BOLU ABANT IZZET BAYSAL UNIVERSITY

THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

DEVELOPMENT OF A SKIN CREAM WITH COENZYME Q AND MILLED RICE BASIS AND INVESTIGATION OF ITS CERTAIN CHEMICAL, PHYSICAL AND MICROBIOLOGICAL PROPERTIES

MASTER OF SCIENCE

SERAP YILDIZ

BOLU, JULY 2019

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APPROVAL OF THE THESIS

DEVELOPMENT OF A SKIN CREAM WITH COENZYME Q AND MILLED RICE BASIS AND INVESTIGATION OF ITS CERTAIN CHEMICAL, PHYSICAL AND MICROBIOLOGICAL PROPERTIES submitted by **SERAP YILDIZ** and defended before the below named jury in partial fulfillment of the requirements for the degree of **Master of Science** in **Department of Biology, The Graduate School of Natural and Applied Sciences of Bolu Abant Izzet Baysal University in 29.07.2019** by

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DECLARATION

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

SERAP YILDIZ

ABSTRACT

DEVELOPMENT OF A SKIN CREAM WITH COENZYME Q AND MILLED RICE BASIS AND INVESTIGATION OF ITS CERTAIN CHEMICAL, PHYSICAL AND MICROBIOLOGICAL PROPERTIES
MSC THESIS
SERAP YILDIZ
BOLU ABANT IZZET BAYSAL UNIVERSITY GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF BIOLOGY
(SUPERVISOR: PROF.DR. SEYHUN YURDUGÜL

BOLU, JULY 2019

The cosmetic products used for hygiene in the Roman public bathrooms were part of the cosmetic industry after some time. Nowadays, cosmetic products containing chemicals, as well as skin-friendly natural cosmetics, are also remarkable.

The whitening effect of rice flour; the cell renewal of coenzyme Q10; the moisturizing, feeding and antibacterial properties of the olive oil have an important role in natural cosmetic products. In our study, we tried to obtain an edible cosmetic product by combining these three components. In addition, the *Pinus sylvestris* extract was used to increase the color, smell, density and antibacterial effect of the cream.

When the control group, rice flour-coenzyme Q10-olive oil and rice flour-coenzyme Q10-olive oil-yellow pine extract were taken into consideration, the third group did not have any microbial increase as well as no change was observed for the pH, viscosity, fat and protein content, where almost appropriate cosmetic standards were obtained.

KEYWORDS: Coenzyme Q10, olive oil, *Pinus sylvestris*, skin cream, milled rice.

ÖZET

DEVELOPMENT OF A SKIN CREAM WITH COENZYME Q AND MILLED RICE BASIS AND INVESTIGATION OF ITS CERTAIN CHEMICAL, PHYSICAL AND MICROBIOLOGICAL PROPERTIES
YÜKSEK LISANS TEZI
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(TEZ DANIŞMANI: PROF. DR. SEYHUN YURDUGÜL)

BOLU, TEMMUZ - 2019

İlk olarak Roma halk banyolarında hijyen amaçlı kullanılan kozmetik ürünleri, gelişen kozmetik endüstrisinin bir parçası olmuştur. Günümüzde, kimyasal madde içeren kozmetik ürünlerinin yanı sıra cilt dostu doğal kozmetik ürünler de dikkat çekmektedir.

Pirinç unu; beyazlatıcı etkisinden, koenzim Q10 hücre yenileyiciliği ve zeytinyağı ise cildi nemlendirme, besleme ve antibakteriyel özelliklerinden dolayı doğal kozmetik ürünlerinde oldukça önemli bir yere sahiptirler. Çalışmamızda bu üç sözünü ettiğimiz bileşen bir araya getirilerek yenilebilir bir kozmetik ürünü elde edilmeye çalışılmıştır. Bunlara ek olarak kremin rengini, kokusunu, yoğunluğunu ayarlaması ve antibakteriyel etkisini arttırmak için sarı çam özütü de kullanılmıştır.

Kontrol grubu, pirinç unu-koenzim Q10-zeytin yağı ve pirinç unu-koenzim Q10-zeytin yağı-sarı çam özütü olmak üzere üç grupta tamamlanan çalışmalar dikkate alındığında üçüncü grupta mikrobiyal artış gözlemlenmemiş olunup pH, viskozite, yağ ve protein oranları bakımından da kozmetik standartlarına hemen hemen uygun sonuçlar elde edilmiştir.

ANAHTAR KELİMELER: Koenzim Q10, zeytinyağı, *Sarı çam,* vücut kremi, pirinç unu

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LIST OF ABBREVIATIONS ANDSYMBOLS

App **:** Appendix

UV **:** Ultraviolet

˚C **:** Degree Celsius

CoQ10 **:** Coenzyme Q10

cm : Centimeter

G : Gram

SLN **:** Solid Lipid Nanoparticles

NLC **:** Nanostructured Lipid Carriers

ETC **:** Electron Transport Chain

AIDS **:** Acquired Immunodeficiency Syndrome

PR **:** Pathogenesis Related

EO **:**Essential Oils

MD **:** Mediterranean Diet

P.sylvestris : *Pinus sylvestris*

EVOO **:**Extra Virgin OliveOil

Log : Logarithm

mL : Milliliter

TMAB : Total Mesophilic Aerobic Bacteria

mm : Millimeter

CO **:**Control(contains only rice flour)

R+CoQ$_{10}$+O **:**Rice flour-CoenzymeQ$_{10}$ - Olive oil

R+CoQ$_{10}$+O+P **:** Rice flour- CoenzymeQ$_{10}$ - Olive oil-*Pinus sylvestris* extract

TRT **:** Treatment

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1. INTRODUCTION

1.1. Cosmetics

The "cosmetic" first mentioned in the ancient Roman public baths which were used for personal hygiene [Johnston,1973]. Some slaves called cosmetae used the cosmetic product for hair dye, facial makeup, manicure and apply ointments to the skin. In Greek ‘cosmetikos’ means to arrange and that was derived from cosmetae. The name reached into our modern period, the word “cosmetic” is “to beautify the body”. Cosmetics are products used to enrich or change the appearance or body odor. Many cosmetics are applied to hair and face. The modern use of cosmetics was mostly improved by a few practical yet powerful innovations such as the toothpaste box, the shaver, the compact makeup.[Vail, 1947]. Consumer demand for more effective cosmetic products has led to an increase in fundamental scientific research and product improvement in the cosmetics industry [JUNCAN and LUNG, 2016].

The cosmetic products are originated from mixed chemical substances or being formed by natural products such as olive oil. The use of inorganic ingredients in cosmetics can cause many diseases. For example, in the Middle Ages, before the lead was discovered to be poisonous, women used the lead in the face cream to have a pale skin and therefore this led to the death of some people [Nardello-Rataj and Bonté, 2008; Alves et al., 2009]. Recently, both the cosmetics industry and research are moving forward natural cosmetic and organic product manufacture, in order to obtain more effective and skin safe products according to the demands of an increasing quantity of consumers, therefore the preference of the consumer move towards natural products.A cosmetic product can be acceptable natural only when it contains natural ingredients. Natural raw materials are those substances extracted from plants, animals, or minerals by physical methods, without any chemical changes occur [Hanno et al., 2015].

A cosmetic product can be designed for its application on different parts of the body including the epiderma of human body, nails, hair and external genital organs, teeth and oral mucosa. Cosmetic studies are based on to clean, smell, change

the appearance in make-up concept, as well as to correct and maintain body odors. Cosmetic is semi-medicinal; cosmetic with medicine is a new concept.

1.1.1 Cosmetology

The science of cosmetics is called as cosmetology.

In cosmetics the products are classified in two ways:

a- Applicability of the product to different parts of the skin

b- According to its ingredients.

Cosmetic creams are in the category of products applied to various parts of the body (Wang et al.,2008).

The creams are defined as the skin care products that must be used to maintain the existing balance of the skin and prevent damage caused by external effects. Studies show that 90% of women complain that their skin is dry. Although healthy skin is supple and soft, the dry skin is hard and rough. From the cosmetic point of view, the significant cause of dry skin is the reduction of epidermal lipids. These are structures that prevent the evaporation of water from the skin and allow water to be kept in the skin.

1.1.1.1. Edible cosmetics

Edible cosmetics is a branch of cosmetics and it gains interest in the recent years, offering a natural basis. Up to now, limited studies has been carried out to verify the widespread use of nutrients involved in the formula of oral supplements and cosmetics to cope with skin problems. Even though the oral and topical applications of this kind is still under debate, the priority is given to ascorbic acid and alpha tocopherol, which are the core parts of vitamin C and E respectively, when an edible combination is considered for design, aiming to get rid of the wrinkles, therefore preventing the oxidation of skin. The edible concept offers an almost non-toxic combination, therefore the consumer attraction is significantly increased (Watts

et al. 2002). For example, if coenzyme Q10 is considered, the risk analysis indicated an amount of less than 1.2 g/day/person is suitable after various clinical trials, that's why it is regarded as safe(Hidaka et al. 2008). The presence of coenzyme Q10 was easily detected by ultra-performance liquid chromatography with electrospray ionization-mass spectrometry as an anti-aging ingredient in edible cosmetics(Lee et al., 2014). In addition, scots pine can able to store various sugars including raffinose, mellibiose, sucrose, glucose, galactose, fructose, arabinose and other pentoses. Throughout the year, arabinose, fructose, glucose, galactose and sucrose were abundant, but on the contrary, raffinose and melibiose were found to disappear during summer. Moreover, the carbohydrate polymer starch and the fatty acids such as diacylglycerols and triacylglycerols are present(Fischer and Höll, 1991). All these food-related characteristics allow these materials mentioned in Section 1.1.1.1. to be used in an edible cream formulation.

1.2. *Oryza sativa*

Oryza sativa, the rice, is a genus having a life period of more than two years' in the grass family (Poaceae) that first emerged in southern China, Thailand and India and currently is being grown in tropical rainy, half-tropical, and mild temperate zones in the world. It is a rare crop characterized by its quite pale color, smooth taste, low sodium content, and easily digestible carbohydrate content and lowered allergic potential. In addition, the rice flour is an appealing foodstuff to be used for producing non-gluten food (Gujral et al., 2003). Rice can exert antioxidant capacity. *Oryza sativa* has hundreds of varieties with distinct cereal color, dimensions, and form, environmental tolerances, and seasonality. The types of *Oryzasativa* are usually classified as valley rice, upland rice, spring rice, and summer rice. *Oryza sativa* is mainly grown in areas that are inundated with water for part of the growing period. It also helps to reduce competition in the use of rainwater or flood bed systems in the majority of cultivated rice. Some rice diversity may be grown out of flooding, but they contain only 4% of rice planted throughout the world. *Oryza sativa* and wheat (Triticum species) are one of the two very important grain product in the world for the nutritional needs of human (Corn, *Zea mays*, is produced in abundance, but a small part of it is used for livestock feed and production of ethanol to biofuel).

Rice is grown on an approximated 3% of the world's agricultural terrain. It serves as a basic resource of calories for over half of the world's population.

Oryza sativa has also been crucial as a model system in botany. Also, it is the first plant species for which the genome has been entirely studied by mapping. *Oryza* weed can be grown in a pinch (cluster) or spread out from rhizomes. They usually have vertical roots up to two meters or taller, with long, straight sheet blades. The flowers grow on broad, separated clump. The oblong spikelets, which each include one flower that are sparse during the root rather than forming intensive clumps.

The edible inner part, called rice paddy, is covered by a hull or husk discarded at milling (Abbas et al,2011).

Rice including a high para aminobenzoic acid concentration (PABA). PABA is a very good sunscreen. When orally ingested PABA is found to increase ascorbic acid content in human body. It is believed that, rice has two important sun protection components, ferulic acid and allantoin. Ferulic acid shows antioxidative characteristic. Incredibly, different studies indicated that when ferulic acid is added to the ascorbic acid and alpha-tocopherol, the sun protection capacity was increased two-folds. The benefits of ferulic acid can be expressed by two important consequences: an enhanced stability was gained to a solution of C and E vitamins and a powerful and double efficient photoprotective synergy is observed. The other sun protective agent of rice, allantoin is known to be an anti-inflammatory substance is applied to sooth sunburn and help promote the repair of skin. Tyrosinase is a very important enzyme and it can able to increase the activity of melanin, a pigment which gives the skin color. Rice flour, which can find its treatment way as a paste, can able to inhibit the tyrosinase activity as well as bearing the rare characteristic of skin whitening and it has been using in this form in Asia-Pacific countries(Pillaiyar et al., 2017).

An oily skin can be easily converted to a very matte type, by brushing the skin gently with rice powder, especially this technique is applied to face by young women and men. For this purpose, rice is kept in a bowl of water, and subsequently the rice is discarded, this water is used to form skin tonic and it has a capacity to get rid of the oil in order to allow a matte skin.(https://whiterskin.info) In make-up technologies, the minerals abundant in rice help absorb the oil at a great extent, in

addition fading of the make-up is prevented. Another impact of rice powder on cosmetics is reported to cover the blemishes and flaws, therefore a smoothy and fresh appearance of skin is provided. The healthy breathing activity is another benefit reported for the rice powder via keeping the pores in open position. It was also reported that many anti-aging skin and wrinkle creams, is made up of ceramides, providing a young and fresh look (Kanlayavattanakul et al, 2016; Abbas et al, 2011).

1.3 Coenzyme Q10

Coenzyme Q10 was initially detached from beef mitochondria by Prof. Fredrick L. Crane in 1957, Dr. Karl Folkers was reported the chemical structure of Coenzyme Q10 in 1958. CoQ10 is a lipid soluble vitamin similar substance. CoQ10, known as ubiquinone and/or ubidecarenone, is a coenzyme that is mainly present in most of the multicellular eukaryotes. Its structure is composed of a 1,4-benzoquinone, where the letter ‘q’ indicates the chemical structure of quinone and 10 represents the number of isoprenyl sub-groups.

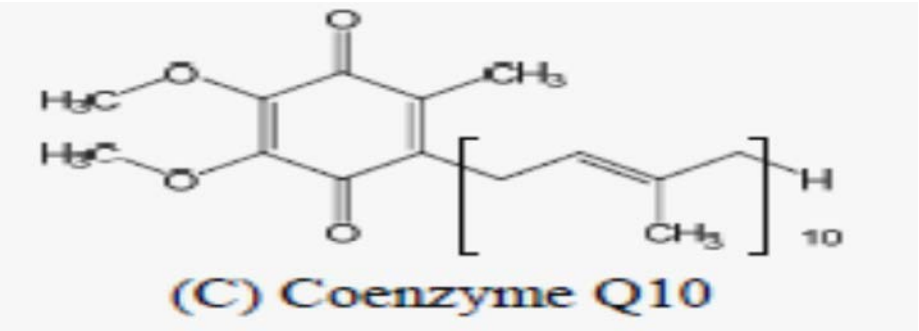

Figure 1.The structure of Coenzyme Q10 (Adapted from Huang et al. 2009).

It is a vitamin soluble in fats present especially in heart, liver, and brain [Banu et al.,2016].CoQ10 structure is 2,3-dimethoxy-5-methyl, 6-polyisoprene parabenzoquinone. It has a polyisoprene chain including 10 isoprene units in human [Crane, 2001]. CoQ10 is an antioxidant functioning as a free radical scavenger, investigated by Lars Emster. Antioxidants such as Coenzyme Q10 can eliminate free radicals by neutralization. It can decrease or even help prevent damaging cell membranes, interfere with DNA, and cell death. (Kapoor and Kapoor, 2013). CoQ10,

working as an antioxidant in the skin, is found to be 10 times more effective on the outer layer of cells than on the underlying of cells. A reduction in the efficacy of antioxidant systems leads to skin aging. Besides, CoQ10 is commonly used as an anti-aging agent in the cosmetic industry. Lipid nanoparticles, solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC), are novel carrier systems for CoQ10 that are derived from oil/water emulsions (Garrido-Maraver et al., 2014)

CoQ10 is manufactured in order to maintain the sustainable functions of organs and reported to be an active chemical reagent in the body involved in enzyme function. CoQ10 is so effective in the production of energy for the cells and has an antioxidative activity[Kapoor and Kapoor, 2013]. It is in water-insoluble, crystallized powder form. Absorption process of CoQ10 is same as a fat and the uptake mechanism resembles that of vitamin E, another lipophilic substance.

CoQ10 absorption process is similar to the lipid uptake mechanism such as vitamin E, other liposoluble nutrients. In human beings, the process is made up of the pancreatic enzymes secretion and gall into the small intestine that helps emulsification and micelle formation, necessary for the absorption of the lipid-soluble nutrient.

CoQ10 has a key role in the electron transport chain (ETC). It functions as an electron transport agent from complex I and complex II enzymes to complex III enzyme which is crucial in the process, since no another substance may behave like that. Besides, coenzyme Q10 functions in all cells of the body to produce energy and it acts as a fat antioxidant, regulating membrane fluidity, recycling radical forms of ascorbic acid and alpha tocopherol, and preserves the phospholipid nature of the membranes against peroxidation. Because of its antioxidative property, a high degree of hydrophobicity and universal presence in biological systems offers a crucial function for ubiquinone and ubiquinol in the defensive mechanism of the cell against oxidation related problems. Thus, it has a key role in cellular mechanism [Banu et al.,2016].

The bioactive anti-oxidant structure, ubiquinol, is thought to be very important in the elder, anyone who may experience higher levels of oxidative stress or individuals who appear no response to regular CoQ10 intake.

CoQ10 is a naturally occurring molecule which can be bought as a dietary supplement and found to be helpful in medical cases due to its vital role in the human body, such as it helps to convert food into energy. If CoQ10 is deficient in human body, 5 major phenotypes are claimed to cover its autosomal recessive state, including: (a) the triple abundance of recurrent myoglobinuria, brain problems and irregular muscle fibre formation in some DNAdisordersleadingto encephalomyopathy. (b) harsh multi-systemic disorders in infants, (c) the ataxia in cerebellum (d) growth problems, ataxia and loss of ability to hear in Leigh syndrome and (e) isolated myopathy. CoQ10 acts a powerful antioxidant and it helps in treating the variety of disorders. The function of antioxidants in our body are to cope with harmful areas in the skin known as free radicals destroying the cell layer, interfere with DNA, and even lead to cell death.

According to some researchers, these free radicals provide to the aging period, as well as a numeral of health issues such as cancer and heart disease. So, antioxidants like coenzyme q10 may deactivate free radicals and can decrease and even support prevent some of the harm they reason. Scientists imagine that CoQ10 can help heart-related problems, as it may increase energy synthesis in cells, stops blood clotting and plays an antioxidative role.

Food intake enhances biliary excretion of gall acids in the body and helps the CoQ10 absorption. Exogenous CoQ10 is easily absorbed from the small intestine if it follows a meal intake. Peak plasma levels are achieved in 2 - 6 hours after oral administration[Kapoor and Kapoor, 1957].

Many illnesses, related to low levels of CoQ10, can be treated by further CoQ10 intake, with the inclusion of cardiovascular problems. Also, Parkinson’s illness, muscular dystrophy, breast and other cancers, *diabetes mellitus*, male infertility, acquired immunodeficiency syndrome (AIDS), asthma, thyroid disorders, and periodontal illness, since such as the heart, immune system, and gingival problems reporting nausea, anorexia, or body eruptions with insufficient Coenzyme Q10 levels can benefit from the further intake of CoQ10.

Moreover, the levels of CoQ10 in skin decrease with age and ultraviolet irradiation, that’s why it is mostly used in anti-aging skin care cosmetics. The epidermis, the outermost layer of skin, is exposed directly to ultraviolet rays and

ultraviolet radiation is known to decrease antioxidants such as CoQ10 in the skin[22].

Therefore the epidermis is a tissue that presumably benefits most from topically applied CoQ10.

CoQ10 may be produced artificially and is regarded as either a food supplement or a medicine at the same time. Coenzyme Q10 was reported to reach the third rank of sales amount in United States after Omega-3 fatty acids and multivitamins[Kapoor and Kapoor, 2013].

1.4. *Pinus sylvestris* (*Scots pine*)

Plant extracts and essential oils (EO) are considered to be one of the potential resources of substances with anticancer, antimicrobial and antioxidant properties, and source of free radical scavenging agents. Most of the Pinus species are trees or shrubs with specific morphological characteristics of leaves (needles) rich in terpene aromatic essential oil. Pine needle essential oils are mainly used in folk medicine for the treatment of respiratory diseases. Up to now, there has been an increased interest in the investigation of its chemical structure as well as biological activity of the essential oils isolated from various pine species.

Although numerous researchers have reported the antimicrobial and antifungal action of plant extracts, the essential oils (EO) and plant secondary metabolites have limited applications in folk medicine, perfumery industry, food flavoring, and conservation but just in recent years, they have been identified for their potential antimicrobial role.[Czerwińska and Szparaga, 2015] .

Figure 2: The structure of *Pinus sylvestris - L.*

Pinus sylvestris synthesize few, mostly homologous, disulfide-rich proteins (Sp-AMPs) in reply to fungal pathogenic factor. Some researchers reported the expression, function, and structure of these proteins. In addition to this, the pristine protein displays antifungal effect on *Heterobasidion annosum* via morphological differences in spores and hyphae.

The research on the chemical structure of the carbohydrates of *Pinus sylvestris* brings to light that it is linked to solvable and unsolvable beta-(1,3)-glucans, the main ingredient of the fungal cell barrier, with high affinity.

Homology research propose a Greek-key-beta-barrel layer having a protection patch on the surface that can establish at least four sugar units. It has been found that these proteins symbolize a recent class of antimicrobial proteins that may be classified as pathogenesis-related (PR) protein family 18.(Sooriyaarachchi et al., 2011).

The essential oil obtained from the leaves is also used in asthma, bronchitis and other respiratory tract infections as well as in digestive disorders such as wind (Chevallier,1996). A volatile oil obtained from seed has diuretic and respiratory stimulatory properties (Chevallier, 1996). Seeds are used in the treatment of bronchitis, tuberculosis and bladder infections (Chevallier, 1996). The plant is used in Bach flower medicines - key words for prescribing 'self-condemnation', 'guilt

feelings' and 'hopelessness' (Chancellor, 1985). Essential oil is used in aromatherapy. His keyword is 'Refreshing' (Westwood, 1993).

1.5 Olive oil

Olive oil, the new member of the nutrition pyramid that reflects the Mediterranean Diet (MD) contains a stable lifestyle, healthy cooking methods, conventional, regional and non-harmful products for environmental, convivial, bodily activity with an enough quantity of relaxation, as well as caloric restriction and nutrient frugality.

It is also known as the main Mediterranean Diet source, extra virgin olive oil (EVOO). EVOO is thought to be an important feature for the healthy features of Mediterranean Diet because of the reason that it contains fatty acid, vitamin, and polyphenol compounds. Moreover, it has extra nutraceutical characteristics, including antioxidant, anti-inflammatory, anti-cancer, antimicrobial, antiviral and hypoglycemic properties, as well as preserving heart and brain, and helping throughout health during pregnancy and lactation. The vitamin E found in olive oil has antioxidant properties and delays cell aging. Since it carries oxygen to the heart and other organs, it prevents fatigue, provides resistance to capillary wall, protects erythrocytes from the burning effect of poisons, prevents blood clotting, can be used to treat the accumulation of Ca^{+} and wall disease and muscle distortion in sterility.

The major phenolic ingredients liable for the nutraceutical properties of EVOO are hydroxytyrosol, tyrosol, and oleuropein. The common oil manufacture and extraction technologies, like extraction at poor oxidative stress, establish the amount of final polyphenol including in virgin olive oil. Limited knowledge on the epigenetic effects of olive polyphenols is currently present, although the epigenetic impacts of many other plant polyphenols have been well reported. In this conditions, it has been found that, if mothers are fed with enough quantity of olive oil throughout pregnancy, their children may be effective to a lower risk of wheezing in the primary period of their lives. Also, EVOO may have a positive effect on many inflammatory diseases, even in the early period of life because of the reason that it has oleocanthal, a natural anti-inflammatory substance (Trapani et al., 2017).

A study pointed out that honey, olive oil and beeswax mixture is found to be useful in the management of dermatitis and *Psoriasis vulgaris* (Al-Waili, 2003). An original product in the armamentarium of the preparations for burns and wounds treatment was developed by Zbuchea and coworkers in 2016 and it was done only on the basis of medicinal plants and natural components. The ointment formularization contains olive oil extract from a mixture of nine medicinal plants with an efficiency on wound improvements (*Calendula officinalis* L., *Matricaria chamomilla L., Symphytum officinale L., Hypericumperforatum L., Achillea millefolium L., Arctium lappa L., Plantago major L., Althaea officinalis L., Quercus robur L.*), sea buckthorn oil, lavender essential oil and as thickening factors, coconut oil, beeswax and conifer resin. The LC-MS analyses of the ethanolic extracts from the plant mixture, together with its ethanolic re-extracts, as well as its ointment have evidenced high levels of polyphenols like caffeic, chlorogenic, gallic and ferulic acids, as well as quercetin and rutin, all of which being known compounds showed good wound healing activity. The neutral red assay has shown no cytotoxic effect on fibroblast NCTC cell line exposed to herbal extracts. Finally, the wound healing action of the submitted ointment has been clinically confirmed and highlighted by some case reports (Zbuchea et al., 2016).

1.6 Dermatological problems

Coenzyme Q10 may display a duty in forming the young look of skin. Coenzyme Q10 content gets lower as human beings get older. The lowered CoQ10 leads to stop the production of collagen and elastin. Collagen is so important that as since it leads to the firmness of the skin, on the other hand elastin leads to a flexible skin. The lowered collagen and elastin levels lead to a skin sag.

Coenzyme Q10 also helps the body by acting as a strong antioxidant. Coenzyme Q10 helps to neutralize the damage free radicals that are one of the major reasons of aging. So, the age-related decline in Coenzyme Q10 means that the skin is more prone to damage by free radicals.

Using topical anti-aging creams that contain CoQ10 can effectively help fight the signs of aging. The CoQ10 can penetrate deeply to provide antioxidants and help create collagen and elastin.

1.7 Foodborne Pathogens in Cream

1.7.1 *Escherichia coli*

*Escherichia coli (E. coli)*is first described by Theodor Escherich, in 1885, that as *Bacterium coli commune*, isolated from the feces of newborns. *E. coli* is apathogenicgram-negative bacteria which has rodshaped, facultatively anaerobic bacterium.Its growth occurs over a wide range of temperature from 15-45°C on EMB agar. *E.coli*is 1-3 x 0.4-0.7 µm in size and 0.6 to 0.7 µm in volume and that is arranged singly or in pairs. *E. coli* is motile due to peritrichous flagella and some strains are non-motile and that may be fimbriated. The antigenicstructure of *E. coli*is composed of a heat stable lipopolysaccharide (LPS) is the major cell wall antigen of *E.coli*. *E. coli* possess 4 antigens; flagellar, somatic, capsular, and fimbrial antigens.

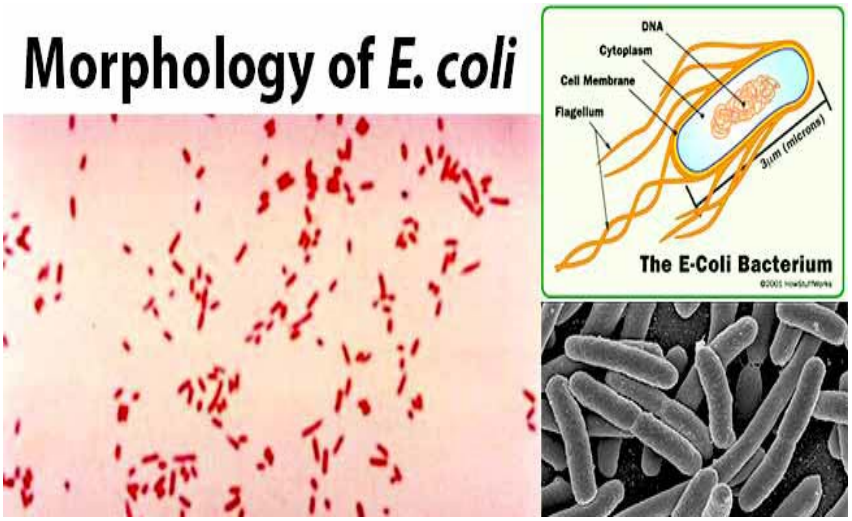

Figure 3: The morphology of *E.coli* gram negative bacteria (https://microbenotes.com).

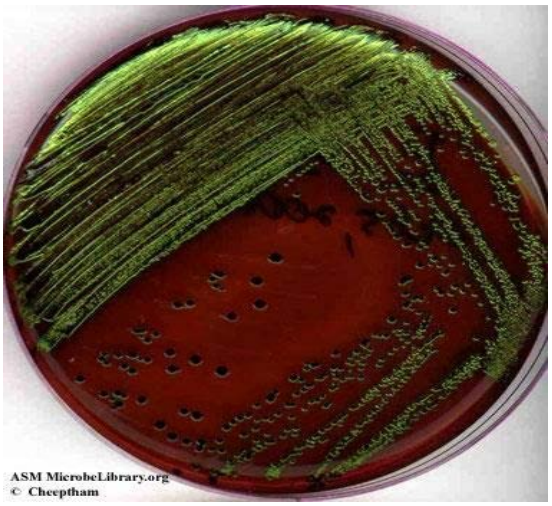

Figure 4 : *Escherichia coli* colonies in Eosin Methylene Blue Agar (EMB)

E.coli is a bacterium that can cause severe foodborne disease in human gastrointestinal tract and also causes illness such as abdominal cramps, vomiting, bloody diarrhea, acute kidney failure in children, adult kidney failure, fever, bleeding, confusion. Primary sources of *E. coli*outbreaks are raw or undercooked ground meat products, fresh vegetables or fruit; unpasteurized milk, unpasteurized fruit juices and yogurt and cheese made from raw milk; swimming pools,lakes and ponds.

1.7.2 *Salmonella*

Salmonella is a group of rod-shaped, gram-negative, non-spore forming, facultatively anaerobic bacteria in the family Enterobacteriaceae that has a diameter around 0.7 to 1.5 µm, length from 2 to 5 µm, and flagella that grade in all direction. Their principal habitat is the intestinal tract of humans and other animals so that is closely related to *E.coli* bacteria.

Primary sources of *Salmonella* outbreaks are the ingestion of contaminated food or water which can result in gastroenteritis, typhoid fever; paratyphoid fever is caused by *S. paratyphi, S. schottmuelleri,* and *S. hirschfeldii,* which are considered variants of *S. enteritidis.*

Figure 5: *Salmonella* colonies in XLD Agar

Factors affecting the growth of Salmonella spp. are average of temperature (ºC) (min.) 5.2, 35 – 43(max.), 46.2, pH (min.) 3.8, 7 – 7.5(max.), 9.5, water activity (aw) (min.) 0.94, 0.99 >0.99(max.), most serotypes fail to grow at < 7ºC.

1.7.3 *Listeria monocytogenes*

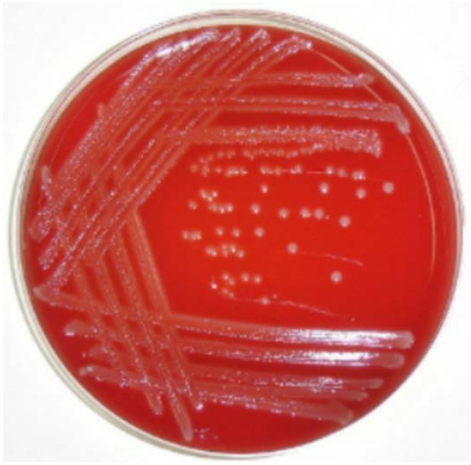

Figure 6: *Listeria monocytogenes*colonies on Blood Agar

*Listeria monocytogenes*are a group of gram-positive, rod-shaped, facultative anaerobic bacterium that food-borne pathogens cause illness in humans and animals because of reasons that are able to grow and multiply at refrigerated temperatures, it gets an important matter in ready-to-eat food.

Listeriosis cases are often occurred by resulting from the foods contaminated with *Listeria monocytogenes*(Yavuz and Mihriban, 2010). *Listeria monocytogenes*are usually found in meat, raw milk, soft cheese, vegetables, pasteurised dairy product and fish. *Listeria g*enus have six subgroups such as *L.monocytogenes, L.ivanovii, L.seeligeri, L.innocua, L.welshimeri,* and *L.grayi.* Out of these only *L.monocytogenes* have been authentically reported by many researchers as human and animal pathogen (Vazquez-Boland et al., 2001).

2. THE AIM AND SCOPE OF THE STUDY

In this study we aim to develop a skin cream(ointment) based on coenzyme Q and milled rice as well as fortified with olive oil and fir (*Pinus sylvestris)* extract and investigated its certain chemical, physical and microbiological properties. The shelf life of the ointment was checked. The literature survey indicated that, the study was original since no any scientist has been working on this title. Up to now, no any toxicity and dermatological tests of this cream were carried out by us, since the formulation and compositional studies took a long time.

3. MATERIALS AND METHODS

3.1. MATERIALS

3.1.1. Rice flour

Rice flour (Piyale) was purchased from a local market in Bolu, TURKEY.

3.1.2.Coenzyme Q 10

Coenzyme Q10 (Sigma-Aldrich Chemical Co.)was used in this study.

3.1.3. *Pinus sylvestris* extract

The extract was obtained from the leaves of fir (*Pinus sylvestris*) trees, located at the wood in Abant Izzet BAYSAL University, Bolu, TURKEY in September 2017. The leaves of plants were weed out and washed twice, after drying at room temperature.

3.1.4. Olive oil

Olive oil (Lio) was purchased from a local market in Bolu, TURKEY.

3.2. METHOD

3.2.1 The treatment groups

Three different cream formulation was made in the study as follows: Control(contains only rice flour) represented as CO, Rice flour-CoenzymeQ_{10} - Olive oil (R+CoQ_{10}+O) combination and Rice flour- CoenzymeQ_{10} - Olive oil-*Pinus sylvestris* extract (R+CoQ_{10}+O+P) combination.

3.2.1.1. The recipe of the control group

25 g of rice flour and 75 mL of distilled water were continuously stirred and treated with mild heat in a beaker for a period of approximately 7-10 minutes. Then the cream was transferred to a deep bowl and that was allowed to cool at room temperature.

3.2.1.2. The recipe of R+CoQ$_{10}$+O

25 g of rice flour, 73 mL of distilled water, 0.5 mg coenzyme Q10 and 2 mL olive oil were thoroughly mixed in a sterile beaker, after that were continuously stirred and treated with mild heat in a beaker for a period of approximately 7-10 minutes. Then the cream was transferred to a deep bowl and that was allowed to cool at room temperature.

3.2.1.3.The recipe of the R+CoQ$_{10}$+O+*Pinus sylvestris* extracts

25 g of rice flour, 70 mL of distilled water, 0.5 mg of coenzyme Q10, 2 mL of olive oil and 2 mL of *Pinus sylvestris* extract were mixed in sterile conditions and then continuously stirred and treated with mild heat in a beaker approximately 7-10 minutes. Then the cream was transferred to a deep bowl and that was allowed to cool at room temperature.

3.2.2. Preparation of *Pinus sylvestris* extracts

The leaves of *Pinus sylvestris* were grinded by a sterile mortar and pestle, before the extracts were obtained. Subsequently, this extract were transferred to the test tubes, and following the centrifugation, the plasma part of this sample was transferred to another test tube and microfiltered via a sterile cellulose membrane filter (0.45 micrometer) (Sartorius Stedim, Minisart 16555-K).

3.2.3. Test microorganisms

The antibacterial activity of *Pinus sylvestris* extracts were tested in *vitro* against common gram positive and gram negative foodborne pathogens, such as *Listeria monocytogenes*, *Staphylococcus aureus* and Salmonella spp. which were provided as a generous gift of Abant Izzet Baysal University, Department of Food Engineering. *Escherichia coli* ATCC 12601 was provided as a reference strain from Abant Izzet Baysal University, Faculty of Medicine, Department of Microbiology.

3.2.4. The microbiological analysis

One gram sample from the cream mixture was weighed and mixed with 9 mL of sterile distilled water, afterwards 1 mL sample was taken from this mixture according to the dilution technique and spreaded onto the appropriate petri plates by

spread plate technique. Salmonella spp. counts were carried out on XLD; *E.coli* on EMB(MERCK, Darmstadt, Germany); the total mesophilic aerobic bacteria (TMAB) on nutrient (MERCK, Darmstadt, Germany; Listeria spp. on blood (MERCK, Darmstadt, Germany); *S.aureus* on Staphylococcus medium 110 (Difco^{TM}) agars by incubating the plates at 37°C for 24 hours. Yeast counts were performed by using yeast extract agar(LAB018) at 30°C for 24 hours. The analysis was repeated on the 0^{th}, 30^{th}, 60^{th}, 90^{th} and 120^{th} days in duplicates.

3.2.5. Antimicrobial screening

The antibacterial activity of *Pinus sylvestris* extract was examined by Kirby-Bauer disc diffusion method in Petri dishes. For this purpose, Whatman filter papers having a diameter of 6 mm, were sterilized at 100° C for a period of 24 hours. The extract was spreaded on the appropriate agars containing previously laid bacteria reported in Section 3.2.3. adjusted to 0,5 McFarland units. 5µL, 10µL, 20µL of this extract were sequentially applied onto sterile paper discs and one disc was left as control. The activity of the *Pinus sylvestris* extract was detected by the diameters of the inhibition zones in millimeters after 24- 48 hours of incubation at 37°C in duplicates.

3.2.7. DPPH

For the assessment of antioxidant activity DPPH was analyzed by the following method: 1 g of each sample was homogenized by 2 mL 50% methyl alcohol at room temperature. After an hour, the mixture was centrifuged (14.000 rpm for 17 minutes), and the supernatant was collected. The pellet was then homogenized in 2 mL of 70% acetone; following one hour, it was re-centrifuged (14.000 rpm for 17 minutes).

The supernatants obtained from the first and last centrifugations were pooled, and the volume was adjusted to 5mL with distilled water (Rufino et al., 2007).

The antioxidant activity was determined based on the extinction of absorption by the 2.2-diphenyl-1-picril hydrazyl (DPPH) radical (Rufino et al., 2007). Approximately0.5 mL of 60 µM DPPH was added to each extract. The control sample was consisted of 0.5 mL of methanol with 0.5 mL of 60 µM DPPH. After 30 minutes, absorbance was measured ina spectrophotometer(Hitachi U-1900) at 517

nm, and the results were expressed as percentage of free radical scavenging (% SRL) according to Equation 1:

$$\% SRL = (Ac - Am) \times 100/Ac$$

Ac = control sample absorbance
Am = sampleabsorbance

3.2.6. The physicochemical analysis

3.2.6.1The pH

The pH of the skin cream samples was measured by using a digital pH meter (WTW Inolab pH 720, Germany).

3.2.6.2 Total Soluble Solids (^{o}Brix)

A portable refractometer (Miiler, RHB-10/ATC, Germany) was used for the detection of total soluble solid content of the skin cream. The measurements were performed at 25ºC ± 0.5ºC and the results were expressed as ^{o}Brix.

3.2.6.3 Viscosity

The viscosity of the samples was measured by a viscometer (AND Vibro Viscometer, SV-10, Japan).

3.2.6.4 Color Determination

The color of the skin cream was determined by a colorimeter according to CIE $L^*A^*B^*$ system (Konica Minolta, CR-400, Japan). The color values were expressed as L^* (whiteness or brightness/darkness), a^* (redness/greenness), and b^* (yellowness/blueness). All measurements were performed in triplicates.

3.2.6.5 Determination of Total Sugar Content

The total sugar content of the skin cream sample was determined by using dinitrosalicylic acid (DNS) (Sigma, USA) method (Miller, 1959). The standard solutions which contained 1, 2, 3 and 4% of D-fructose (Merck, Germany) were prepared. 1 mL from each of these fructose solutions were treated with 3 mL DNS (Sigma, USA). Subsequently 1 g of skin cream was added to this mixture in test tubes and then these samples were incubated in boiling water bath for 30 minutes. Following the cooling of the test tubes, 1 mL aliquot from the incubated sample was completed to 40 mL with distilled water and the absorbance was determined by using a spectrophotometer at O.D.540 nm (Shimadzu, PharmaSpec UV-1700, UV-Visible Spectrophotometer, Japan). A linear standard curve for fructose was constructed using the absolute amounts of fructose plotted against spectrophotometric values to determine the amount released from each sample.

3.2.6.6 Preparation of DNS Solution

10.6 g of DNS (Sigma, USA) (2-hydroxy-3,5 dinitro benzoic acid), 19.8 g of sodium hydroxide, 7.6 g of phenol, 8.3 g of sodium metabisulphide, 306 g of sodium potassium tartarate were dissolved in 1416 g distilled water.

3.2.6.7 Fat Determination by Soxhlet Method

The fat content of the skin cream was determined by Soxhlet method (Soxhlet, 1879). The samples were placed in a porous cellulose thimble. Petroleum ether was used as an organic solvent in this experiment. The thimble was placed in an extraction chamber, which is suspended above in a flask, previously dried at 105ºC, weighed and coded as W1, containing the solvent and below a condenser. After heating the flask, the solvent evaporates and moves up into the condenser where it is converted into a liquid that trickles into the extraction chamber containing the sample. The extraction chamber is designed so that when the solvent surrounding the sample exceeds a certain level it overflows and trickles back down into the boiling flask. At the end of the extraction process, which lasts six hours, the flask

containing the solvent and lipid was removed. The solvent in the flask was then evaporated and the mass of the remaining lipid was measured. The flasks were placed in an oven at 105ºC and dried until a constant weight was reached (1-2 hours). Then the flasks were cooled in a desiccator and the flask and its content were weighed. It was evaluated as W2. The percentage of lipid in the initial sample was calculated according to the equation (Eq.1):

Eq.1: % Crude fat = $\frac{W2-W1}{m}$ x100

Weight of empty flask (g) = W1

Weight of flask and extracted fat (g) = W2

Weight of sample = m

3.2.6.8 Determination of Total Nitrogen (Kjeldahl method)

The Kjeldahl method was used to determine nitrogen content (Magomya et al., 2014). The skin cream samples; weighed in a Kjeldahl digestion flask by boiling with 25 mL of concentrated H_2SO_4 and a Kjeldahl digestion tablet (catalyst) was stirred until the mixture was clear. After digesting the mixture by observing light green color, for a period of 30 minutes, 25 mL of 4% boric acid solution was put into a 250 mL volumetric flask. The digest was then filtered into a 250 mL volumetric flask and the solution was made up to mark with distilled water and connected for distillation. Ammonia was steam distilled from the digest to which it had been added to 50 mL of 40% sodium hydroxide solution and released from the acid digest by the addition of sodium hydroxide. 150 mL of the distillate was collected in a conical flask. Distillate was titrated with 0.1 N HCl solution. The amount of hydrochloric acid used was proportional to the amount of nitrogen originally present in the test portion, calculated according to Eq.2.

% Nitrogen was calculated as follows:

$$\text{Eq. 2: \% N} = \frac{(V_2 - V_1)\text{mL x Normality x 0.014 g Nitrogen x 100}}{\text{Weight of sample (g)}}$$

V_1 = mL of 0.1 N HCl consumption for the blind

V_2 = mL of 0.1 N HCl consumption for the sample

Normality = normality of HCl solution

% Protein= % N x 6.25

3.2.6.9. The Statistical Evaluation

The data were shown as means and analyzed by the SPSS program. One – way Anova Scheffe test and Duncan's test was used to detect the significance between treatment groups and different concentration and process times of each treatment group. Kruskal-Wallis test was used in the physicochemical analysis of skin cream to detect the significance between treatment groups. Pearson correlations was used to correlate related chemical parameters.

The significant level was set to $p<0.05$ at the beginning of the study.

4.RESULTS AND DISCUSSIONS

4.1. The Microbial Assay

In this experiment, three different cream formulation was made as follows; Control(contains only rice flour and sterile distilled water), represented as CO; Rice flour-CoenzymeQ10 - Olive oil (R+CoQ10+O) combination, Rice flour-CoenzymeQ10 - Olive oil- *Pinus sylvestris* extract (R+CoQ10+O+P) combination. The microbial characteristics of these samples were evaluated by direct enumeration using spread plate technique and it was found that the rice flour-CoenzymeQ10 - Olive oil-*Pinus sylvestris* extract (R+CoQ10+O+P) combination is the most effective treatment on the total mesophilic aerobic bacteria(TMAB), especially when the results of the nineteeth day was considered. No microbial growth was determined when the results of 0-90th day interval was evaluated in this combination (Figure 7) (Appendix A1, A2 and A3).

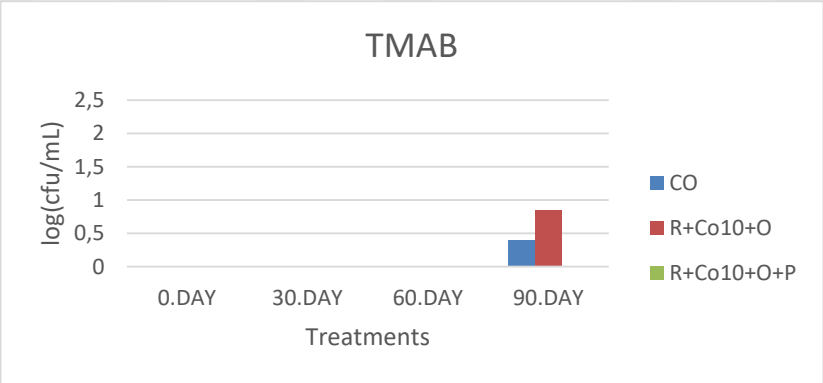

Figure 7. The effect of CO, R+CoQ10+O, and R+CoQ10+O+P combinations on the total mesophilic aerobic bacteria (TMAB) levels of natural skin cream, with respect to control.

When the total mesophilic aerobic bacteria (TMAB) counts were evaluated, it was observed that approximately 0,397 log cycle was obtained in control group (CO) while in the Rice flour-CoenzymeQ10 - Olive oil (R+CoQ10+O) combination it was found to be 0,845, This increase may be due to the lipophilic character of microorganisms, as since the lipids in olive oil may promote the growth of these

microorganisms at the end of 90 days(Shine et al., 1993). No TMAB was observed in Rice flour- CoenzymeQ10 - Olive oil- *Pinus sylvestris* extract (R+CoQ10+O+P) combination, even for a period of 90 days' storage at 4° C during three months. This may be due to the antibacterial activity of *Pinus sylvestris.*

In a study, conducted by Tamendjari et al.(2018), various characteristics of oils obtained via extraction from tree oleaster producing fruits and a variety (var. Chemlal) subjected to cultivation of *Olea europaea L. var. oleaster* was investigated including the quality indices, phenolics and tocopherol, antioxidative potential and the antimicrobial activity. The oleaster oils from olive trees are also reported not only to have a nutritional, but also protective effect against food borne pathogens.

In order to characterize this cream according to its microbial characteristics, *Staphylococcus aureus*, the total mesophilic aerobic bacteria and yeast-mold content was enumerated by using appropriate growth medium and results were reported in Figures 7,8 and 9 as cfu/mL. No Salmonella spp., *Escherichia coli*, *Listeria monocytogenes* were observed. In our study, it was found that one of the ingredients, the *Pinus sylvestris* extract of this cream was displaying its antibacterial effect against the bacteria reported above including a methicilin-resistant *Staphylococcus aureus*, as well as yeast and mold. Another reason is, probably due to the possible antimicrobial activity of oils of oleasters, and the high phenolic and tocopherol content of olive oil, another ingredient of olive oil, when compared to the oils of Chemlal variety. The oils of oleasters were also found to show a high antioxidative activity as well (Tamendjari et al., 2018). These findings are in line with our study, when *Staphylococcus aureus* was considered, since no any growth of this bacteria was found. Although, *Staphylococcusaureus* is a gram positive organism, *Staphylococcus aureus* was reported to be inhibited in both Tamendjari et al.'s work and our studies(Figure 8.).

In addition, no *S.aureus* were observed in both R+CoQ10+O+P and R+CoQ10+O combinations (Figure 8). *Staphylococcus aureus* counts were observed as 1,807 log at 60. days and 2,159 log cycles at nineteeth day in control (CO) while it did not grow in the R+CoQ10+O+P and R+CoQ10+O combinations.

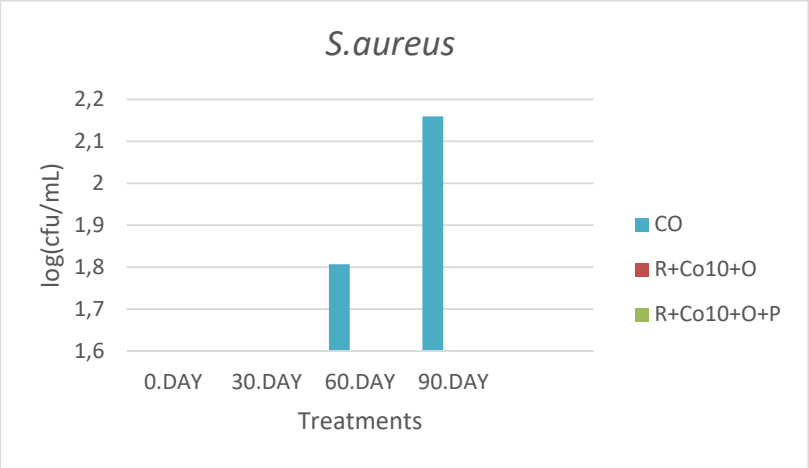

Figure 8. The effect of CO, R+CoQ10+O, and R+CoQ10+O+P combinations on the *S.aureus* levels of natural skin cream, with respect to control.

The elimination of *Staphylococcus aureus* was found to be important by this cream since it is one of the frequently existing microorganism on skin. Some reserachers pointed out that the presence of *Staphylococcus aureus* on skin, may lead to severe foodborne infections due to improper handling of certain food materials. This is probably due to the contact of the food materials, mainly the dairy products via dirty hands especially sold in the market (Arslan and Özdemir, 2012).

A study carried out by Yakhlef et al. in 2018 indicated that *Pseudomonas fluorescens* (CECT 378), *Staphylococcus aureus*(CECT 239), *Escherichia coli* (CECT 434), and *Enterococcusfaecalis* (CECT 481) was subjected to the treatment of virgin olive oil, pomace, and olive mill wastewater of the three different Algerian cultivars, including Blanquette, Rouguette and Sigoise and it was found that, due to the phenolic content of these derivatives, especially glutaraldehyde-like compounds were reported to be active on these four strains.The olive mill waste waters include hydroxyglycol, hydroxytyrosol 1-0-glycoside, hydroxytyrosol, Hydroxytyrosol 4-O-glucoside, salidroside, tyrosol, caffeic acid, ester of caffeic, comselogoside, decarboxymethyl elenolic acid free, elenolic acid, total phenols and the olive oils contain hydroxytyrosol, hydroxytyrosol, Glicol, oleuropeinaglycon, 4-ethylphenol, Pinoresinol, 1-Acetoxypinoresinol, Luteolin, Apigenin, Total phenols, and the pomace is believed to contain hydroxytyrosol, hydroxytyrosol-1-O-glucoside, hydroxytyrosol-4-O-glucoside, tyrosol, salidroside,, rutin, luteolin, luteolin 7-glucoside, p-coumaric acid, vanillic acid, cafeic acid, ester of cafeic acid, and

comselogoside. The possible antimicrobial effect is probably due to the inhibitory action of these substances (Yakhlef et al., 2018).

Otherwise, olive oil was reported to show antibacterial activity against *Clostridium perfringens, E.coli, Lactobacillus acidophilus, Bifidobacterium bifidum* and it was reported that no growth was observed just after one hour of treatment against *Listeria monocytogenes, Staphylococcus aureus, Salmonella enterica, Yersinia spp., and Shigella sonnei* (Medinaet al., 2006). On the other hand, in our study the similar effect of olive oil may possibly indicate an antimicrobial action against *E.coli*, *Staphylococcus aureus, Listeria monocytogenes* and *Salmonella spp.*

When the antibacterial effect of Coenzyme Q10 was considered, no any inhibition against a microorganism of Coenzyme Q10 was found in literature. A study performed by Brauner et al. (2014) indicated that other than the antioxidative function, CoQ10 was reported to adjust immune function by mainly unknown mechanisms (Brauner et al.,2014).The effect of CoQ10 on the structure of different antimicrobial peptides and natural killer (NK) cells was analyzed on both immune components existed in the pathogenic behavior of diabetes and diabetes-related long-term problems like cardiovascular diseases and it was found that, when they were present together with CoQ10,the serum levels of two antimicrobial peptides, including human cathelicidin and the human beta defensin 1, were reduced in patients suffering from type one *diabetes mellitus*.

Moreover, in a study carried out by Trang and Pasuwan(2018), the protein hydrolysate of Thai jasmine variety rice bran was investigated and no any inhibitory effect was observed against certain important human pathogens leading to severe foodbome illnesses, including Staphylococcus aureus, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella typhimurium* (Trang and Pasuwan, 2018).

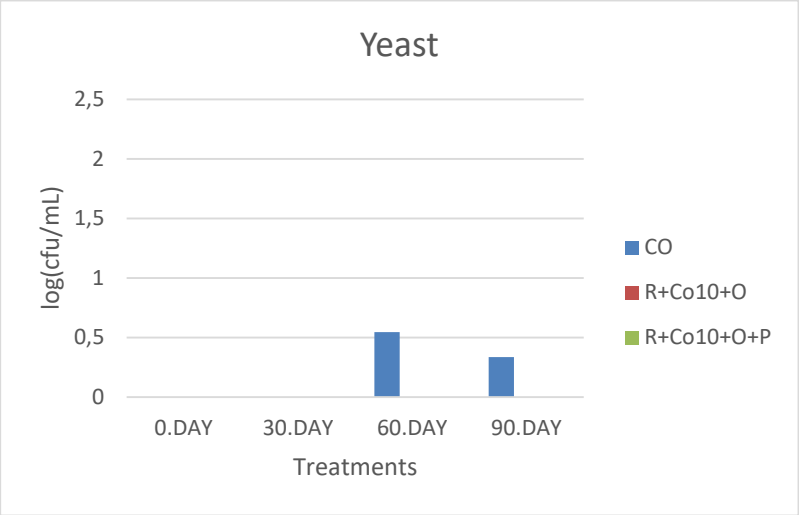

Figure 9. The yeast-mold counts of CO, R+CoQ10+O, and R+CoQ10+O+P combinations of natural skin cream, with respect to control.

When the yeast-mold counts were evaluated, it was observed that R+CoQ10+O+P and R+CoQ10+O combinations were found to be the most effective on the yeast-mold more than CO combination (Figure 4.3). In control, at sixteeth and nineteeth day, 0,544 and 0,243 log cycle growth of yeast-mold were respectively observed but no growth was seen in R+CoQ10+O+P and R+CoQ10+O treatments.

In addition, no bacterial growth was observed in *Listeria, Salmonella spp.* and *E.coli* bacteria in these three groups' combinations, including the control as because of the reason that olive oil and *Pinus sylvestris* extracts may show antibacterial activity (Brenes et al.,2007; Schanz et al., 1992). Besides, olive oil has vitamin E and since the main component of this vitamin is alpha-tocopherol at a rate of 95% (Naegele, 2017), this substance was reported to show antibacterial activity in a recent study carried out by Harper et al. in 2018; in which the hypothesis of the study was based upon the screening of the antibiofilm effects of soft nanomaterials of this component. In mouth, the alpha-tocopherol (alpha-T) and alpha-tocopherol phosphate (alpha-TP) was found to release soft nanomaterials and found to display against retardation of the biofilm formation of *Streptococcus oralis* at a high extent, especially with the alpha-tocopherol phosphate (Harper et al.,2018). I would like to claim that the antimicrobial effect of olive oil may also be due to the possible antimicrobial activity of alpha-tocopherol based substances.

Considering all enumerations, as since no confluent proliferation of bacteria was observed, so no data were available to compare the treatment groups from the statistical point of view.

4.2. The Antibacterial activity of *Pinus sylvestris*

The antibacterial activity of *Pinus sylvestris* extract against several gram(-) and gram (+) bacteria in disc-diffusion method was analyzed before the cream was formulated (Table 4.1). *Pinus sylvestris* extracts were found to be effective against *E.coli, Salmonella spp. Listeria spp. and S.aureus.*

According to a study conducted by Czerwińska and Szparaga, *Pinus sylvestris* extracts were found to be effective against *Listeria monocytogenes, Micrococcus luteus, Staphylococcus aureus* and *Escherichia coli*, ranging between 12 to 8 mm inhibition zone diameters respectively, which was found to be in line with our study carried out by 20µLs of the same species against *Listeria spp., S.aureus* and, *Escherichia coli*, between 11-17 mms after an incubation period at 37° C for 48 hours. In addition this extract was found to be the most effective on *Salmonella spp.* (12 mm) (Table 4.1).

The interaction between the ingredients may thought to show antagonistic effect when used in combination, but unexpectedly a synergetic effect on the major foodborne pathogens were observed. Further studies are required to understand the presence of these pathogenic strains in the cream that was formulated in our study.

According to the statistical analysis, one way Anova Duncan's test was found to be more suitable for determining the significant difference among the zone diameters recorded in testing the effect of *Pinus sylvestris* on all bacteria used in this study, except *Staphylococcus aureus* as since one way Anova Scheffe's test indicated no significant difference (Appendix A.4).

4.3. The DPPH Assay

2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) is a popular standard antioxidant assay considered as a free radical calculation method based on electron-transfer and this method is basic and sensible. The use of the DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry, so it can be useful to assess various products at a time. In this study, three groups were tested.

According to the results of DPPH analysis, there were no significant difference between the groups, indicating a low antioxidative activity with respect to control(Appendix A.5.).

4.4. The Physicochemical Analysis

The physicochemical analysis of the three treatment groups were carried out(Table 4.1 and 4.2) and it was found that in the protein and viscosity analysis, there was no significant difference between the treatment groups with respect to control. While preparing the cream, mild heat treatment was applied when cream is subjected to mixing with distilled water in the coffee pot. High temperatures eventually lead to the denaturation of proteins present probably at a few amount in the rice powder, indicating that the secondary and tertiary structures containing hydrogen bonds and hydrophobic interactions are disrupted but the primary structure is still yet to be stable.

When the oil content was evaluated, group 3, which contains the same amount of olive oil compared to other treatment group (Group 2) was found to be significantly different. The reason is that, unlike the other two groups, the essential oil content of *Pinus sylvestris* extract probably increases the amount of oil present in the treatment group (Venskutonis et al., 2011).

Also, the pH analysis indicated that there were no significant difference between the groups 1 and 2 but group 3 was different from the others (Ali, and Yosipov, 2013). The skin care industry mostly prefers these cosmetic products at a pH optimum of 5.5, where the interval of 3-8 is accepted.

If the interval is exceeded in both above and below directions it causes serious skin problems including irritation and rash (Wang et al.,2008). When the acidity analysis was evaluated, a significant difference was observed in between the three groups. When Brix^{o} analysis was evaluated, the first and second treatment groups showed no significant difference in themselves, the other group were different from the others. Since the third group contains Scots pine, this would decrease the soluble solid content, probably this would help our cream easily absorbed from the skin.

When the color of the cream was considered, three important parameters were evaluated. L* value represents whiteness or brightness/darkness, a* value represents redness/greenness and b* value represents yellowness/blueness. There were no significant difference between L* values, but, on the other hand, a* values indicated significant difference among the treatments, probably the olive oil and *Pinus sylvestris* extracts increased greenness via chromoplasts and chlorophyll pigment inherent, respectively. A significant difference was observed between control and the treatment groups in b* values, it may be due to the discoloration of the *Pinus sylvestris* extract while centrifugation, as well as their combination together with olive oil increased discoloration.

Table 4.1. The results of the physicochemical analysis

Protein	1	0,11±0,007[a]
	2	0,11±0,014[a]
	3	0,09±0,000[a]
Viscosity	1	93,85±1,909[a]
	2	90,12±1,223[a]
	3	86,3±4,101[a]
Oil	1	0,00±0,000
	2	0,20±0,000[a]
	3	0,80±0,000[b]
pH	1	6,18±0,014[a]
	2	6,15±0,007[a]
	3	5,82±0,007[b]
Acidity	1	0,067±0,004[ab]
	2	0,061±0,004[a]
	3	0,08±0,004[b]
Brix	1	12,25±0,354[a]
	2	12,75±0,354[a]
	3	8,25±0,354[b]
L*	1	85,29±3,14[a]
	2	89,99±0,113[a]
	3	87,09±0,721[a]
a*	1	-0,91±0,000[a]
	2	-1,58±0,014[b]
	3	-1,16±0,113[c]
b*	1	4,25±0,262[a]
	2	7,57±0,028[b]
	3	7,79±0,686[b]

Group 1 : rice flour and distilled water mixture ; Group 2 : rice flour, distilled water, coenzymeQ_{10} and 2ml olive oil mixture ; Group 3 : rice flour, distilled water, coenzymeQ_{10} , 2ml olive oil and 2ml *Pinus sylvestris* extracts .

Table 4.2: The antibacterial activity of *Pinus sylvestris* extracts carried out by disc-diffusion method, inhibition zones(mm) at 37° C after 48 hours.

Pinus sylvestris	After 48 hours	Bacteria	*E.coli*	Salmonella spp.	Listeria spp.	S.*aureus*	Mean value
		5µL	*11*	10	9	9	9.75
		10µL	12	12	10	10	11
		20µL	17	18	14	12	15.25

Table 4.3 : The result of the antibacterial activity of *Pinus sylvestris* extracts analysis

TREATMENTS	*E.coli*	Salmonella spp.	Listeria spp.	*S.aureus*
1	11± 0,577367[b]	10±1,154734[b]	9± 0,577367[b]	9± 0,577367[b]
2	12±0,577367[b]	12± 0,577367[b]	10± 0,577367[b]	10± 0,577367[b]
3	17±0,577367[a]	18± 0,577367[a]	14± 0,577367[a]	12± 0,577367[a]

5.CONCLUSIONS AND RECOMMENDATIONS

In our study, various natural ingredients are used because of their anti-bacterial effects as well as the anti-aging properties and skin whitening. Some non-natural creams used in cosmetic technology can cause systematic damage to the body. However, since this kind of a natural cream does not contain any harmful ingredients, it does not pose a threat to health, that's why this topic is chosen. In addition, a homogeneous cream was obtained by adding the Coenzyme Q10, *Pinus sylvestris* extract and rice flour during the production phase. As a result, it was determined that the two samples were thought to display antibacterial properties against gram (+) and gram (-) bacteria, since no microorganisms were detected in the counts.

We tried to produce a skin cream with coenzyme Q and milled rice basis and three combinations were produced as CO, R+CoQ10+O and R+CoQ10+O+P combinations. It was found that R+CoQ10+O+P is the most durable combination with highest shelf life period, extended via olive oil, Coenzyme q10 and *Pinus sylvestris* extracts. [Kapoor and Kapoor, 2013]. In group 3 no microbial growth was observed, including the most important foodborne pathogens. It can be concluded that, the shelf life of the cream is extended up to a period of ninety days. The pH plays a role in the barrier of skin and defence to some microorganisms such as *S.aureus*. Normally, the skin pH is a bit acidic, between an interval of 4.0- 6.0 (Iwanicka et al., 2017). In addition, continuous increase and decrease in skin pH causes skin irritation. Therefore, the pH values of a cream should be between 3.0 and 8.0 according to cosmetic standards. (Iwanicka et al., 2017). In our study, the pH of Group 3 is the most convenient group that meets the cosmetic standards ($5,82\pm0,007^{b}$).

According to result of DPPH assay, all three samples of creams made in our study have no antioxidative activity.

Therefore, the sticky property in this cream may show a disadvantage and this should be extensively studied in further research, including the human trials, the

presence of microorganism, the kinetics of the metabolites formed after the combination, and the edible properties supported by a taste panel in future.

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APPENDICES

1. APPENDICES

APPENDIX A.1.: The microbiological analysis of the group (Group 1-Control)
ND: Not Detected

Rice flour and distilled water	Dilution(average no. of m/o was reported)	Salmonella spp.	*E.coli*	*Listeria monocytogenes*	TMAB	Yeast-Mold	*S.aureus*
0. day	10^{-4}	ND	ND	ND	ND	ND	ND
	10^{-5}	ND	ND	ND	ND	ND	ND
30. day	10^{-4}	ND	ND	ND	ND	ND	ND
	10^{-5}	ND	ND	ND	ND	ND	ND
60. day	10^{-4}	ND	ND	ND	ND	1/ND	ND
	10^{-5}	ND	ND	ND	ND	6/ND/ ND	ND
90. day	10^{-4}	ND	ND	ND	3/ND/ ND	3/2/2	+300
	10^{-5}	ND	ND	ND	3/ND/ ND	ND	100/100

APPENDIX A.2.: The microbiological analysis of the group (Group 2- Rice flour, distiled water, CoQ_{10}, oil)
ND: Not Detected

Rice flour, distiled water, CoQ_{10}, oil	Dilution(average no. of m/o was reported)	Salmonella spp.	*E.coli*	*Listeria monocytogenes*	TMAB	Yeast-Mold	*S.aureus*
0. day	10^{-4}	ND	ND	ND	ND	ND	ND
	10^{-5}	ND	ND	ND	ND	ND	ND
30. day	10^{-4}	ND	ND	ND	1/ND/ ND	ND	ND
	10^{-5}	ND	ND	ND	ND	ND	1/ND/ ND
60. day	10^{-4}	ND	ND	ND	ND/1	ND	ND
	10^{-5}	ND	ND	ND	ND	ND	ND
90. day	10^{-4}	ND	ND	ND	6/8/ND	ND	ND
	10^{-5}	ND	ND	ND	ND	ND	ND

APPENDIX A.3. The microbiological analysis of the group (Group 3- Rice flour, distilled water, CoenzymeQ$_{10}$, oil, 2mL*Pinus sylvestris*)
ND: Not Detected

Rice flour, distilled water, CoenzymeQ$_{10}$, oil, 2mL*Pinus sylvestris*	Dilution(average no. of m/o was reported)	Salmonella spp.	*E.coli*	*Listeria monocytogenes*	TMAB	Yeast	*S.aureus*
0. day	10^{-4}	ND	ND	ND	ND	ND	ND
	10^{-5}	ND	ND	ND	ND	ND	ND
30. day	10^{-4}	ND	ND	ND	ND	ND	ND
	10^{-5}	ND	ND	ND	ND	ND	ND
60. day	10^{-4}	ND	ND	ND	ND	ND	ND
	10^{-5}	ND	ND	ND	ND	ND	ND
90. day	10^{-4}	ND	ND	ND	ND	ND	ND
	10^{-5}	ND	ND	ND	ND	ND	ND

APPENDIX A.4. The statistical analysis of the microbial counts of fir extract.

Salmonella spp.

ANOVA

zone

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	104,000	2	52,000	26,000	,001
Within Groups	12,000	6	2,000		
Total	116,000	8			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: zone

	(I) trt	(J) trt	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Scheffe	1,00	2,00	-2,00000	1,15470	,296	-5,7034	1,7034
		3,00	-8,00000*	1,15470	,001	-11,7034	-4,2966
	2,00	1,00	2,00000	1,15470	,296	-1,7034	5,7034
		3,00	-6,00000*	1,15470	,006	-9,7034	-2,2966
	3,00	1,00	8,00000*	1,15470	,001	4,2966	11,7034
		2,00	6,00000*	1,15470	,006	2,2966	9,7034

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

zone

	trt	N	Subset for alpha = 0.05	
			1	2
Duncan[a]	1,00	3	10,0000	
	2,00	3	12,0000	
	3,00	3		18,0000
	Sig.		,134	1,000
Scheffe[a]	1,00	3	10,0000	
	2,00	3	12,0000	
	3,00	3		18,0000
	Sig.		,296	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3,000.

E.coli

ANOVA

zone

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	62,000	2	31,000	31,000	,001
Within Groups	6,000	6	1,000		
Total	68,000	8			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: zone

	(I) trt	(J) trt	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Scheffe	1,00	2,00	-1,00000	,81650	,512	-3,6187	1,6187
		3,00	-6,00000*	,81650	,001	-8,6187	-3,3813
	2,00	1,00	1,00000	,81650	,512	-1,6187	3,6187
		3,00	-5,00000*	,81650	,003	-7,6187	-2,3813
	3,00	1,00	6,00000*	,81650	,001	3,3813	8,6187
		2,00	5,00000*	,81650	,003	2,3813	7,6187

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

zone

	trt	N	Subset for alpha = 0.05	
			1	2
Duncan[a]	1,00	3	11,0000	
	2,00	3	12,0000	
	3,00	3		17,0000
	Sig.		,267	1,000
Scheffe[a]	1,00	3	11,0000	
	2,00	3	12,0000	
	3,00	3		17,0000
	Sig.		,512	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3,000.

Listeria

ANOVA

zone

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	42,000	2	21,000	21,000	,002
Within Groups	6,000	6	1,000		
Total	48,000	8			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: zone

	(I) trt	(J) trt	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Scheffe	1,00	2,00	-1,00000	,81650	,512	-3,6187	1,6187
		3,00	-5,00000*	,81650	,003	-7,6187	-2,3813
	2,00	1,00	1,00000	,81650	,512	-1,6187	3,6187
		3,00	-4,00000*	,81650	,008	-6,6187	-1,3813
	3,00	1,00	5,00000*	,81650	,003	2,3813	7,6187
		2,00	4,00000*	,81650	,008	1,3813	6,6187

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

zone

	trt	N	Subset for alpha = 0.05	
			1	2
Duncan[a]	1,00	3	9,0000	
	2,00	3	10,0000	
	3,00	3		14,0000
	Sig.		,267	1,000
Scheffe[a]	1,00	3	9,0000	
	2,00	3	10,0000	
	3,00	3		14,0000
	Sig.		,512	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3,000.

S. aureus

ANOVA

zone

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14,000	2	7,000	7,000	,027
Within Groups	6,000	6	1,000		
Total	20,000	8			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: zone

	(I) trt	(J) trt	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Scheffe	1,00	2,00	-1,00000	,81650	,512	-3,6187	1,6187
		3,00	-3,00000*	,81650	,029	-5,6187	-,3813
	2,00	1,00	1,00000	,81650	,512	-1,6187	3,6187
		3,00	-2,00000	,81650	,125	-4,6187	,6187
	3,00	1,00	3,00000*	,81650	,029	,3813	5,6187
		2,00	2,00000	,81650	,125	-,6187	4,6187

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

zone

	trt	N	Subset for alpha = 0.05	
			1	2
Duncan[a]	1,00	3	9,0000	
	2,00	3	10,0000	
	3,00	3		12,0000
	Sig.		,267	1,000
Scheffe[a]	1,00	3	9,0000	
	2,00	3	10,0000	10,0000
	3,00	3		12,0000
	Sig.		,512	,125

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3,000.

APPENDIX A.5. DPPH Scavenging Activity In Percentage

CO	R+CO+O	R+CO+O+P
90,94	93,6	89,7
91,24	93,6	88,94

CO: Control
R+CO+O: Rice flour-Coenzyme Q_{10}-Olive oil
R+CO+O+P: Rice flour-Coenzyme Q_{10}-Olive oil-*Pinus sylvestris*

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