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INVESTIGATION OF MONGOLIAN SEA BUCKTHORN

((Hippophae rhamnoides spp. Mongolica)

- NUTRITIONAL STUDY -

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Introduction

Functional foods abundant in antioxidants have garnered increasing scientific and commercial attention due to their capacity to mitigate illnesses associated with oxidative stress. Sea buckthorn (*Hippophae rhamnoides L.*), a deciduous shrub indigenous to Europe and Asia, yields berries renowned for their remarkable concentrations of vitamins, flavonoids, and polyphenols. The Mongolian subspecies, *Hippophae rhamnoides spp. mongolica*, is esteemed for its resilience in extreme conditions and its distinctive nutritional composition. Nonetheless, despite the growing use of sea buckthorn in nutraceuticals and food items, thorough data on the antioxidant activity of its freeze-dried forms remains insufficient. This study seeks to assess the antioxidant capabilities of freeze-dried Mongolian sea buckthorn by DPPH, FRAP, Total Polyphenol Content (TPC), Total Flavonoid Content (TFC) test as well as nutritional animal study modelled on fruit flies the *Drosophila melanogaster* on three different diet conditions utilizing the sea buckthorn. Furthermore, a functional food product incorporating the lyophilized sea buckthorn was developed. The comparison of these values with those of fresh fruits and current literature will enhance the knowledge of the functional potential of this underused berry in health-related applications.

1. Raising a topic

Sea buckthorn have been increasingly gaining interests of many researchers and health advocated around the world. The plant by itself have been shown to have many health benefits through various studies and investigations. It also has a rich historical background. Our focus of research today is focusing on a sub species grown in Mongolia. I believe this subspecies is quite unique from other variants grown elsewhere. Firstly, Mongolia has a very dry climate and overall high altitude which gives its unique quality.

In this Diploma Thesis, we investigate a sample of fresh Sea buckthorn fruit obtained from a local store in Ulaanbaatar, Mongolia to see what kind of benefits this sample has and further develop a bakery product containing the sea buckthorn with gluten free, lactose free and vegetarian muffin as many consumers invested in their nutritional intake poses dietary restrictions.

We conducted Fruit-Fly nutritional experiments using the prepared sea buckthorn samples that compares the viability of the flies on normal diet, high sugar diet and zero media diet only including the sample with set concentrations to determine if there is a dose-response curve or any significant result. Due to its known high antioxidant contents positive results are to be expected.

2. Literature review

Since 2005, Mongolia has had a temperature increase of 1.59°C, a rate roughly thrice the global average (FETCH, 2021). This warming is altering Mongolia's summers, rendering them hotter and drier, which depletes grasslands and diminishes feed supply (ALTANSUKH et al., 2024). Mongolia exhibits a distinctly continental climate, characterized by significant temperature changes, little precipitation, and pronounced regional differences influenced by latitude and altitude. Average temperatures fluctuate between around -4°C and -8°C in mountainous areas, rising to about 2°C in the steppe desert region and roughly 6°C in the southern desert adjacent to China. Temperature fluctuates significantly over the year. Historically, maximum temperatures have reached around 24°C in July, whilst minimum temperatures in January decline to about -28°C. Annual precipitation seldom surpasses 400 mm and is generally significantly lower in the southern and central desert and steppe areas. The Gobi Desert receives an annual precipitation of just 40 mm. Nationally, around 85% of precipitation occurs from April to September. Minor inter-annual fluctuations in precipitation can result in significant drought occurrences, with certain areas receiving no rainfall whatsoever (THE WORLD BANK GROUP, 2021). With these harsh conditions one can infer that the plant have obtained tremendous resilience and further defence mechanisms to protect itself which gives us its potent phytonutrients.

To add to the importance of this study, Mongolia possesses the highest cancer-related mortality rate globally (RAJAPPA et al., 2023). The incidence and death rates of colorectal cancer (CRC) in Mongolia were 6.3 and 4.0 per 100,000 individuals, respectively. The incidence of CRC did not differ statistically between men and women and steadily increasing with age (TSEDENDORJ et al., 2024). According to the American Cancer Society, they mention a prolonged diet rich in red meats (including beef, hog, lamb, or liver) and processed meats (such as hot dogs and some deli meats) elevates the risk of colon cancer. Cooking meats at elevated temperatures (frying, broiling, or grilling) generates compounds that may increase your cancer risk. A deficiency in vitamin D may elevate your risk. Adhering to a nutritious dietary regimen rich in fruits, vegetables, and whole grains, while restricting or eliminating red and processed meats and sugary beverages, likely reduces risk (AMERICAN CANCER SOCIETY., 2025). In the article *Diet is the Number One Killer in Mongolia,* BROMAGE (2021) recounts a study conducted by the former Health Sciences University of Mongolia and Harvard University in which researchers travelled across eight provinces in

Mongolia to train 200 medical students to analyse everyday diets of local populations. In their findings revealed most of the population regardless of their lifestyle or region, they consumed similar foods – primarily red meat, whole milk and unfortified flour. Which is the leading contributor to widespread vitamin deficiencies across the country. With this being said, it is evident that the diet of the people heavily influence their risk of CRC. Hence a supplementing local nutraceuticals to remedy this negative effect on their health is recommended for people in rural areas as well as creating more functional foods with added fiber.

Sea buckthorn

Sea buckthorn (*Hippophae L.)* is classified under the family Elaeagnaceae, order Elaeagnales, superorder Celastraneae, subclass Rosidae, class Magnoliopsida, and division Magnoliophyta. (LINNAEUS, 1753) Sea buckthorn is a plant that is temperate of the Euroasian contitent widespead and distributed throughout 38 countries like Afghanistan, Azerbaijan, Belarus, Bhutan, Britain, Bulgaria, Canada, China, Czech Republic, Denmark, Estonia, Finland, France, Germany, India, Iran, Italy, Kyrgystan, Latvia, Lithuania, Moldova, Mongolia, Nepal, Netherlands, Hungary, Norway, Pakistan, Poland, Portugal, Russia, Slovakia, Sweden, Switerland, Turkey, Ukraine and Uzbekistan (RAJKAL, 2009).

Sea buckthorn thrives in multiple lands that feature extreme environmental circumstances, including seacoasts, riverbanks, and frigid deserts. It is seen to grow naturally at elevated altitudes in the temperate regions of Asia and Europe (ROUSI, 1971). The species flourishes in temperatures ranging from −43 °C to +40 °C (RONGSEN, 1992) in regions receiving 400–600 mm of precipitation (LI - SCHROEDER, 1996) The plant is also deemed to be resistant to drought (LI - SCHROEDER, 1996) which is another specialty making it suitable for harsh environments like Mongolia.

A key adaptive feature of sea buckthorn is its extensive root system, with about 80% of its roots concentrated in the topsoil, contributing to soil stabilization and erosion control. Additionally, the presence of nitrogen-fixing nodules containing *Frankia* enhances soil fertility (CLAWSON - CARÚ - BENSON, 1998). Furthermore due to the extensive root system of the Sea buckthorn plant, there are several benefits at play for example it can mitigate soil erosion and its effect of desertification on agroecosystems, decrease the water loss of the soil, improvement of the land reclamation as well as create more habitable land for the wildlife species (LI - SCHROEDER, 1996).

The leaves are small, lanceolate, and covered with silvery scales, displayed on Figure 1, which help reduce water loss by reflecting sunlight (RONGSEN, 1992). The plant produces small, yellowish flowers and berries that vary in shape (from round to elongated) and color, ranging from yellow to orange-red. Each berry contains a single ovoid to elliptical seed measuring approximately 2.8–4.2 mm with a dark brown coat (SURYAKUMAR - GUPTA, 2011). Typical sea buckthorn plants bear rigid thorns along their stems, with thorn density ranging between 1 to 5 thorns per cm^2, varying by species. While *H. rhamnoides* is generally dioecious, rare occurrences of monoecious forms have been mentioned (SEN -TAMCHOS - KAUL, 2014).

The term *Hippophae* is from the Latin word "*hippo*" and "*phao*s" which mean horse and shine respectively where there is a legend of Alexander the Great left his horses in a field of Sea buckthorn and when he later returned they appeared to have shiny coats and much improved health which lead to the naming of the plant. This story can also depict the therapeutic aspects of the plant. The *rhamnoides* which comes from "like Rhamnus" (another thorny plant genus).

The field smells pungent from ripe seabuckthorn berries. Mature berries are collected either by hand or by mechanized means. The harvesting method is arduous and time-intensive due to the softness, diminutive size, and great delicacy of the berries. Furthermore, the plant's thorniness complicates the harvesting process. Given the challenges associated with hand berry harvesting, mechanized harvesters are becoming essential for the seabuckthorn sector. Mechanical harvesting can be categorized as either direct or indirect (FU et al., 2014).

In Mongolia, medicinal extracts from the leaves and branches of the plant are utilized to treat colitis and enterocolitis in both humans and animals (GULIYEV et al., 2004). In Russia, oil derived from seeds and fruits is utilized in the treatment of chronic dermatoses, eczema, psoriasis, thrombosis, lupus erythematosus, burns, frostbite, and cervical erosion. Seabuckthorn berries are processed into various products, including squash, syrup, jam, jellies, savory chutney, pickles, candies, pies, and both alcoholic and nonalcoholic beverages, as well as for flavoring dairy products (BALet al., 2011; DHYANI et al., 2010)

Figure 1: **“Sea buckthorn in different periods”**

Source: WANG, et al., (2022)

Bioactivity

Every part of the plant, such as the leaf, seed, rood, thorn, bark, stem, and fruit (berry) has some nutraceutical or medicinal value and due to this fact its known as a "multipurose-wonder plant" and "golden bush" (LI - SCHROEDER, 1996). From the different parts of the plant more than 190 bioactive compounds have been noted (BAL - MEDA - NAIK - SATYA, 2011).

However, factors like geographical location, climate, genome, harvesting time, extraction method and method of processing can heavily influence variations in vitamin contents of the products derived.

Our focus of study for this thesis is the berries of the sea buckthorn, which are highly nutritious and essential part of the plant. Typically the berries composition consists of 68% pulp, 23% seed and 7.75 peel. As mentioned earlier its is a rich source of bioactive compounds that varies based on species, origin, maturity, fruit size, fruit colour, geographic location and method of extraction. The berries are highly acidic and sour to taste and have a distinct aroma unique enough for flavouring food products. It has been noted that the berries contain glucose and fructose as carbohydrates, vitamins like C, E and K, along with essential fatty acids, amino acids, organic acids, flavonoids like isorhamnetin, quercetin, and kaempferol, tocopherols, carotenoids and mineral elements (BAL - MEDA - NAIK - SATYA, 2011). Table 1 demonstrates the components of the sea buckthorn along side its therapeutic effects, derived from SURYAKUMAR - GUPTA, 2011)

Table 1. "Major-phytochemicals in Sea buckthorn and their medicinal properties." (SURYAKUMAR - GUPTA, 2011)

SBT phytoconstituents	Medicinal properties	References
Tocopherols	Acts as antioxidant, minimizes lipid oxidation, helps to relieve pain	(KALLIO-YANG, 2002)
Carotenoids	Acts as antioxidant and helps in collagen synthesis and epithelialization	(ANDERSSON et al., 2008)
Vitamin K	Prevents bleeding; promotes wound healing; anti-ulcer effect	(JAMYANSAN-BADGAA, 2006)
Vitamin C	Acts as antioxidant and sustain cell membrane integrity Accelerates collagen synthesis	(KALLIO - YANG, - PEIPPO, 2002)
Vitamin B complex	Stimulate cell repair and nerve regeneration	(JAMYANSAN - BADGAA, 2006)
Phytosterols	Improves microcirculation in the skin, anti-ulcer, anti-atherogenic, anti-cancer, regulate inflammatory process	(YANG - KARLSSON - OKSMAN - KALLIO, 2001)
Polyphenolic compounds	Antioxidant, cytoprotective, cardioprotective, wound healing	(UPADHYAY - KUMAR - GUPTA, 2010)
Polyunsaturated fatty acids (PUFA)	Immunomodulatory, neuroprotective, anti-tumor	(YANG - KALLIO, 2001)
Organic acids	Lower the risk of heart attack and stroke, anti-ulcer, wound healing, anti-arthritic	(YANG - KALLIO, 2001)
Coumarins and triterpenes	Control of appetite, sleep, memory and learning	(GREY et al., 2010)
Zinc	Strengthen the blood circulation, anti-tumor Aids in cell proliferation, acts as a cofactor for enzymes, and enhances utilization of vitamin A	(GUPTA - SINGH, 2005)

Hippophae rhamnoides spp. mongolica, like other subspecies of sea buckthorn, demonstrates notable morphological variability influenced by species traits and environmental conditions.

In addition to their nutritional value, berries offer protective benefits against cardiovascular illnesses, mucosal injuries, dermatological issues, cancer, and enhance immune system functionality (RAJKAL, 2009). In ancient times, sea buckthorn berry juice was utilized in various pharmacological formulations to facilitate expectoration, enhance blood circulation, eliminate blood stasis, and invigorate spleen function (ZEB, 2004). Flavonoids, particularly isorhamnetin, have been documented to have cardioprotective, hepatoprotective, antitumor, and anticancer properties (TENG et al.,2006; MAHESHWARI et al., 2011; LI, et al., 2014, 2015)

Carotenoids, particularly zeaxanthin and beta-cryptoxanthin esters derived from sea buckthorn berries, have been utilized as food additives, cosmetic components, and nutraceuticals (SURYAKUMAR-GUPTA, 2011).

There have been several comprehensive studies on the nutraceutical and medicinal importance of sea buckthorn (SHARMA - KALKAL, 2018) as well as reviews summarizing its nutrients, phytochemistry, health benefits and food applications (WANG, et al., 2022). In this section we will mention some prominent results sea buckthorn has shown over the years regarding its health benefits.

The function of *Hippophae* in cancer prevention and management is predominantly founded on research utilizing laboratory animals and various cancer cell lines. The ethanol extract from the leaves of *Hippophae rhamnoides L.* exhibited antiproliferative effects on human acute myeloid leukemia cells (KG-1a, HL60, and U937), resulting in the activation of the S-phase checkpoint, which slowed the cell cycle and induced apoptosis (ZHAMANBAEVA et al., 2014). The hydroalcoholic extract from dried berries of *Hippophae rhamnoides* was observed to diminish carcinogen-induced papillomagenesis in the stomach and skin of mice. Elevated activities of phase II enzymes (GST and DTD) and antioxidant enzymes (SOD, CAT, GPX, and GR), along with the upregulation of the DNA-binding activity of the IRF-1 transcription factor, a recognized antioncogenic factor that induces growth suppression and apoptosis, may be associated with the anticancer effects (PADMAVATHI, et al., 2005). GREY et al., (2010) investigated the antiproliferative effects of ethyl acetate and ethanol:water (1:1) extracts from berries with differing compositions on Caco-2 (colon) and Hep G2 (liver) cancer cell lines. The ethyl acetate extract had the most potent inhibitory impact on Caco-2 cells, whereas the ethanol: water extract demonstrated significant inhibition on Hep G2 cells. Furthermore, photochemical examination revealed that the ethyl extract was rich in urosolic acid but contained few phenolic compounds, whereas the ethanol:water extract exhibited elevated quantities of phenols and proanthocyanidins. An ethanol fruit extract yielded a water-soluble *Hippophae rhamnoides* antifatigue polysaccharide (HRWP-A), which was investigated for its capacity to prevent the growth of Lewis Lung Carcinoma

(LLC) in tumor-bearing mice. Results indicated that the anticancer effects mediated by HRWP-A may be attributed to its role in enhancing lymphocyte proliferation and amplifying the phagocytic and cytotoxic activities of macrophages. Additionally, levels of NO and TNF-α were elevated concurrent with enhanced NK cell activity and CTL cytotoxicity in LLC tumor-bearing mice (WANG, et al., 2015)

The methanol extract of sea buckthorn berries shown antibacterial activity against Staphylococcus aureus, Bacillus subtilis, Streptococcus pneumoniae, Pseudomonas aeruginosa, Escherichia coli, and Klebsiella pneumoniae (CHAMAN et al., 2011). Hiporamin, a refined tannin fraction derived from sea buckthorn, demonstrated extensive antiviral efficacy against influenza virus strains A and B, herpes simplex virus type 1, adenovirus type 2, and HIV-1, along with modest antibacterial activity against both gram-positive and gram-negative bacteria (SHIPULINA et al., 2006).

The hypolipidemic and hypoglycemic effects of total flavonoids from seed residues (FSH) of *Hippophae rhamnoides* were shown in a high-fat diet mice model (WANG et al., 2015), demonstrated by a notable reduction in total cholesterol levels in both blood serum and liver. Furthermore, the content of triglycerides in the liver and the density of low-density lipoprotein cholesterol (LDL-C) were reduced. The findings were also corroborated by transmission electron microscopy studies. FSH therapy effectively prevented the increase in blood glucose and improved poor glucose tolerance.

Multiple animal studies have substantiated the antiulcer properties of sea buckthorn, emphasizing its antioxidative and mucosal restorative activities (SULEYMAN et al., 2001; XING et al., 2002; WANG -CHEN, 2007; DOGRA et al., 2013; YILMAZ et al., 2014)

SHARMA et al. (2011) indicated that the ingestion of commercially available sea buckthorn pulp significantly decreased blood glucose levels and thiobarbituric acid reactive compounds (TBARS). Administration of sea buckthorn pulp restored reduced glutathione (GSH) levels to near normal and mitigated degenerative alterations of pancreatic beta cells in STZ-diabetic mice. Furthermore pure sea buckthorn juice and l-quebrachitol-enriched sea buckthorn juice were assessed for its impact on a type-2 diabetes db/db mouse model. Pure sea buckthorn juice decreased feed intake, weight gain, random blood glucose levels, and insulin receptor β expression in the liver, while dramatically enhancing glucose tolerance and pancreatic tissue integrity. Sea buckthorn juice fortified with l-quebrachitol elevated fasting plasma insulin levels and had comparable effects on random blood glucose levels, glucose tolerance, and pancreatic tissue, as noted with pure sea buckthorn juice (XUE et al., 2015).

LIM et al. (2013) investigated the antioxidant and cytoprotective properties of sea buckthorn fruit extract (SFE) against oxidative stress in mouse embryonic fibroblast cells, attributing its efficacy to potent radical scavenging activity and modified cell cycle regulation, which inhibit apoptotic cell death induced by oxidative stress.

3. Material and method

3.1Sample preparation

To prepare our sample of fresh 500g of Sea buckthorn imported from Mongolia were first stored in the freezer then sectioned carefully to 250g and then split into 2 large glass petri dishes and was lyophilized (see Figure 2.A). Shown in Figure 2, are the steps that took place to prepare the sample before each experiment before being carried out then stored in a 50ml tube. As shown in Figure 1. B is after the lyophilization to weigh to determine the dry weight which was then later used to determine the fruits total water content. Then the samples were sectioned and crushed using a ceramic mortar and pestle and the seeds separated to have a more homogenous sample (Figure 2. C.). As shown in Figure 2.D is the complete sample used in the experiments.

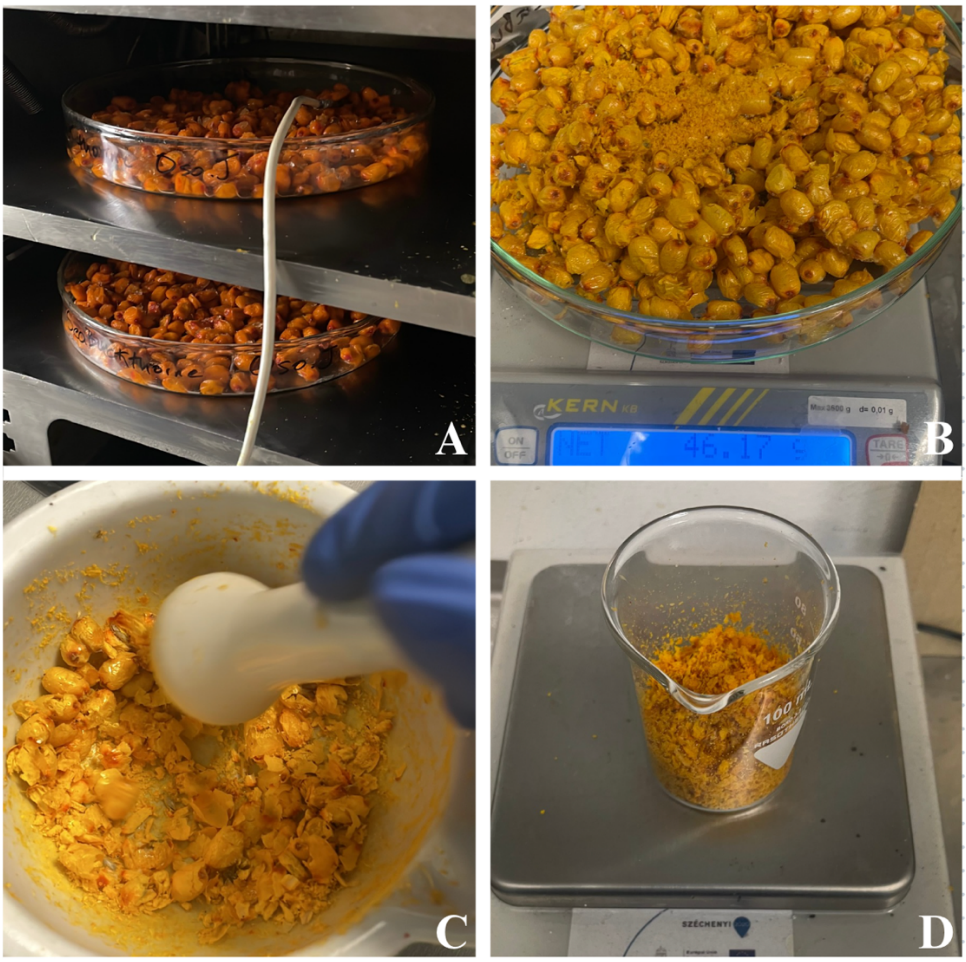

Figure 2**. A. Lyophilization of 250g of Sea buckthorn. B. Lyophilized Sea buckthorn fruits C. Crushing Freeze dried samples with mortar and pestle. D. End of sample preparation with seeds removed, scaled for each experiment.**

Source: Author (2025)

3.2. Fly Experiments

Table 2. Materials used to make various media mixtures for the Fly experiments.

Media type	Distilled Water (mL)	Yeast paste (g)	Sugar (sucrose) (g)	Wheat Flour(g)	Agar (g)	Nipagin (g)	Charcoal (g)	Sea buckthorn (g)
Normal sugar	500	35	26	15	5	0.5	-	3.2
High sugar	500	35	128	15	5	0.5	-	3.2
Zero	300	-	-	-	3	0.5	3	4.8

For the Fly experiments we followed the method mentioned in the article of ALEYA, et.al., 2023 but slightly altered to fit the sample. For this thesis, in all the *Drosolphila melanogaster* experiments a wm4h or the white mottled 4) strain of flies from the Bloomington Stock Center. Three types of media with different dietary circumstances were used to observe the developmental effects of the flies labelled as Zero media as Z, normal media as NM, and high sugar media as HS.

Stock solutions and culture media:

For Zero media, the values demonstrated in Table 2, the media was prepared with 3g charcoal powder, 3g agar, 300ml distilled water homogenized on a hot plate and boiled for 10 seconds until the agar dissolved and 0.5g NIPAGIN from Thermofisher was added after cooling down to 45 C to prevent any mold growth in the media. The sea buckthorn sample was added in a small 100ml glass beaker to 60ml as shown in Figure 2 and the 4.8g of sea buckthorn was homogenized to make the concentration of 80g/L then added to 5 viles, each having about 3ml of media, leaving behind 30ml then 30ml more of the initial media was added for serial dilution making the second concentration of 40g/L of sea buckthorn. This process repeated until the last concentration contained 5g/L. This media type contains no nutrients (SANG-KING, 1961) except our sample for the flies. This way we can observe what will happen to the development of the flies if their only source of nutrition is sea buckthorn berries without the seeds.

For the normal media and high sugar diet, 35g of yeast, 500ml of distilled water, 15g of wheat flour, 5g of agar powder, 0.5g of NIPAGIN and for the NM 26 of sugar (sucrose) alternatively for the HS media 128g of sucrose as this concentration is 0.75M. The cooking of the media starts with homogenizing the yeast and distilled water on a hotplate in a large glass beaker heating and constantly stirring with a magnetic stirrer. As the temperature rises, add the sucrose and wheat flour and bring to a boil. As it is boiling, slowly add the agar powder to avoid clumping, and give it a thorough mix for complete homogenization. Keep the stirring and the boiling continuing for 30 minutes then place in a water bath to gradually cool the media to 50 Celcius and add in the NIPAGIN. As previously mentioned in the zero media, add the sea buckthorn sample in the 100ml glass beaker until 80ml and homogenize 3.2g of our sea buckthorn to create 40g/L of maxium concentration and add into 5 viles each then leave the 40ml of media in the beaker to add more of the stock solution until 80ml again to half the initial concentration into 20g/L concentration and repeat the process until we have 5 concentrations each consisting of 40g/L, 20g/L, 10g/L, 5g/L and 2.5g/L respectively for both media types NM and HS. The viles where then cooled completely to 25 Celcius and are ready for harboring embryos.

For labelling and annotations, zero media concentrations will be noted as Z1, Z2, Z3, Z4 Z5 starting from the highest concentration (80g/L) to lowest concentrations (5g/L) respectably. Similarly for the Normal media highest concentration (40g/L) as N1, N2 (20g/L), N3 (10g/L), N4 (5g/L) and N5(2.5g/L). The same structure is used for the High sugar media only changing the letter N to H. For the denotation of controls, normal media controls labelled CN following the plate number in which the eggs were collected. Similarly for high sugar media controls, CH is used.

Figure 3: **Zero media stock mixing with SBT sample before filling into vials.**

Source: Author (2025)

Collection of embryos:

For the *Drosophila melanogaster* embryos they needed to be 0-2hours old and about 200 five day old male and female wm4h flies were placed in an embryo collection chamber positioned on top of a plate containing normal medium yeast paste. Over a 48-hour period, the egg collection plates were replaced every 2 hours to collect the embryos within the 0-2 hour developmental window. Then the embryos were carefully transferred from the collection plate to the experiment vials we prepared either the Zero media, Normal media or High-Sugar media supplemented with the sea buckthorn sample. The eggs were carried on fine forceps under a light microscope. 50 embryos were counted and placed into each vial with 5 repeats for zero media, 3 each for normal and high sugar medias per sea buckthorn concentration.

Monitoring viability and development of the *Drosophila melanogaster*:

After egg collection, the vials were each plugged with a clean cotton ball for air tight seal and always at constant temperature of 25 C and constant humidity. The vials were stored in an incubator that kept the mentioned parameters constant and only taken out during monitoring once a day same time every day. When the larvae turned into pupa which is the third instar larvae state were marked and counted until there were no more pupa. When the pupae hatch into flies the flies are extracted from the vials carefully and counted. Any flies stuck in the vials are marked to avoid any error in counting. The vials are monitored every-day until there are no more flies are found 3 days after the last flies were counted.

Along side the medias with our sample, a control is also counted for to make comparison of any effect the sea buckthorn. The control is made whenever there is a new plate from the egg collector so we can ensure if the effect is from the certain group of embryo collector plate or the sea buckthorn itself.

Drosophila melanogaster eggs' for 3 media types Normal sugar, High Sugar, and Zero Media with 3, 3, and 5 repeats respectively containing 50 eggs per vile as well as controls used.

In total 3050 eggs were counted and used.

3.3 Sea buckthorn muffin product development:

Materials:Glutton-free, Lactose-free, vegetarian muffin:

Corn flour, banana, honey, coconut yoghurt, freeze-dried Seabuckthorn and seed husks

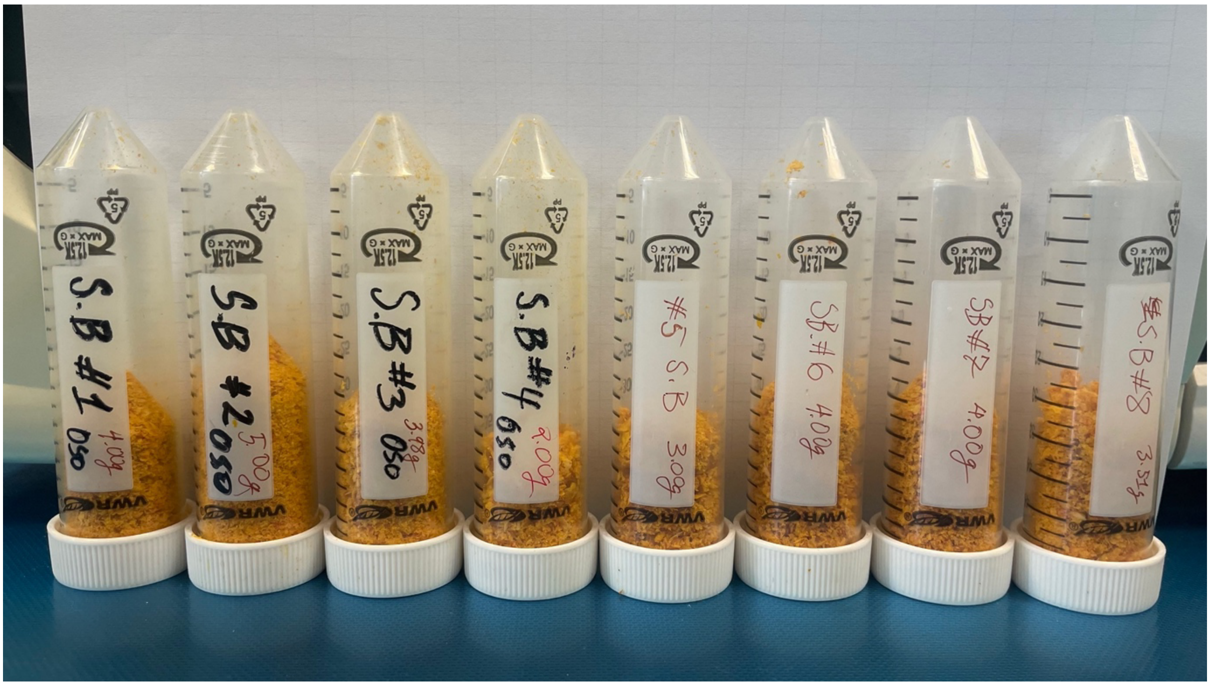

Figure 4: **Sea buckthorn samples cut into different weights for product development.**

Source: Author (2025)

Steps:

1. Preparation of dry ingredients: on a kitchen grade scale the dry ingredients were weighed individually then combined in a bowl, these include the gluten free corn flour, salt, and baking powder and seed husk (second development of the muffins)
2. Preparation of the sea buckthorn flakes: from the sample preparation of the freeze-dried fruits, samples were weighed into 2g, 3g, 3.51g, 3.98g, 4g, 4g 4g, and 5g as shown in Figure 3 and frozen in storage until used for baking
3. Hydration of the sea buckthorn: to prevent the bioactive substance from degrading due to baking, the sea buckthorn flakes were hydrated into 100g of coconut yoghurt as part of the wet ingredients and homogenized and left for at least 10 minutes.
4. Preparation of wet ingredients: bananas were smashed and homogenized then the honey, melted coconut oil, and vanilla extract were mixed in.
5. Dividing the batter: the dry and the wet ingredients were cut into 2 equal parts respectively for one being control and the other with the sea buckthorn samples

6. Batter preparation: the sea buckthorn infused coconut yoghurt was mixed with the one of the wet ingredients as batter B and for the other half of the wet batter plain coconut yoghurt were incorporated as batter A.
1. 7.Combining wet ingredients with dry: batter A and batter B had their respective dry ingredients gently incorporated. As batter A had beige coloring and batter B having distinct yellow/orange coloring from the sea buckthorn
7. Portion into muffin molds
8. Baking: the muffins were baked at 170 Celcius for 16minutes and 30 seconds.
9. Cooling: after baking the muffins were tested with toothpick to ensure it was cooked all the way through and stored in the fridge until tasting and TPC, TFC and organoleptic studies.
10. TPC and TFC test: the samples were homogenized and prepared according to the TPC and TFC methods that is mentioned later in the thesis.

An Organoleptic survey will be conducted, henceforth the following image is the survey questions asked from contestants in Figure 4. Which can determine any improvements to be made, consumer data which can determine the target audience for the product and what the target audience would like to consume.

Texture analyzer:

The muffin samples will be sent to the central laboratory for further investigations of its quality to analyze its firmness and stringiness. The methods and the procedure will be done by the laboratory. The following procedure is from their methods utilizing the Stable Micro Systems Ltd. Texture Analyzer.

Sample preparation: Extract samples from the storage location just before testing to preserve moisture and freshness. Remove the bottom and top crusts, along with a little portion from the sides, to create a cube-like shape, ensuring that only the softer inside of the muffin is evaluated, excluding the tougher outer crust.

Test set-up: Position the muffin sample centrally under the cylinder probe, steering clear of any irregular or non-representative regions. This test presupposes that the sample's surface area exceeds the diameter of the used probe.

A Force-Time curve is constructed from that demonstrates the attributes of a muffin firmness and springiness assessment. The probe compresses the sample until it achieves a reduction of

25% in product height. It maintains this distance for 30 seconds before retracting from the sample and returning to its initial location.

Firmness is defined as the force (measured in grams, kilograms, or Newtons) necessary to compress the product by a predetermined distance, such as 25%. A straightforward method to assess the springiness attribute involves measuring the force after 30 seconds, dividing this value by the maximum force, and subsequently multiplying by 100%.

$$\frac{F}{F} * 100 = \%\, recovery$$

If the value is closer to 100% the product behaves more like a “spring”.

Sea buckthorn Gluten free, Lactose free, Vegetarian Muffins – Organoleptic survey

Personal Information

Gender:
◻ Male ◻ Female ◻ Other ◻ Prefer not to say

Nationality:

How old are you?
◻ 18–24 ◻ 25–34 ◻ 35–44 ◻ 45–54 ◻ 55–64 ◻ 65+

Do you usually consume gluten-free or lactose-free products?
◻ Yes ◻ No ◻ Occasionally

If yes, what is the main reason?
◻ Celiac disease / Lactose intolerance
◻ Lifestyle choice
◻ Curiosity
◻ Other: ___________

How often do you consume muffins?
◻ Regularly (weekly)
◻ Occasionally
◻ Rarely
◻ Never

Do you prefer fruit-based muffins?
◻ Yes ◻ No ◻ Depends on the fruit

Sensory Evaluation of Muffins

Please evaluate the samples based on the following attributes. Use the scale below to rate each characteristic.

Sample Code	Appearance	Odor	Texture/Mouthfeel	Taste	Aftertaste	Overall Impression
Muffin A						
Muffin B						

Hedonic Scale:
1. Dislike extremely
2. Dislike very much
3. Dislike moderately
4. Neither like nor dislike
5. Like slightly
6. Like moderately
7. Like very much
8. Like extremely

Additional Questions

1. Which muffin did you prefer overall?
◻ Muffin A (Without Sea Buckthorn)
◻ Muffin B (With Sea Buckthorn)
◻ No preference

2. Comment on the taste of Muffin B (Sea Buckthorn):
What did you think about the fruity/tart flavor?

____

3. Would you purchase a gluten-free, lactose-free muffin like these?
◻ Yes ◻ No ◻ Maybe

4. Any suggestions for improvement?

____

Figure 5: **Organoleptic survey for SBT product development.**

Source: Author (2025)

3.4. DPPH Radical Scavenging activity

For the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, method of (BLOIS, 1958) was utilizes to measure the antioxidant effects of sea buckthorn, hence the samples were prepared according to the following steps:

Materials

- DPPH reagent (2,2-diphenyl-1-picrylhydrazyl)
- Methanol (analytical grade, Sigma-Aldrich)
- Trolox standard (1 mg/mL in methanol)
- Distilled water
- Pipettes and cuvettes
- UV-Vis spectrophotometer (set at 517 nm)

Steps

1. 1 ml extract was dissolved in 9 ml methanol and distilled water respectively, and then further diluted as required for measurement.
2. DPPH reagent (9 % m/V) was prepared on day of measurements.
3. 100 µl sample/methanol/standard, 1400 µl methanol, and 1500 µl DPPH of the reagent was mixed together.
4. Absorbance values were measured at 517 nm after 30 minutes incubation.
5. Trolox was used as a standard (1mg.ml -1) for calculation, and values were given as trolox equivalent (µmol TE.ml -1).

3.5. FRAP Ferric Reducing Antioxidant Power

The BENZIE-STRAIN, 1996 method was used for FRAP antioxidant assay modified by NEMES et. al. (2018) which introduces a simple and quick method to assess the antioxidant capacity of sample by measuring the ability of the reducing ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions.

Materials

- Sodium acetate buffer (300 mM, pH ~3.6)
- Ferric chloride ($FeCl_3$) (20 mM)
- TPTZ (*2,4,6-Tris(2-pyridyl)-s-triazine*, 10 mM)
- Distilled water
- Ascorbic acid standard (1 mg/mL in distilled water)
- FRAP reagent (prepared fresh before each measurement)

- Glass bottle covered with aluminum foil (for light protection)
- UV-Vis spectrophotometer (set at 593 nm)
- Pipettes, microtubes, and vortex mixer

Steps

1. FRAP reagent was prepared freshly before the measurement: 50 ml 300mM sodium acetate buffer, 5 ml 20 mM Ferric chloride, and 10 mM TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) were mixed together, and stored in a glass bottle covered by aluminium foil until measurement.
2. During measurement, 65 µl distilled water (75 µl for blank), 10 µl of samples and standards, and 2250 µl FRAP reagent were mixed together, vortexed, and incubated at 25 °C for 8 minutes.
3. Absorbance values were measured at 593 nm wavelength on a spectrophotometer (UV-Vis).
4. Ascorbic acid was used as standard (1mg.ml -1), and values were calculated as ascorbic acid equivalent (µmol AAE. ml -1).

3.6. Total Phenol Content (TPC)

The following were used to carry out the total polyphenol content of the lyophilized (freeze dried) Sea buckthorn. According to SINGLETON et al., (1999) the phenolic content was determined using the Folin-Ciocalteu colorimetric method. The phenolic compounds reduce a phosphomolybdic-phosphotungstic acid complex and make a blue color which is measured bt the spectrophotometer. Gallic acid was used as calibration which the calculations indicate gallic acid equivalents (GAE) per gram of dry weight.

Materials

- Gallic acid (standard, 10 mg/100 mL in 80:20 methanol–distilled water)
- Folin–Ciocalteu reagent (0.2 N, prepared by diluting 2 N solution 10×)
- Sodium carbonate (Na_2CO_3) (75 g/L)
- Methanol (analytical grade)
- Distilled water
- Volumetric flasks, test tubes (10 mL), pipettes, filter paper
- Centrifuge (3,000–4,000 rpm)

- UV-Vis spectrophotometer (set at 760 nm)

Sample Preparation:

The sample preparation for TPC and flavonoid measurement is the same.

First weigh 5/10 g of sample and dilute to 50/100 ml (centrifuge tube/volumetric flask). Once all samples are prepared, let them stand for half an hour (vortexing/shaking every 10 minutes). Finally, filter them through filter paper into 100 ml Erlenmeyerflasks/centrifuge at 3–4000 rpm

Preparation of Calibration Solutions:

Stock solution preparation: Dissolve 10 mg of gallic acid in methanol–distilled water (80:20) and dilute to 100 ml. (100 mg/l) Use the gallic acid stock solution to prepare the calibration series, diluted with methanolic distilled water to a final volume of 10 ml, as follows:

- 0 mg/l: 0 ml gallic acid stock solution + 10 ml methanolic distilled water
- 5 mg/l: 0.5 ml gallic acid stock solution + 9.5 ml methanolic distilled water
- 10 mg/l: 1 ml gallic acid stock solution + 9 ml methanolic distilled water
- 20 mg/l: 2 ml gallic acid stock solution + 8 ml methanolic distilled water
- 50 mg/l: 5 ml gallic acid stock solution + 5 ml methanolic distilled water
- 100 mg/l: 10 ml gallic acid stock solution + 0 ml methanolic distilled water

Preparation of Reaction Mixtures:

- Prepare two 10 ml test tubes for each sample (repeat from here onwards), and one 10ml test tube for each calibration solution.
- Pipette 0.5 ml of each sample filtrate and calibration solution into the test tubes.
- Add 2.5 ml of 0.2 N Folin reagent to all solutions (including calibrators).
- Let it stand for 5 minutes.
- Add 2 ml of 75 g/l $Na2CO3$ solution.
- Let the samples rest in a dark cabinet for 2 hours.

Measurement:

Set the wavelength of 760 nm and measure using methanolic distilled water (blank, 0 mg/ml) to zero the UV-Vis, and wash the cuvette with the sample.

Solution preparation:

- **0.2 N Folin-Ciocalteu Reagent:** Pipette 10 ml of 2 N Folin-Ciocalteu reagent into a 100 ml volumetric flask and dilute to the mark with distilled water. Shake well.

- **75 g/l Na2CO3 Solution:** Weigh 7.5 g of Na2CO3 into a beaker, dissolve in distilled water, and quantitatively transfer to a 100 ml volumetric flask.
    - Dilute to the mark with distilled water and shake well.

3.7. Total Flavonoid Content (TFC)

The total flavonoid content was determined using the methods described by KIM - JEONG - LEE, 2003 using aluminium colorimetric methods with catechin as the standard.

Materials

- Catechin (standard, 10 mg/100 mL in 80:20 methanol–distilled water)
- Sodium nitrite ($NaNO_2$) (5% w/v)
- Aluminum chloride ($AlCl_3$) (10% w/v)
- Sodium hydroxide (NaOH) (1 M)
- Methanol and distilled water
- Volumetric flasks, pipettes, test tubes (10 mL), filter paper
- Centrifuge (3,000–4,000 rpm)
- UV-Vis spectrophotometer (set at 510 nm)

Methods

Determination of Total Flavonoid Content

Stock solution preparation: Dissolve 10 mg of catechin in methanol–distilled water (80:20) and dilute to 100 ml. (100 mg/l)

- 0 mg/ml (blank): 10 ml methanolic distilled water
- 20 mg/l: 2 ml catechin stock solution + 8 ml methanolic distilled water
- 40 mg/l: 4 ml catechin stock solution + 6 ml methanolic distilled water
- 60 mg/l: 6 ml catechin stock solution + 4 ml methanolic distilled water
- 80 mg/l: 8 ml catechin stock solution + 2 ml methanolic distilled water
- 100 mg/l: 10 ml catechin stock solution + 0 ml methanolic distilled water

Preparation of Reaction Mixtures:

- Prepare two 10 ml test tubes for each sample (repeat from here onwards), and one 10ml test tube for each calibration solution, plus one test tube for the blank.
- Pipette 4 ml of pure distilled water into each test tube, except for the blank, which gets 5 ml (instead of the sample, it contains distilled water).
- Pipette 1 ml of each sample filtrate and calibration solution into the test tubes.

- Add 0.3 ml of 5% NaNO2 solution to all solutions (including calibrators and the blank).
- Let it stand for 5 minutes.
- Add 0.3 ml of 10% AlCl3 solution.
- Let it stand for 1 minute.
- Add 2 ml of 1 M NaOH solution.
- Add 2.4 ml of distilled water.

The samples can now be measured immediately.

Measurement:

Measure at a wavelength of 510 nm, using methanolic distilled water as the blank for calibration.

Solution Preparation:

- **5% NaNO2 Solution**: Weigh 5 g NaNO2 into a beaker and dissolve in approximately 20 ml of distilled water. Quantitatively transfer the solution to a 100 ml volumetric flask and dilute to the point.
- **10% AlCl3 Solution:** Weigh 10 g of AlCl3 into a beaker and dissolve in approximately 20 ml of distilled water. Transfer the solution quantitatively to a 100 ml volumetric flask and dilute to the point.
- **1 M NaOH Solution:** Weigh 40 g of NaOH into a beaker and dissolve in approximately 200 ml of distilled water. Transfer quantitatively to a 1000 ml volumetric flask and dilute to the mark. Alternatively weigh 4g of NaOH and dissolve it 100 ml distilled water.

4. Results and their Evaluation

4.1 Contents of the sample

From 250.01g of total weight, 46.19g were recorded for its freeze-dried result. From this we can conjure its water content which is visible on Figure 6. This was calculated by deducting dry weight of 46.19g from the fresh fruit sample weight of 250.01g we get 203.82g which is divided by the raw fruit sample and multiplied by 100 which gives us 81.52% moisture content.

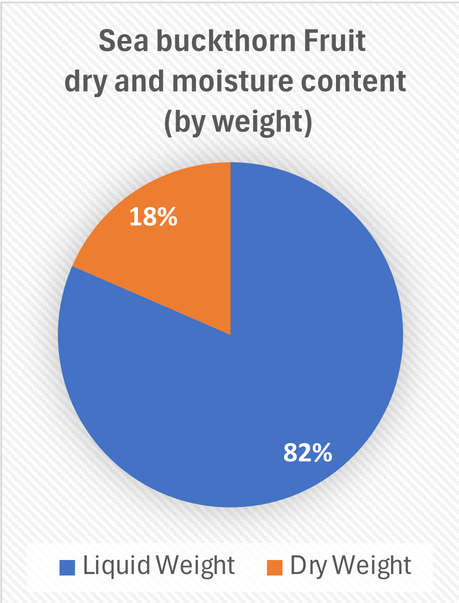

Figure 6: **Sea buckthorn Fruit sample dry and moisture content by weight.**

Source: Author (2025)

4.2. Fly experiments.

4.2.1. Zero Media

For this media type since it did not contain any nutrition to the flies except our sample of sea buckthorn, it is evident that based on the composition of the berries, it contains all the necessary nutrients for the flies to carry out their development. We can observe on Figure 6 that as the concentration is increased their viability surely increases as there are more nutrients to go around. The Table 3 shows the data of the pupae recorded on the different concentrations. Each concentration had a repeat of 5 and shown on the table below is the average number of the 5 datasets. As we can see the highest amount of pupae emerged are from the concentration of 80g/L Z1 being 28.2 pupae cumulatively following the 40g/L Z2 at 25.8, 20g/L Z3 at 21.6, 10g/L Z4 at 8.8 and finally 5g/L Z5 at 1.2 pupae emerging. Similar pattern can be observed from the Table 3 Fly data. From this data we derived the viability of the flies by dividing the values by 50 then multiplied by 100 to get the viability percentage of the flies. The results are visualized on Figure 7.

Table 3: Daily Pupa and Fly count data of the Zero Media (Z) experiments.

Day	Z1 (80g/L) Pupa	Z2 (40g/L) Pupa	Z3 (20g/L) Pupa	Z4 (10g/L) Pupa	Z5 (5g/L) Pupa	Z1 (80g/L) Fly	Z2 (40g/L) Fly	Z3 (20g/L) Fly	Z4 (10g/L) Fly	Z5 (5g/L) Fly
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0
5	0	9.8	0	0	0	0	0	0	0	0
6	12.8	20.2	12.0	0.0	0	0	0	0	0	0
7	26.2	24.8	17.6	0.8	0	0	0	0	0	0
8	28.2	25.2	20.0	3.0	0.2	4	0.2	0.2	0	0
9	28.2	25.8	21.0	4.8	0.4	23	3.8	1.6	0	0
10	28.2	25.8	21.6	6.8	0.6	23.4	13.4	5	0	0
11	28.2	25.8	21.6	7.2	0.6	24.4	18.2	10.6	0.4	0
12	28.2	25.8	21.6	7.2	0.6	24.6	19.2	12.4	1.4	0.2
13	28.2	25.8	21.6	7.4	0.8	24.6	20	13.4	2.4	0.2
14	28.2	25.8	21.6	7.6	0.8	24.6	20	13.8	3	0.2
15	28.2	25.8	21.6	7.6	1	24.6	20.2	14	3.2	0.2
16	28.2	25.8	21.6	8.8	1	24.8	20.2	14.8	4.4	0.2
17	28.2	25.8	21.6	8.8	1	24.8	20.4	14.8	4.8	0.2
18	28.2	25.8	21.6	8.8	1.2	24.8	20.4	14.8	4.8	0.4

On Figure 5, is an example of Z2-04, 04 being repeat number 4. Stated in methods sections, these zero media trials concentrations each had 5 repeats to compare and average between them. When counted, the pupa was marked with one colour on the glass and another colour the next day and so on so that there were no double counting. Furthermore to ensure the numbers were correct, each of the vials were counted 3 times during monitoring hours then once again once the experiment has finished.

Figure 7: **Zero Media Z2 vial markings on pupa and flies after counting.**

Source: Author (2025)

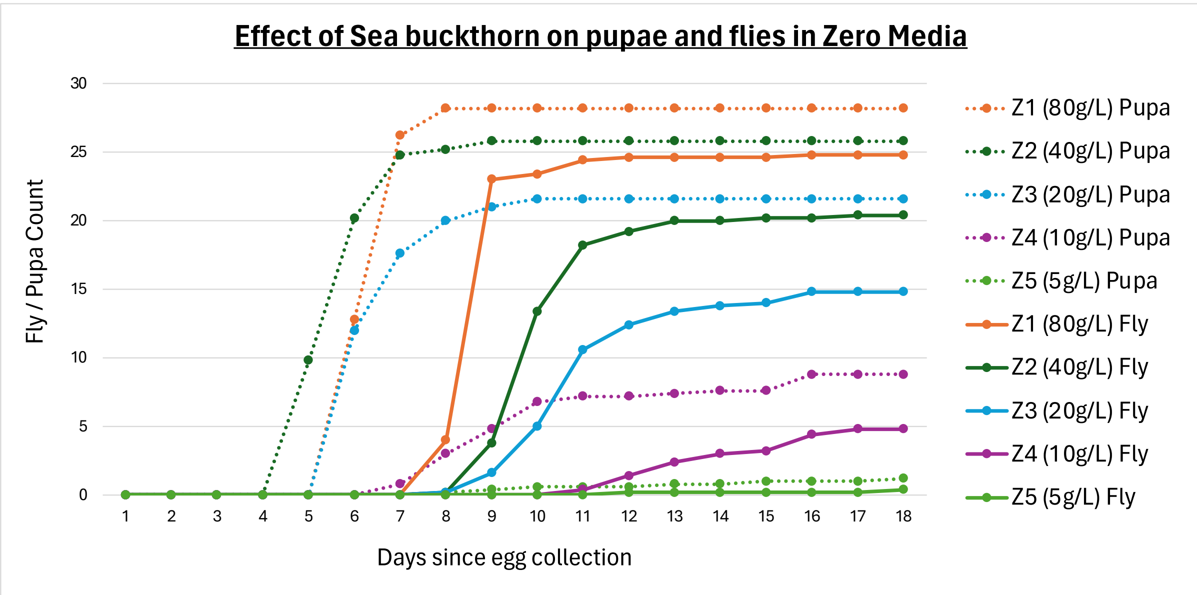

Figure 8: **Effect of Sea buckthorn on pupae and flies in Zero Media**

Source: Author (2025)

For the visualization of the pupa/fly count, Z1 is in orange the pupas as dotted lines and the flies regular lines. Z2 is dark green, Z3 blue, Z4 purple, and Z5 as light green

The viability calculations, the pupa to fly viability was calculated. This calculation shown in Figure 7 green bars represents the percentage of flies hatched from the pupae. The percentage of the green bars was calculated by the average fly viability of a concentration divided by the pupa viability then multiplied by 100. The standard deviation was calculated from the repeats and shown in Table 4. From this data we can see an ascending pattern as the concentration is doubled showing the biggest increase in viability between Z4 and Z3 by 20% in flies and 25.6% in pupae.

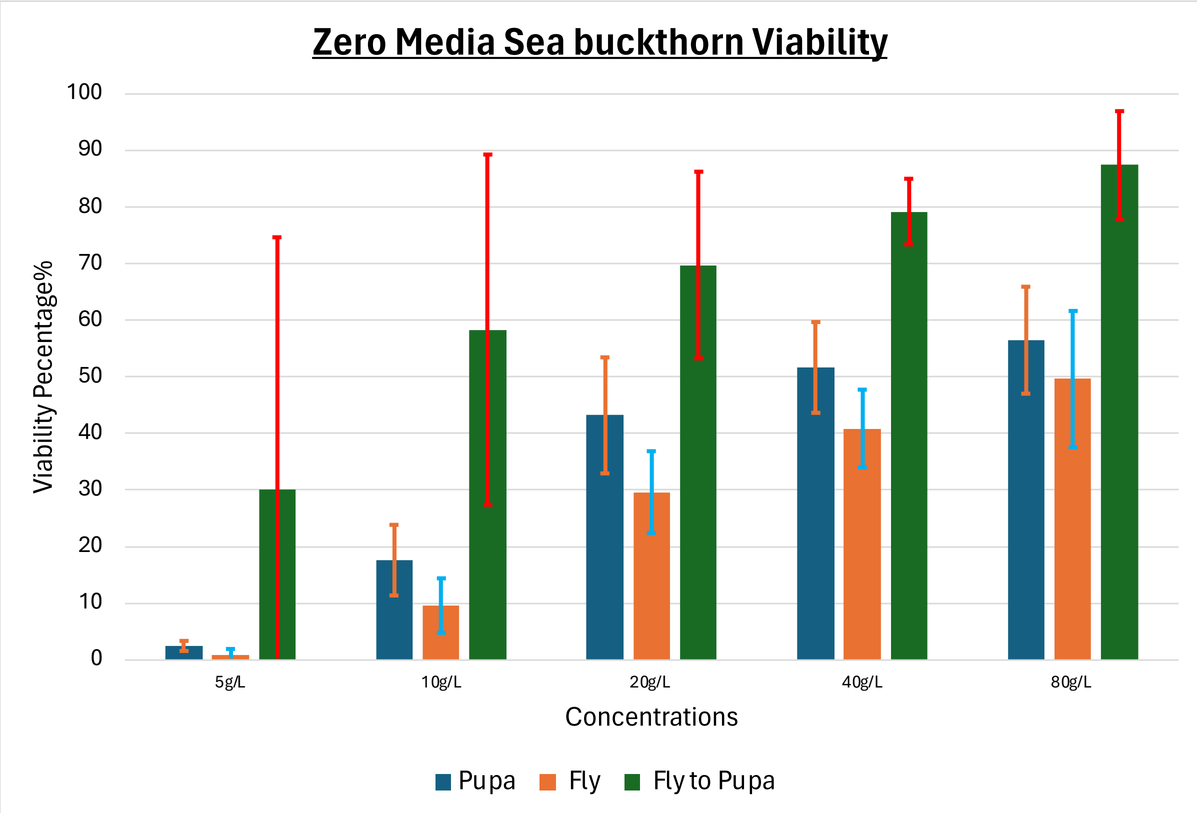

Figure 9: **Total Viability visualization of Zero Media fruit flies.**

Source: Author (2025)

Table 4: Total Viability Data of Zero Media fruit flies with Standard Deviations.

Concentration	Pupa	Fly	Fly to Pupa	σ Pupa	σ Fly	σ Fly to Pupa
Z5 (5g/L)	2.4	0.8	30	0.89	1.1	44.72
Z4 (10g/L)	17.6	9.6	58.3	6.2	4.8	30.9
Z3 (20g/L)	43.2	29.6	69.7	10.3	7.3	16.5
Z4 (40g/L)	51.6	40.8	79.1	8.0	6.9	5.8
Z5 (80g/L)	56.4	49.6	87.4	9.4	12.1	9.6

4.2.2. Normal Media

The Normal Media (NM) results show the most prominent results from the other experiments. As shown in Figure 9, the different concentrations in columns are set from lowest concentration (2.5g/L) to highest concentration left to right. The colour of the sea buckthorn fruit pigment gets stronger as the concentration increases. In table 5, the daily pupa count and fly count are recorded and averaged from the 3 repeats of each concentration. The average of the 2 controls are also calculated in the same table, thus used in the line graph in Figure 10. The controls are shown in distinct red colour bold lines in flies and dotted lines in pupae to show the comparison of their development apart from the rest of the concentrations.

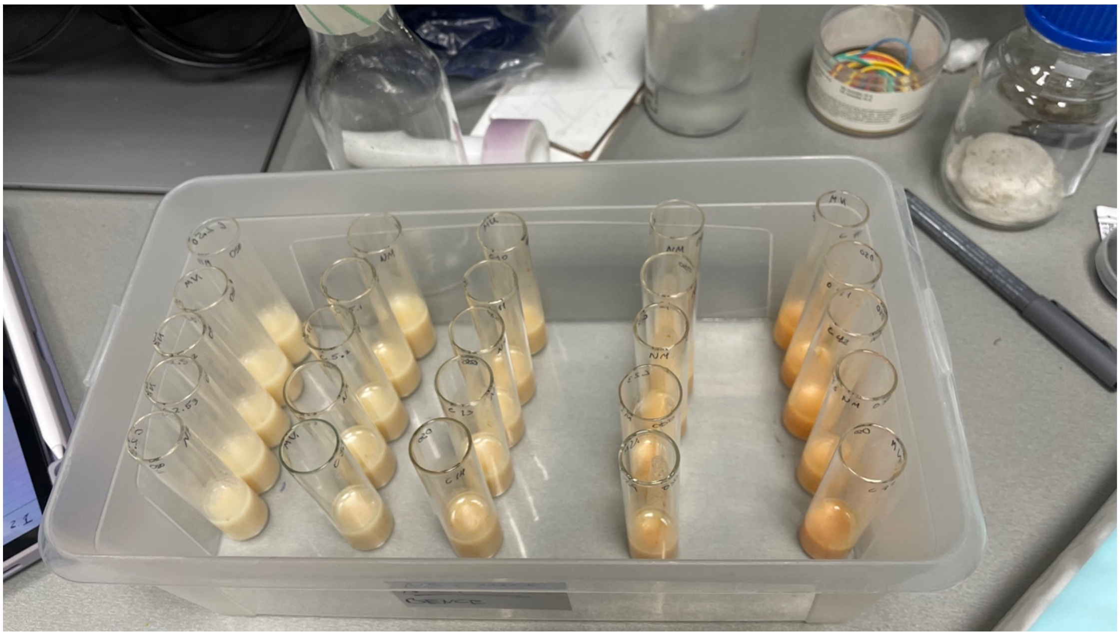

Figure 10: **Normal Media concentrations distributed into vials. 5 repeat concentrations per column order from left to right N1, N2, N3, N4, N5 respectively.**

Source: Author (2025)

Table 5: Daily Pupa and Fly count of Normal media.

Day	CN 01 Pupa	CN 02 Pupa	CN Pupa Average	N1 (40g/L) Pupa	N2 (20g/L) Pupa	N3 (10g/L) Pupa	N4 (5g/L) Pupa	N5 (2.5g/L) Pupa
1	0	0	0	0.0	0.0	0.0	0.0	0.0
2	0	0	0	0.0	0.0	0.0	0.0	0.0
3	0	0	0	0.0	0.0	0.0	0.0	0.0
4	0	0	0	0.0	0.0	0.0	0.0	0.0
5	3	4	3.5	0.0	0.0	0.7	0.3	1.7
6	23	26	24.5	8.7	26.3	25.3	25.0	28.3

7	24	28	26	32.7	33.7	32.3	33.0	34.7
8	24	28	26	33.7	34.3	33.7	33.3	35.0
9	24	28	26	33.7	34.3	34.0	33.7	35.7
10	24	29	26.5	34.7	35.7	34.3	33.7	36.3
11	24	29	26.5	34.7	35.7	34.3	33.7	36.7
12	24	29	26.5	34.7	35.7	34.3	33.7	36.7
13	24	29	26.5	34.7	35.7	34.3	33.7	36.7
14	24	29	26.5	34.7	35.7	34.3	33.7	36.7
15	24	29	26.5	34.7	35.7	34.3	33.7	36.7
Day	CN 01 Fly	CN 02 Fly	CN Fly Average	N1 (40g/L) Fly	N2 (20g/L) Fly	N3 (10g/L) Fly	N4 (5g/L) Fly	N5 (2.5g/L) Fly
1	0.0	0.0	0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0	0.0	0.0	0.0	0.0	0.0
4	0.0	0.0	0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0	0.0	0.0	0.0	0.0	0.0
8	1.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0
9	21.0	2.0	11.5	0.0	0.0	1.0	1.0	1.7
10	23.0	23.0	23	15.7	27.0	25.7	24.0	27.0
11	23.0	26.0	24.5	31.3	32.0	33.0	30.3	33.7
12	23.0	26.0	24.5	32.7	32.3	33.7	31.3	33.7
13	23.0	26.0	24.5	33.3	33.0	34.0	32.0	33.7
14	23.0	26.0	24.5	33.7	33.0	34.0	32.3	34.7
15	23.0	26.0	24.5	33.7	33.0	34.3	32.3	34.7

Figure 10 shows how much the sea buckthorn helped the development of the *Drosophila melanogaster.* We can observe a one day delay from the samples possibly due to the extra carbohydrate content from the sea buckthorn however all of the concentrations looks like they did significantly better according to the increase in number of pupa and flies **7.2 to 10.2 more pupae** than control pupae. Similar to pupae the emergence of flies showed the same **one day delay** as well as **7.8 to 10.2 more flies** than the control. Samples show higher viability than the control. T-test will be performed to check for significance in the next subchapters.

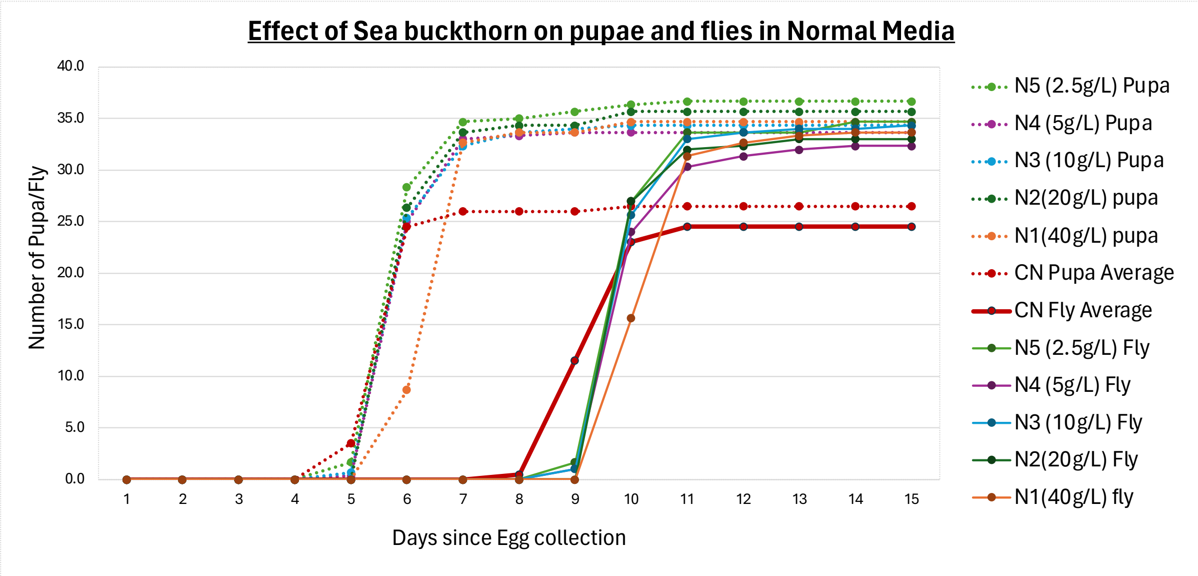

Figure 11: **Effect of Sea buckthorn on Pupae and Flies in Normal Media**

Source: Author (2025)

Figure 10 only shows the count of the pupa and fly and it does not reveal its viability percentage. Table 6 shows the actual viability of the normal media on sea buckthorn. The viability was calculated according the following formulas and the results in Table 6.

$$Viability\ of\ Pupa = \frac{cumulative\ pupa}{50\ (total\ number\ of\ eggs)} * 100$$

$$Viability\ of\ fly = \frac{cumulative\ flies}{50\ (total\ number\ of\ eggs)} * 100$$

$$Viability\ from\ Pupa\ to\ Fly = \frac{Viability\ of\ Fly}{Viability\ of\ Pupa} * 100$$

Then, to calculate the relative viability to visualize how much greater or less the viability was compared to the control the following formula was utilized.

$$Relative\ Viability = \frac{Pupa\ viability(e.g\ N1) OR\ Fly\ viability}{own\ control\ pupa\ OR\ fly\ viablity\ (e.g\ CN\ 01)} * 100$$

To elaborate on the formula, using the specific control used per each concentration is crucial in determining the comparison between the control and the different media types. The same type of calculations were also carried out in High sugar media concentrations viability concentrations. The control was divided by itself and multiplied by 100 to get the 100%. Then the results of this calculations can be found on Table 7 of the relative viability of the normal media flies on sea buckthorn. For example N1's pupa viably is 144.44% which is 1.4 times higher than the its' control. From our results, all of the concentrations' viability were higher than the control, meaning the sea buckthorn showed positive results we were expecting. The visualization is available on Figure 11. The red line drawn across represents the control line as 100%, the blue bar represents the relative pupa viability, and the orange bar represents the hatched flies' viability.

Table 6: Total Viability of Normal media on Sea buckthorn

Concentrations	Pupa %	Fly %	Fly to pupa %
CN	53.00	49.00	92.74
N1 (40g/L)	69.33	67.33	97.25
N2 (20g/L)	71.33	66.00	93.84
N3 (10g/L)	68.67	68.67	100.00
N4 (5g/L)	67.33	64.67	95.37
N5 (2.5g/L)	73.33	69.33	94.83

Table 7: Relative viability of Normal Media flies on Sea buckthorn

Concentrations	Pupa %	Fly %	Fly to Pupa %	σ Pupa %	σ Fly %	σ Fly to Pupa%
CN	100.00	100.00	100.00	7.07	4.24	4.37
N1 (40g/L)	144.44	146.38	101.47	8.08	8.08	7.50
N2 (20g/L)	122.99	126.92	104.67	16.29	8.72	9.44
N3 (10g/L)	129.56	140.14	107.82	8.08	10.07	9.38
N4 (5g/L)	140.28	140.58	99.52	6.43	12.86	10.52
N5 (2.5g/L)	152.78	150.72	98.95	5.03	2.31	6.76

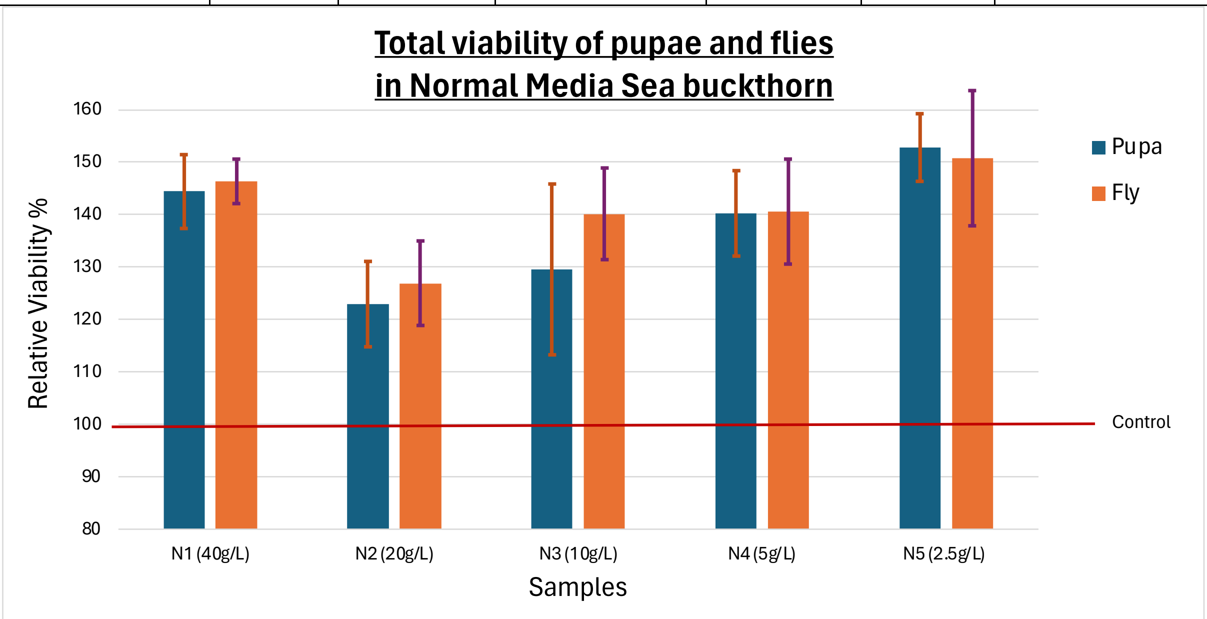

Figure 12: **Relative Viability of Pupae and Flies in Normal Media Seabuckthorn**

Source: Author (2025)

4.2.3. High-sugar Media

The high-sugar media trials were run the same way as the normal media trials. The calculations of the viability were done the same way as the formulas mentioned in normal media results. One difference we can see it that Figure 12 which visualizes the viability of the fruit flies in the HS diet supplemented with sea buckthorn is instead of the count of fly to pupa the viability percentage is shown. The curves will ultimately look the same. In Figure 12 we can clearly see any curve that is above the red line for fly viability and the dotted red line for pupa suggests a "rescue" effect as the HS media induces a diabetic like state for the development of the flies. To observe, H5 both pupa and fly did significantly better than their corresponding controls. Further specific details can be taken from Table 8. An outlier that seems to appear was H1 fly. Although the pupae viability was higher than the control the flies that hatched seemed to have a stunt in their development. Ultimately, the T test revealed the H1 results to be significant, it still is not convincing data hence a repeat experiment is highly likely.

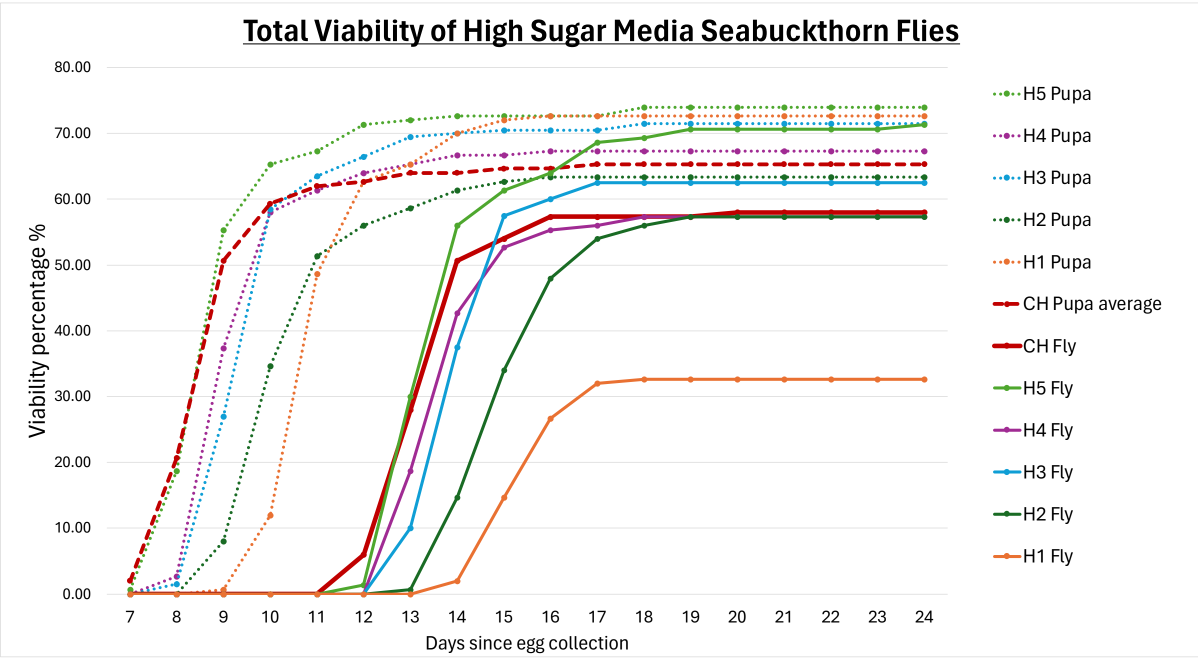

Figure 13: **Effect of Sea buckthorn on High Sugar Media (HS) Pupa and Fly**

Source: Author (2025)

Table 8: Daily Count of Pupa and Fly of High Sugar Media

Da	CH	CH	CH	CH	H1(40g/	H2(20g/	H3	H4	H5

y	11 Fly	21 Fly	04 Fly	Average	L) Fly	L) Fly	(10g/L) Fly	(5g/L) Fly	(2.5g/L) Fly
6	0	0	0	0.0	0	0	0	0	0
7	0	0	0	0.0	0	0	0	0	0.0
8	0	0	0	0.0	0	0	0	0.0	0.0
9	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0
10	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0
11	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0
12	8	0	1	3.0	0.0	0.0	0.0	0.0	0.7
13	18	10	14	14.0	0.0	0.3	5.0	9.3	15.0
14	24	30	22	25.3	1.0	7.3	18.8	21.3	28.0
15	24	32	25	27.0	7.3	17.0	28.8	26.3	30.7
16	25	32	29	28.7	13.3	24.0	30.0	27.7	32.0
17	25	32	29	28.7	16.0	27.0	31.3	28.0	34.3
18	25	32	29	28.7	16.3	28.0	31.3	28.7	34.7
19	25	32	29	28.7	16.3	28.7	31.3	28.7	35.3
20	25	33	29	29.0	16.3	28.7	31.3	28.7	35.3
21	25	33	29	29.0	16.3	28.7	31.3	28.7	35.3
22	25	33	29	29.0	16.3	28.7	31.3	28.7	35.3
23	25	33	29	29.0	16.3	28.7	31.3	28.7	35.3
24	25	33	29	29.0	16.3	28.7	31.3	28.7	35.7
Day	**CH 11 Pupa**	**CH 21 Pupa**	**CH 04 Pupa**	**CH Average**	**H1(40g/L) Pupa**	**H2(20g/L) Pupa**	**H3 (10g/L) Pupa**	**H4 (5g/L) Pupa**	**H5 (2.5g/L) Pupa**
6	0.0	0	0	0.0	0	0	0	0	0
7	3.0	0	0	1.0	0	0	0	0	0.3
8	16.0	8	7	10.3	0	0	0.8	1.3	9.3
9	25.0	32	19	25.3	0.3	4.0	13.5	18.7	27.7
10	28.0	34	27	29.7	6.0	17.3	29.3	29.0	32.7
11	28.0	36	29	31.0	24.3	25.7	31.8	30.7	33.7
12	28.0	36	30	31.3	31.3	28.0	33.3	32.0	35.7
13	28.0	36	32	32.0	32.7	29.3	34.8	32.7	36.0
14	28.0	36	32	32.0	35.0	30.7	35.0	33.3	36.3
15	28.0	37	32	32.3	36.0	31.3	35.3	33.3	36.3
16	28.0	37	32	32.3	36.3	31.7	35.3	33.7	36.3
17	28.0	37	33	32.7	36.3	31.7	35.3	33.7	36.3
18	28.0	37	33	32.7	36.3	31.7	35.8	33.7	37.0
19	28.0	37	33	32.7	36.3	31.7	35.8	33.7	37.0
20	28.0	37	33	32.7	36.3	31.7	35.8	33.7	37.0
21	28.0	37	33	32.7	36.3	31.7	35.8	33.7	37.0

22	28.0	37	33	32.7	36.3	31.7	35.8	33.7	37.0
23	28.0	37	33	32.7	36.3	31.7	35.8	33.7	37.0
24	28.0	37	33	32.7	36.3	31.7	35.8	33.7	37.0

The result of the fly viability calculations can be seen on Table 9 and for Table 10 the relative viability, explained in NM, where the controls are averaged and is set 100% to show the comparison between the different concentration and their corresponding controls. We can see the difference in their viability on Figure 13. Where, again the control is marked as the red line anything higher or lower indicate their performance. The most prominent result is H5 with the smallest concentration of 2.5g/L seemed to have the most positive effect on the development of the *Drosophila melanogaster*. The H5 flies had 1.22 times more viability than the controls. No dose-response curve seemed to be in place.

Table 9: Total Viability of High Sugar Media flies on Sea buckthorn

Concentrations	Pupa	Fly	Fly to Pupa
CH Average	**65.33**	**58.00**	**88.78**
H1	72.67	32.67	44.74
H2	63.33	57.33	90.86
H3	71.50	62.50	87.34
H4	67.33	57.33	85.08
H5	74.00	71.33	96.42

Table 10: Relative Viability of the flies under High sugar media

Concentrations	Pupa	Fly	Fly to Pupa	σ Pupa	σ Fly	σ Fly to Pupa
CH Average	100.00	100.00	100.00	9.02	8.00	0.79
H1	111.22	56.32	50.39	4.13	14.00	13.01
H2	96.94	98.85	102.34	12.77	14.95	15.37
H3	109.44	107.76	98.37	9.41	9.44	5.50
H4	103.06	98.85	95.83	8.75	9.95	1.39
H5	113.27	122.99	108.60	6.19	6.11	1.63

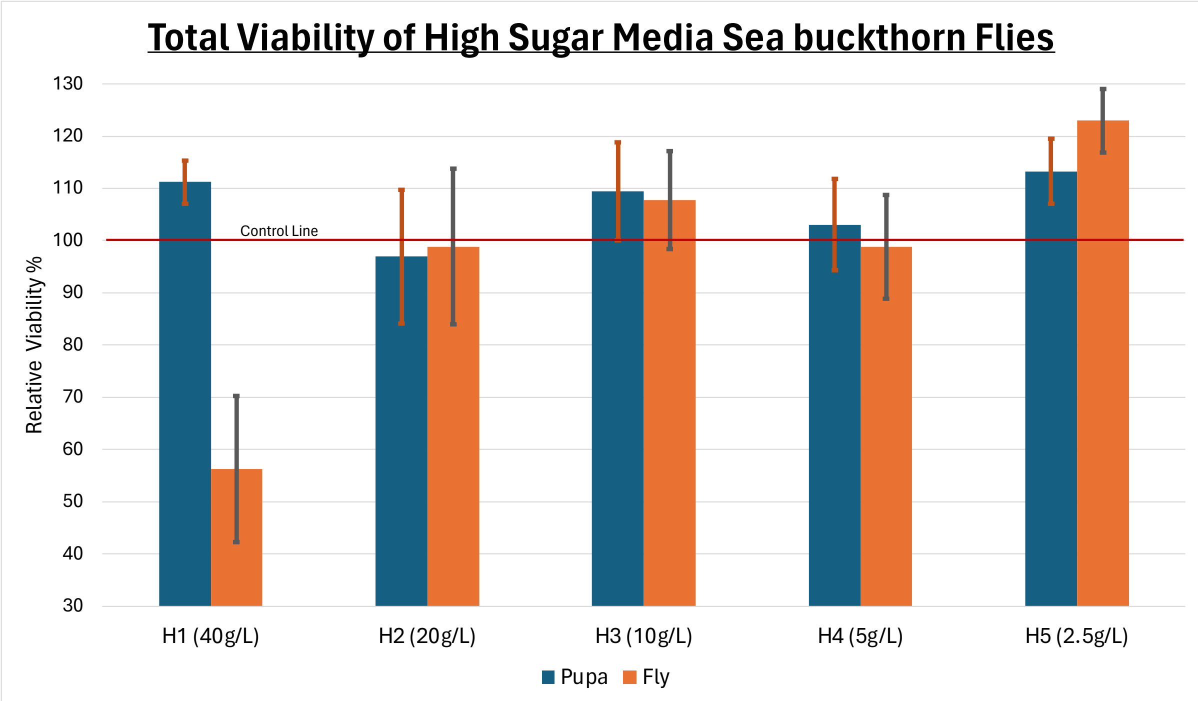

Figure 14: **Relative Viability of High Sugar Media Sea buckthorn**

Source: Author (2025)

4.2.4.T-test for Significance:

A T-test for the p-value was conducted as calculated, the results shown in Table 11 and Table 12 Normal media and High sugar media respectively can be witnessed. In these results, the values indicated in bold show significant values being $p<0.05$ indicating the results are reliable and due to coincidence comparing the results to the controls indicated at CN for normal media and CH for high sugar media. As shown in the Tables 11 and 12 the key starts with SB as Sea buckthorn then UJ as the authors initials, the date written in year-month-day order then the media concentration and the last number indicating the number of repeat. The calculation was performed on Microsoft excel using the p-value formula. From the calculations, concentration N5 showed significant values of 0.03 and 0.01 on cumulative pupa and cumulative fly viability respectably. For the High sugar, Table 12, shows a different result. Only on the Cumulative Fly concentrations H5 and H1 showed significant figures being 0.05 and 0.02 respectably.

There were no T test carried out on Zero media due to there are no control to compare the results to.

Table 11: Result of the T-test on Normal Media Sea buckthorn Viability

Key	Time(day)	Cumulative Pupa	Cumulative Fly
SB-UJ-241120-N5-03	15	39.00	34
SB-UJ-241120-N5-02	15	37.00	36
SB-UJ-241120-N5-01	15	34.00	34
p-value		**0.03**	**0.01**
SB-UJ-241120-N4-03	15	30.00	25
SB-UJ-241120-N4-02	15	36.00	37
SB-UJ-241120-N4-01	15	35.00	35
p-value		0.10	0.21
SB-UJ-241120-N3-03	15	39.00	39
SB-UJ-241120-N3-02	15	32.00	35
SB-UJ-241120-N3-01	15	32.00	29
p-value		0.11	0.09
SB-UJ-241120-N2-03	15	30.00	31
SB-UJ-241120-N2-02	15	45.00	38
SB-UJ-241120-N2-01	15	32.00	30
p-value		0.24	0.09
SB-UJ-241120-N1-03	15	34.00	36
SB-UJ-241120-N1-02	15	39.00	36
SB-UJ-241120-N1-01	15	31.00	29
p-value		0.10	0.06
SB-UJ-241120-CN-01	15	24.00	23
SB-UJ-241120-CN-02	15	29.00	26

Table 12: Result of the T-test on High Sugar Media Sea buckthorn Viability

Key	Time(day)	Cumulative Pupa	Cumulative Fly
SB-UJ-241122-H5-03	24	38.00	37
SB-UJ-241122-H5-02	24	38	36
SB-UJ-241122-H5-01	24	35	34
p-value		0.20	**0.05**
SB-UJ-241126-H4-03	19	32.00	27
SB-UJ-241126-H4-02	19	37.00	32
SB-UJ-241126-H4-01	19	32.00	27
p-value		0.76	0.91
SB-UJ-241126-H3-04	21	41.00	38
SB-UJ-241126-H3-03	21	36.00	31
SB-UJ-241126-H3-02	21	36.00	29
SB-UJ-241126-H3-01	21	30.00	27
p-value		0.41	0.54

SB-UJ-241127-H2-03	20	37.00	31
SB-UJ-241127-H2-02	20	28.00	23
SB-UJ-241127-H2-01	20	30.00	32
p-value		0.80	0.93
SB-UJ-241127-H1-03	19	38.00	19
SB-UJ-241127-H1-02	19	36.00	19
SB-UJ-241127-H1-01	19	35.00	11
p-value		0.25	**0.02**
SB-UJ-241127-CH-21	20	37.00	33
SB-UJ-241126-CH-04	18	33.00	29
SB-UJ-241122-CH-11	18	28.00	25

4.3 Phytochemical analysis

Based on the assays performed on the sea buckthorn berry samples results look quite promising in which the results of the tests are shown on Table 13. Using the moisture content from 4.1 81.52% liquid content of the fruit, we can calculate in reverse how much of the values are for the fresh fruit sample for the performed tests. If 1132.87 was in 18.47% of lyophilized sample the following equations shows how it was calculated.

$\frac{100\%}{18.47\%} = 5.4126 \;\; (test\,)1132.87 \div 5.4126 = 209.3$ mgGAE/ml per 100g fresh fruit

The rest of the values were calculated the same only substituting the test value.

From the FRAP and DPPH assays recalculated fresh fruit values have quite considerable antioxidant activity 3.85 µmol AAE/g and 7.04 µmol TE/g. In Figure 15, the potency of the freeze dried sample is notable having a much higher peak. When compared with other common fruits, sea buckthorn shows strong results. For example, fresh blueberries show FRAP values between 2.0-4.0 µmol AAE/g and DPPH values of 5-9 µmol TE/g (WU et al., 2004). Alternatively, strawberries and raspberries show 2.5-4.5 µmol AAE/g and DPPH of 6-10 µmol TE/g.

Table 13: Phytochemicals and Antioxidant properties of Sea buckthorn sample

Test	Unit	Sea buckthorn lyophilized sample	Fresh fruit value per 100g
FRAP	µmol AAE/g	20.84±0.62	3.85
DPPH	µmol TE/g	38.12±0.79	7.04
TFC	mg RE/mL	414.22±55.05	76.53
TPC	mg GAE/mL	1132.87±55.05	209.30

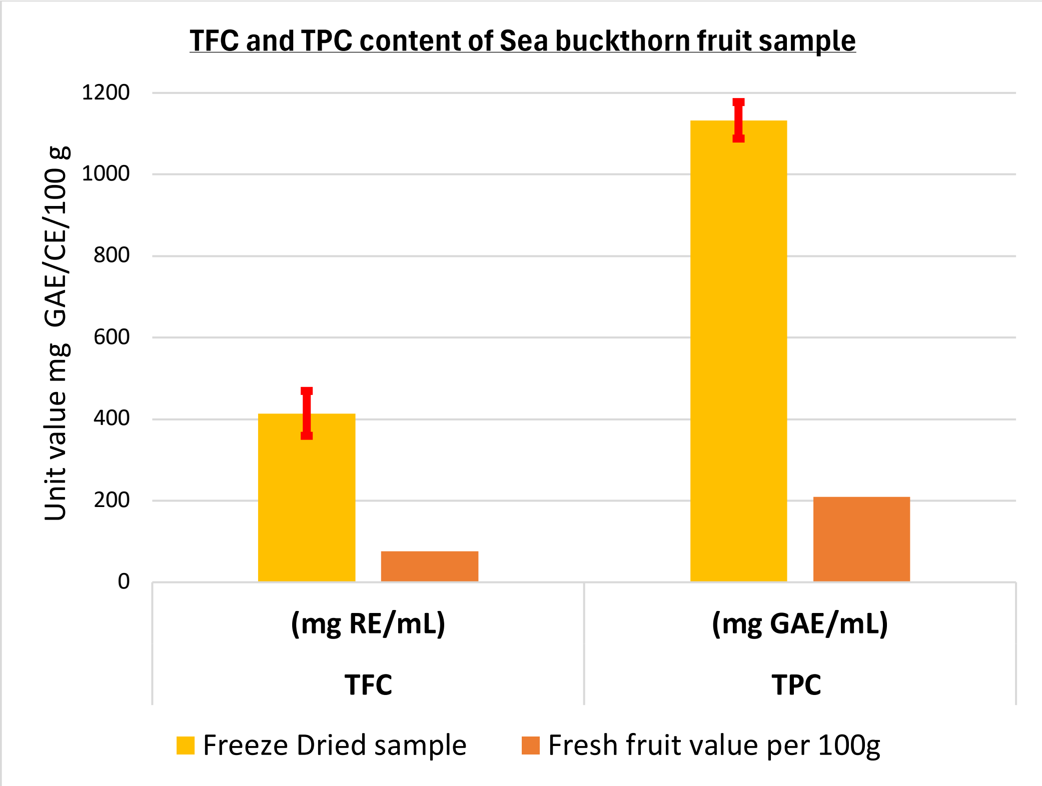

Figure 15: **TPC and TFC content of Sea buckthorn fruit sample**

Source: Author (2025)

The total phenolic content as well as flavonoid contents of Mongolian sea buckthorn were assessed to enhance the understanding of its antioxidant potential. The lyophilized sample showed a TPC of 1132.87±55.05 mg GAE/mL and TFC of 414.22±55.05 mg RE/mL and the recalculated values of the fresh fruit value are 209.30 mg GAE/mL and 76.53 mg RE/mL found in Table 13 and visualized in Figure 14. These values imply that the sea buckthorn sample is exceptionally rich in polyphenolic and flavonoid compounds which both play key roles in neutralizing free radicals and reducing oxidative stress which were mentioned in chapter 2 of its notable benefits.

Compared with other fruits, sea buckthorn marks a superior polyphenol and flavonoid concentrations. For instance, blueberries and black berries usually contain TPC values of 150-250 mg GAE/100g fresh weight and TFC of 20-50mg RE/100g (SCALBERT et al., 2005; NILE-PARK, 2014) in which the recalculated fresh sea buckthorn fruit samples could exceed these values. Once again reinforcing its stance as a strong source of natural antioxidants given the harsh climate and limited agricultural biodiversity in Mongolia.

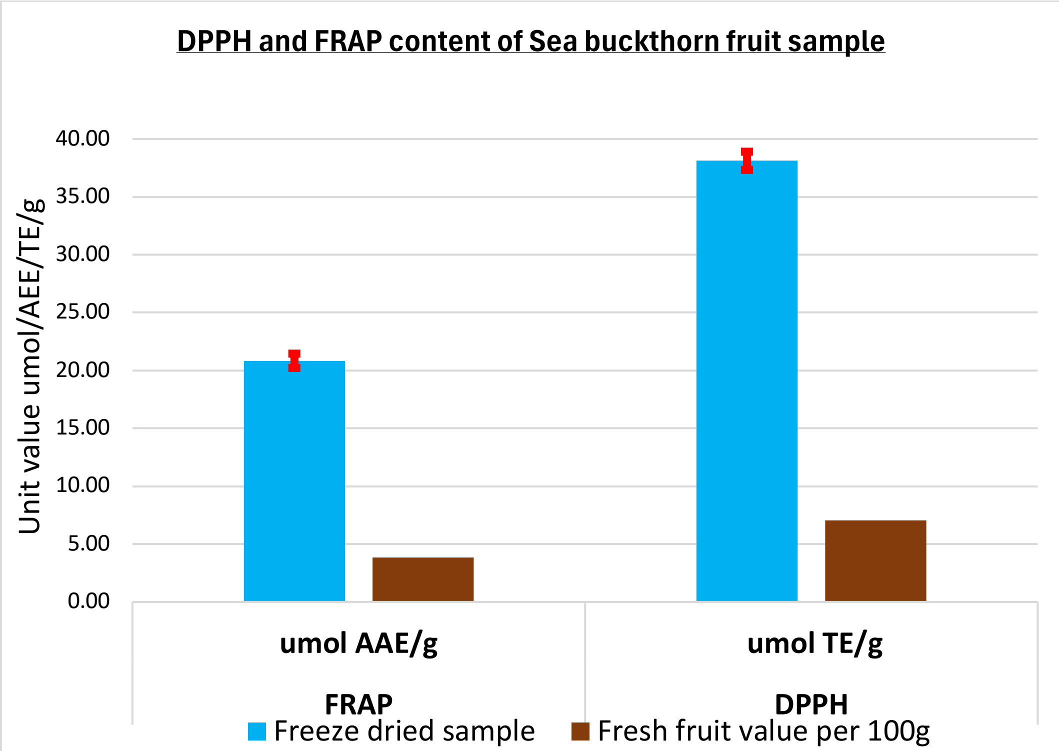

Figure 16: **DPPH and FRAP content of Sea buckthorn fruit sample**

Source: Author (2025)

4.4. Product Development

The muffins were baked according to the steps, Figure 13 displays the end result. On the lower right-hand side of each muffin indicates "A" as the control muffin which does not contain any sea buckthorn and "B" is our sea buckthorn infused muffin.

Figure 17: **Lactose-free, Gluten-free, Vegetarian muffins: A: control muffin B: Sea buckthorn muffins**

Source: Author (2025)

4.4.1. Texture Analysis

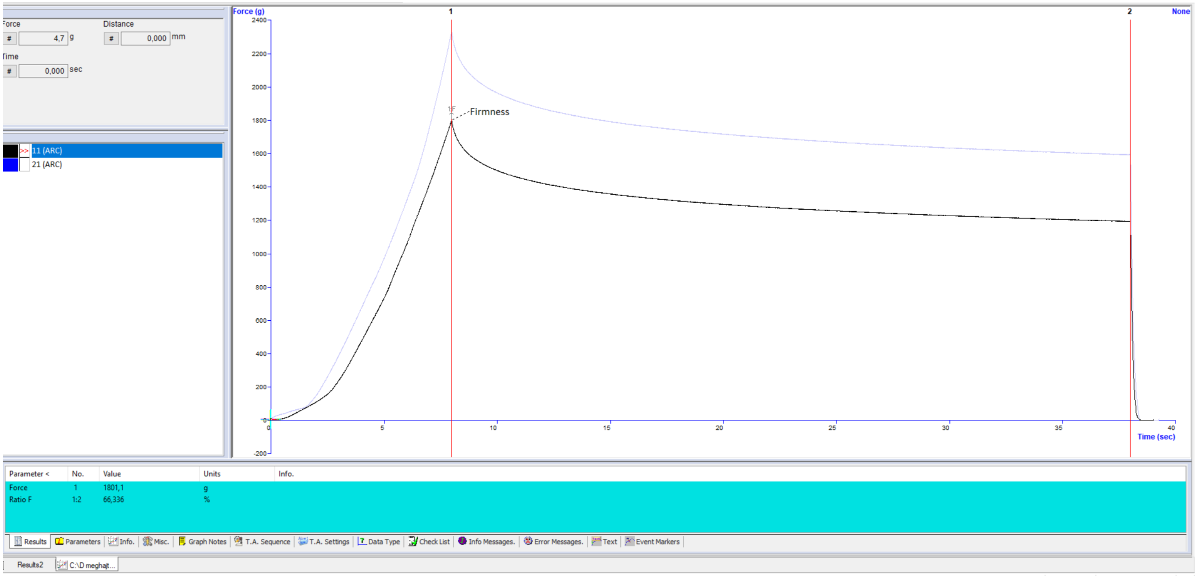

Figure 18: **Texture analyzer Force-Time curve performed on Muffins A and B**

Source: (Agricultural Instrument Center) Agrárműszerközpont (2025)

Table 14: Measurement of the firmness and springiness of muffins

Test ID	Firmness	Springiness
	g	%
	Force 1	Ratio F 1:2
Muffin A	**1801.05**	**66.34**
S.D.	0	0
C.V.	0	0
Muffin B	**2333.27**	**68.32**
S.D.	0	0
C.V.	0	0

The texture analysis shows that the addition of sea buckthorn improves overall firmness by 532.22g and springiness by 1.98% deducted from Table 14. Furthermore Figure 17 shows the Force-Time curve where Muffin A is the darker curve and Muffin B with the sea buckthorn slightly above it with to have a higher peak.

4.4.2 Phenolic compounds

Table 15: TPC and TFC content of muffin samples

Parameter	Muffin A	Muffin B
TPC (mg GAE/mL)	24.39 ±0.2	36.14 ±1.9
TFC (mg RE/mL)	N/A	5.07 ±1.1

From the TPC and TFC test the phenolic compounds were greatly reduced possibly due to the cooking process or simply the concentration added to the muffins was insufficient to be a potent source of neutralizing free radicals and reducing oxidative stress.

4.4.3 Organoleptic survey results

Due to the low number of contestants, sharing the results of the organoleptic survey is insufficient. To summarize the overall score for muffin A was 5 as like slightly for the sensory evaluation and 6 for Muffin B to like moderately. Muffin B was preferred among participants, indicating that Sea buckthorn enhanced overall appeal. Muffin A as the control received overall lower score Recommendations of improving texture and appearance were unanimous.

5. Conclusions and recommendations

Hippophae rhamnoides spp. mongolica has shown some significant results in this investigation. In the fly experiments the normal media diet with different sea buckthorn(SB) concentrations showed higher viability both pupa and fly than their controls with the N5 samples displaying most significant improvements. The high sugar media diet results show H5 and H1 flies are significant although a repeat is recommended to improve consistent results. Zero media results indicate that SB alone provides sufficient macronutrients to support both pupation and hatching in flies, with higher sea buckthorn concentrations resulting in better developmental outcomes suggesting potential dose response curve. Additionally, adding a genetic research from the effect of dieting sea buckthorn would be the next step to this research, tracking for biomarkers, metabolic pathway changes could be essential in understanding how effective it is.

From the phytochemical analysis, the lyophilized fruit sample is a potent source of bioactive compounds. Furthermore for the product development utilizing SB, contributed to a better texture and overall better evaluation than without.

As this investigation only uses one sample of 500g of sea buckthorn, a repeat in the experiments is highly favored as it will strengthen the results. Thus striving for statistical significance in the experiments recommended to prove the results did not conjure from coincidence.

From a broader perspective, this thesis contributes to the documentation and scientific validation of a culturally and ecologically important Mongolian plant. Given the global trend towards natural and functional ingredients, spp. *Mongolica* is well positioned for application in health food products, dietary supplements, and therapeutic food formulations.

In conclusion, this study validates the functional food potential of Mongolian sea buckthorn and encourages multidisciplinary research to explore its clinical efficacy, processing stability, and economic potential. With proper development and promotion, the fruit can gain recognition for its nutraceutical excellence and prevent many ailments.

Summary

This thesis explored the nutritional composition and antioxidant potential of *Hippophae rhamnoides* spp. *Mongolica*, a sea buckthorn species native to Mongolia. The study sought to assess whether the particular sample of Mongolian sea buckthorn has qualities that justify its inclusion in health-promoting diets and nutraceutical applications given the increasing interest in natural antioxidants and functional foods. Investigated through both biological tests and chemical analysis.

The freeze-dried and powdered fruit samples were quantified and compared to calculated fresh fruit equivalents using combination of Total Polyphenol Content (TPC), Total Flavonois Content (TFC), DPPH (2,2-diphenyl-1picrylhydrazyl), and FRAP (Ferric Reducing Antioxidant Power) tests. TPC reached 1132.87 mg GAE/mL, TFC reached 414.22 mg RE/mL, and DPPH and FRAP values were 38.12 µmol TE/g and 20.84 µmol AAE/g, respectively. These values were incredibly high, which exceed typical antioxidant profiles of many well-known fruits.

To explore the biological relevance of these results, *Drosophila melanogaster* (fruit flies) were used in sugar manipulation experiments to simulate metabolic stress conditions. The sea buckthorn supplementation improved developmental outcomes in the flies with high sugar dies implying a protective antioxidative or metabolic effects.

The results taken together add to an increasing body of evidence that supports the sea buckthorn's health benefits, particularly in relation to oxidative stress and maybe metabolic diseases. The study not only highlights the scientific worth of a native Mongolian crop but also creates comparison for the sub species, creates more possibilities for its growing application in food technology, public health and international trade.

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Annex No. 3

List of tables and figures

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Figures

Annex No. 1

Thesis preparation form

The name of the student, Neptune code:..

Thesis topic: ....

....

Name of institute / department:..

Name of supervisor: ....

……………………..… ………………….…………… …………….……………..……

supervisor head of department/institute head of program

Number	Date of consultation	Subject of the consultation	Signature of the supervisor

The result of the similarity test of the diploma thesis: ………………………

The thesis can be submitted and defended.

Debrecen, ……………………………

……………………………………………

signature of the supervisor