T) of cottage cheese fortified with 0.025% dihydroquercetin increased from 82±1 to 115±2 after 54 days of storage, while that of the control increased from 82±2 to 117±2. The analysis of the product’s fatty acid composition, fortified with dihydroquercetin and stored for 54 days, showed that this composition remained unchanged during the period of storage. In addition, there were no changes in the microbiological, heavy metal, pesticide,
and radionuclide profile at the end of the experiment. The author concluded that fortification with 0.025% dihydroquercetin can increase storage time of cottage cheese to 60 days [16].

As a result of VNIMI’s and VNII’s (the Russian Federation) collaborated work, a technological process for manufacturing fortified with dihydroquercetin cottage cheese was developed. The cottage cheese fortified with dihydroquercetin has storage time of two years as opposed to 1 year of cottage cheese of sublimed drying without dihydroquercetin [12].

Thus, in dairy industry Dihydroquercetin can be added to:

<table>
<thead>
<tr>
<th>Name of dairy product</th>
<th>Dose of Dihydroquercetin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry milk</td>
<td>0.02% per fat mass (200 mg per 1 kg of fat)¹ ²</td>
</tr>
<tr>
<td>Cream condensate</td>
<td>0.02% per fat mass¹</td>
</tr>
<tr>
<td>Dry milk with 15% fats</td>
<td>at 0.035 grams per 1 kg of the product⁴</td>
</tr>
<tr>
<td>Dry milk with 20% fats</td>
<td>at 0.046 grams per 1 kg of the product⁵</td>
</tr>
<tr>
<td>Dry milk with 25% fats</td>
<td>0.056 grams per 1 kg of the product⁶</td>
</tr>
<tr>
<td>Cream, sour cream, yogurt, kefir made of dry whole milk</td>
<td>0.056 grams per 1 kg of the dry whole milk³</td>
</tr>
<tr>
<td>Fermented milk desserts</td>
<td>0.056% per net weight⁴</td>
</tr>
<tr>
<td>Butter with 82.2% fats</td>
<td>0.025% per 100 g. of product⁵</td>
</tr>
<tr>
<td>Curd dessert with 5.5% fats</td>
<td>0.025% per 100 grams of product⁶</td>
</tr>
<tr>
<td>Sour cream with 15% fats</td>
<td>0.025% per 100g. of product⁶</td>
</tr>
<tr>
<td>Yoghurt with 7.5% fats</td>
<td>0.025% per 100g of product⁶</td>
</tr>
<tr>
<td>Sour milk products (kefir and yoghurt) made of sterile milk</td>
<td>0.02% per fat mass⁷</td>
</tr>
<tr>
<td>Condensed milk</td>
<td>not more than 1% per fat mass⁸</td>
</tr>
<tr>
<td>Mayonnaise with up to 50% fats</td>
<td>0.25% per mass of product⁹</td>
</tr>
<tr>
<td>Enriched dairy product</td>
<td>0.01 – 0.1% per mass of product¹⁰</td>
</tr>
<tr>
<td>Dry milk product</td>
<td>0.05% per 100 g. of product¹⁰</td>
</tr>
<tr>
<td>Milk-containing concentrated sweet product</td>
<td>0.05 kg. per 100 kg of dromt¹¹</td>
</tr>
</tbody>
</table>

¹ - SanPin 2.3.2.1293-03 (3.4.9.)
² – [15]
³ – [12]
⁴ – [6]
⁵ – [17]

Lavitol (Dihydroquercetin)
5.3. Application of Dihydroquercetin in confectionery

Antioxidant properties and application of dihydroquercetin in confectionery were evaluated in grated cacao; cacao butter; kernel nuts; confectionery fat; chocolate and chocolate candies [42; 43].

Dihydroquercetin was shown to display antioxidant activity regarding lipid oxidation taking place in a variety of fat-containing confectionery products. The greatest inhibition of Fe 2+-induced lipid peroxidation was observed when dihydroquercetin was added to the lipids of kernel nut, cacao powder, and cacao butter. The degree of inhibition in these products was equal to 50-55% at 0.2% dihydroquercetin, 70-75% at 0.5% dihydroquercetin, and 90-95% at 1.0% dihydroquercetin.

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. There was no effect of dihydroquercetin on the chemoluminescence intensity when it was added at the concentrations of 0.05%, 0.5%, 1.0% and 2.0% per lipid weight.

At a concentration of 0.2% per lipid weight, dihydroquercetin inhibited lipid peroxidation by 3%; no inhibition was observed for other concentrations [42].

Cacao butter
The study was conducted to examine the oxidation of cacao butter induced by Fe 2+ ions and the antioxidant activity of dihydroquercetin added to this product. The addition of dihydroquercetin at 0.05%, 0.2%, 0.5%, and 1.0% per the lipid weight 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. There was no effect of dihydroquercetin on the chemiluminescence intensity when it was added at the concentration of 2.0% per lipid weight. 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. No inhibition was observed when dihydroquercetin was added at 2.0% [42].
Cacao powder

The study was conducted to examine the oxidation of cacao powder induced by Fe 2+ ions and the antioxidant activity of dihydroquercetin added to this product. The addition of dihydroquercetin at 0.2% resulted in the chemoluminescence intensity equal to 77.5±3.5 as compared to 158.5±4.9 in the control. There was no effect of dihydroquercetin on the chemoluminescence intensity when it was added at the concentrations of 0.05%, 0.5%, 1.0% and 2.0% per lipid weight. At the concentration 0.2% per lipid weight, dihydroquercetin inhibited lipid peroxidation in cacao powder by 51%; while no inhibition was observed when dihydroquercetin was added at concentrations of 0.05%, 0.5%, 1.0% and 2.0% per lipid weight [42].

Kernel nuts

The study was conducted to examine the oxidation of kernel nuts induced by Fe 2+ ions and the antioxidant activity of dihydroquercetin added to this product. Lipids of kernel nuts are less susceptible to oxidation as compared to the other studied products. The addition of dihydroquercetin at 0.05%, 0.2%, 0.5%, and 1.0% per lipid weight resulted in the following chemoluminescence intensity of the lipids: 185±1.4; 99.8±6.9; 54±5.6; and 15±1.4, respectively, as compared to 222.5±3.5 in the control. There was no effect of dihydroquercetin on the chemiluminescence intensity when it was added at the concentration of 2.0% per lipid weight. Increase in the dihydroquercetin concentration from 0.05 to 1.0% resulted in an increase in the degree of inhibition of peroxidation from 17 to 93%. 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. No inhibition was observed when dihydroquercetin was added at the concentration of 2.0% per lipid weight [42].

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. There was no effect of dihydroquercetin on the chemiluminescence intensity when it was added at the concentration of 0.5% per lipid weight. 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. No effect was observed when dihydroquercetin was added at the concentration of 0.5% per lipid weight [42].

Chocolate

**Lavitol (Dihydroquercetin)**
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 chemiluminescence, 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. The authors concluded that the addition of dihydroquercetin at 0.2-0.5% per lipid weight could increase storage time of confectionery products with a fat base in 2-2.5 times [42].

The objective of this study was to describe products of lipid peroxidation of confectionery products and to examine the impact of dihydroquercetin on the process of their accumulation. The high-performance lipid chromatography showed the absence of malondialdehyde in the product without dihydroquercetin. This phenomenon was observed in all tested samples in spite of the storage time. The spectral analysis has indicated the presence of compounds related to the saturated aldehydes. Tests on oxidation products in the confectionery products with dihydroquercetin showed that with an increase in the concentration of the added dihydroquercetin, there was a decrease in the amount of oxidation products in the samples. Thus, 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. In addition, there was a tendency towards the accumulation of carbonyl compounds with extended storage time in the tested products. The addition of dihydroquercetin to the product at 0.05-1.0% per lipid weight 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). The authors concluded that as an antioxidant, dihydroquercetin lowers the level of oxidation products (such as saturated aldehydes and carbonyl acids) in the confectionery products [43].

Sugar-containing confectionery products on fat-base
A new methodology with the use of high-performance lipid chromatography showed that the level of dihydroquercetin in sugar-containing confectionery products on a fat-base within the first month of storage decreased by 10-15% compared to the initial amounts. Thereafter, the rate of decrease slowed down and by the end of 8 months of storage, it remained to be 20-25% lower than in the initial product. It was also shown that the level of alpha-tocopherol in this product decreased by the 1st month of storage and no traces of this confectionery were found within 1.5-2 months of storage. Therefore, dihydroquercetin was found to be much more stable than alpha-tocopherol in confectionery products with a fat-base [42].

**Chocolate candies**

The chocolate candies containing dry milk, cacao powder, confectionery fat, and kernel nuts were exposed to Fe $^{2+}$-induced chemiluminescence. 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 [42].

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. In addition, there was a tendency towards the accumulation of carbonyl compounds with extended storage times in the tested products. 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). The authors concluded that as an antioxidant, dihydroquercetin lowers the level of oxidation products (such as saturated aldehydes and carbonyl acids) in the confectioneries [42].

The study aimed at evaluating the antioxidant activity of dihydroquercetin added to a biological model or given to animals as a part of a lipid fraction of the confectionery products. The rate of lipid peroxidation and antioxidant potency of dihydroquercetin-containing lipid fractions were evaluated by the quantity of malondeldehyde. Two methods were utilized to determine the antioxidant potency of dihydroquercetin-containing lipid fraction: *in vitro* and *in vivo*. The *in vitro* experiment was conducted with the application of the Fe $^{2+}$-ascorbate system-induced lipid peroxidation in the homogenate. The addition of a lipid fraction derived from the confectionary mass of receipt I (without dihydroquercetin) to the homogenate, resulted in a dose-dependent decrease of the MDA amount by 15-25%, while the addition of a lipid fraction from confectionery mass of receipt II (without dihydroquercetin) decreased the rate of peroxide lipid oxidation by 7% (when added at 10 mg/g) and 16.6% (when added at 100
Lavitol (Dihydroquercetin)

mg/g). This antioxidant activity of the lipid fraction was partially related to its endogenous levels of α-tocopherol. Since the lipid fraction from receipt II contained no cacao beans, the amount of endogenous antioxidants in this fraction was much lower than in the lipid fraction in receipt I. The addition of the lipid fractions with dihydroquercetin to the homogenate resulted in a dose-dependent decrease in the rate of lipid peroxidation as compared to both the control and the lipid fraction without dihydroquercetin. The antioxidant effect of the dihydroquercetin-containing lipid fraction (receipt I) reached its maximum when dihydroquercetin was added at a concentration of 100 mg lipid fraction with 0.5% dihydroquercetin per 1 gram of the liver and 100 mg of lipid fraction with 1% dihydroquercetin per 1 g of the liver. The results were 53% and 49%, respectively. In the lipid fraction from receipt II, the maximal antioxidant effect was achieved when dihydroquercetin was added at 400 mg/g liver, with the resulting 41% inhibition of the lipid peroxidation as compared to the control.

The lipid fractions derived from both receipts I and II were given orally to the experimental mice for 7 days at 200 mg/mouse (100 mg/kg for dihydroquercetin). On the 8th day, the mice were injected with CCL4 with a resulting 3-fold increase in the rate of lipid peroxidation. The 7-day supplementation with the lipid fractions I and II without dihydroquercetin prior to the drug administration, preserved the liver cells from the peroxidation process by 20.5% and 4.3%, respectively. Administration of the lipid fractions I and II with 1.0% dihydroquercetin increased the antioxidant protection by 15% and 17%, respectively [43].

Thus, in dairy industry Dihydroquercetin can be added to:

<table>
<thead>
<tr>
<th>Name of meat product</th>
<th>Dose of Dihydroquercetin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chocolate</td>
<td>0.02% per fat mass (200 mg per 1 kg of fat)¹</td>
</tr>
<tr>
<td>Cacao powder</td>
<td>0.2% per lipid mass²</td>
</tr>
<tr>
<td>Cacao butter</td>
<td>0.2-0.5% per lipid mass²</td>
</tr>
<tr>
<td>Kernel nut</td>
<td>0.2-1.0% per lipid mass²</td>
</tr>
<tr>
<td>Confectionery lipids</td>
<td>1.0-2.0% per lipid mass²</td>
</tr>
<tr>
<td>Beaten cream filling for farinaceous confectioneries with more than 50% fats</td>
<td>0.25% per mass of product³</td>
</tr>
<tr>
<td>Sugary confectioneries on fat basis</td>
<td>0.05% - 1% per fats mass⁴</td>
</tr>
</tbody>
</table>

¹ - SanPin 2.3.2.1293-03 (3.4.9.)
² – [42]
³ – Patent RU 2345545 C2
⁴ – Patent RU 2097977 C1

Lavitol (Dihydroquercetin)
5.4. Application of Dihydroquercetin in beverages

At 10-20 mg per 0.5 liters of a non-alcoholic beverage, dihydroquercetin was shown to neutralize and block free radicals. When used in kvass, dihydroquercetin is used at 10-20 mg per one liter of the beverage [32; 33]. In the case of weak-alcoholic beverages (i.e., beer), dihydroquercetin is added at 20-25 mg per one liter of the finished product [7].

The addition of 5-10 mg of dihydroquercetin to 0.5 liters of non-alcohol soft drink improved organoleptic characteristics of the product [32].

The added to kvass dihydroquercetin (under the private label name of Vezalarix) at 20 mg/100 cm³, suppressed the function of the yeast (*Saccharomyces cerevisiae*, strain Mariobru Lager 497) reproduction and decreased its viability. 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 [33].

In order to use Lavitol (Dihydroquercetin) in low-alcohol-containing products (i.e., beer), take 1-2 liters of the finished product (beer), dissolve a certain amount of Lavitol (Dihydroquercetin), based on 20-25 mg/l of the finished product, while mixing. Conduct a visual inspection of the dissolving Lavitol (Dihydroquercetin) in the beer, and pour the received concentrate into the vessel with the finished product. For example, to prepare 1,000 liters of beer with 20 mg/l Lavitol (Dihydroquercetin), calculate the amount of Lavitol (Dihydroquercetin) to be added to the product (20 mg/l × 1,000 liter = 20,000 mg/l or 20 g/l). Weigh 20 g of Lavitol (Dihydroquercetin) on the analytical scale, dissolve this amount in 1-2 liters of beer while constantly mixing.

Conduct a visual inspection of the dissolution process and upon its completion, pour the prepared concentrate into the vessel with 1,000 liters of the beer. The finished product contains 20 mg of Lavitol (Dihydroquercetin) per liter of beer [7].

“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).
With the aim of studying an influence of potable water Aqua Minerale Beauty upon skin of a healthy person an influence of potable water Aqua Minerale Beauty upon morpho-functional characteristics of skin, body weight, relation of tissues and water balance of an organism were studied.

The test results were following:
- high organoleptic properties of this water (according to the opinion of the patients);
- regulating influence upon a water balance of an organism. This is confirmed by a tendency towards the increase of bound water in an organism of a health person, clinically revealed increase of skin humidity, increase of its turgor and leveling of the skin micro-relief. Besides, patients taking this water pointed out a subjective diuretic effect of this water, reduction of soft tissues pastosity and a tendency towards a reduction of body mass;
- improvement of skin micro-circulation, which is indirectly confirmed by restoration of uniform echogenicity of skin and reduction of sub-epidermal hypoechogetic zone in a smoking woman;
- a tendency towards restoration of hydro-lipid skin mantel, which is certified by ultra sound signs of reduction of skin desquamation, clinically revealed increase of skin humidity and its turgor, restoration of skin micro-relief, shown both clinically and by ultra sound skin scanning, increase of epidermis thickness and reduction of its echogenicity.

It is also important to point out that based on ultra sound signs of the improvement of skin traffic under the influence of potable water Aqua Minerale Beauty, it is probably perspective to conduct further investigation of the possibility of the use of this water in premature ageing prevention programs.
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frozen in dough semi-finished products during storage.” // Department of Meat and Meat Products Technology. Kemer Institute of Technology of Food Products. Internal report., 2006


