DEVELOPMENT OF A FUNCTIONAL SNACK

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by
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ABSTRACT

DEVELOPMENT OF A FUNCTIONAL SNACK

Nowadays, with the increasing animal food prices, many people in our country are malnourished because they cannot get the necessary protein in their bodies. Additionally, with the Corona virus, which entered our lives in 2019, many people had to struggle with various mental disorders. Although we have left behind the pandemic period, negative emotional states such as depression and unhappiness are still observed in many people today. In this direction, vegetables rich in protein were investigated and used in developing functional snacks.

In this study, vegetable chips dough was prepared with 40% pea flour content 15, 17.5, 20, 22.5, and 25% radish, 20, 22.5, 25, 27.5, and 30% zucchini, and 10, 12.5, 15, 17.5, and 20% pumpkin seeds. Each vegetable chip mix was cooked at 50°C, 55°C, and 60°C with the aid of a tray dryer. The proximate analyses (moisture, ash, fat and protein), chemical analyses (antioxidant activity and total phenolic content), physical analyses (color and texture) and sensory analysis were carried out.

Mixture design was created with the help of MINITAB 16.0 (Minitab Inc., State College, PA, USA) program, the results of the analyses were evaluated statistically. Based on the design, it was decided to bake thirteen different mixtures at three different temperatures and to perform all the analyzes mentioned above. As a result of the evaluation of all analyses results, the optimized sample was determined as the vegetable chips sample with 40% pea flour, 22.5% zucchini, 25% radish, and 12.5% pumpkin seeds baked at 60°C. The vegetable chips, with this mixture, were baked at 50°C, 55°C and 60°C in the a tray dryer, and in addition to all analyses, sensory analysis and antioxidant activity analysis for all samples baked at 50°C, 55°C and 60°C were conducted. The sample baked at 60°C was the most liked sample.

ÖZET

FONKSİYONEL ATIŞTIRMALIK GELİŞTİRİLMESİ

Günümüzde artan hayvansal gıda fiyatlarıyla beraber başta ülkemizdeki insanlar olmak üzere birçok insan yeterli proteini alamadıklarından dolayı iyi beslenememektedir. Bununla beraber 2019 yılında hayatımıza giren korona virüs ile birçok insan, çeşitli mental rahatsızlıkla mücadele etmek durumunda kalmıştır. Pandemi dönemini geride bırakmamıza rağmen hala günümüzde birçok insanda depresyon, mutsuzluk gibi olumsuz duygu durumları gözlenmektedir. Bu doğrultuda proteince zengin sebzeler incelenerek fonksiyonel atıştırmalık geliştirilmesinde kullanılmıştır.

Bu çalışmada, %40 oranında bezelye unu içeriğine sahip sebze cips hamuruna %15, %17.5, %20, %22.5 ve %25 oranında turp, %20,%22.5,%25, %27.5 ve %30 oranında kabak ve %10, %12.5, %15, %17.5 ve %20 oranında ise kabak çekirdeği eklenmiştir. Her bir sebze cipsi karışımı 50°C, 55°C ve 60°C de tepsili kurutucu yardımıyla pişirilmiştir. Sebze cipsinde temel analizler (nem, kül, yağ ve protein), kimyasal analizler (antioksidan aktivite ve toplam fenolik içerik), fiziksel analizler (renk ve tekstür) ve duyusal analiz gerçekleştirilmiştir.

Mixture design, MINITAB 16.0 (Minitab Inc., State College,PA,USA) programı yardımıyla oluşturulmuştur, yapılan analizlerin sonuçları istatistiksel olarak değerlendirilmiştir. Yapılan tasarım sonucunda on üç farklı karışımı üç farklı sıcaklıkta pişirilmiştir. Tüm analiz sonuçlarının değerlendirilmesi sonucunda ise en verimli örnek olarak 60°C de %40 oranında bezelye unu, %22.5 oranında kabak, %25 oranında turp ve %12.5 oranında kabak çekirdeği içerikli cips belirlenmiştir. Bu doğrultuda bu karışıma sahip sebze cipsleri 50°C, 55°C ve 60°C de tepsili kurutucu da pişirilerek, tüm analizlere ek olarak duyusal analizine ve antioksidan aktivite analizi de yapılmıştır.

To My Mother

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LIST OF ABREVIATIONS

db:	dry basis
DPPH:	2,2 diphenyl-1-picryhydrazil
EVMD:	Extreme vertices mixture design
GAE:	Gallic acid equivalents
PS:	Pumkin Seed
R:	Radish
TSI:	Turkish Standards Institution
Z:	zucchini

CHAPTER 1

INTRODUCTION

Functional foods are products fortified with ingredients having a positive health influence, products cleared from anti-nutritional compounds, raw materials improved, and novel foods with an improved health benefit (Alongi and Anese, 2021). Functional food contains biologically active ingredients by enhancing health and reducing risk of diseases (Geraldi et al., 2018). Functional foods play an important role in preventing some metabolic diseases as a part of the routine diet (Fu et al., 2022).

Poor eating habits are noticed with increased snacking with comfort foods which food bringing emotional comfort (Singh et al., 2021). Stress is also seen as an important factor among young people that tends to affect snacking as well as eating patterns (Sominsky and Spencer, 2014). Junk food may increase the risk for psychiatric distress, mental disorders and violent behaviors, behavioral problems and mental distress such as anxiety worthlessness, and dizziness (Zahedi et al., 2014). Micronutrients like trace elements, antioxidants, and also vitamins play coping with existing oxidative stress in the body tissues and providing immunity against pathogens (Chapple et al., 2007; Enwonwu et al., 2002).

In response to stress, adults with obesity are more susceptible to consuming unhealthy snacks (Cleobury and Tapper, 2014; Zellner et al., 2007). During functional food design, habits and preferences, also must be taken into account to develop successful products (Sijtsema et al., 2020). As philosopher Ludwig Feuerbach said that 'You are what you eat' in 1848, it is important to what we eat for our life quality. In this direction, the question of whether the consumption of snacks consisting of feedstocks rich in protein can make consumers happy or not has been one of the starting points of the thesis topic.

The aim of this thesis was to develop a functional snack containing pea flour, zucchini, radish, pumpkin seed, and spice mixtures and to determine the optimum amount of ingredients to produce the snacks having high protein content and liked by panelists who attended the sensory panels.

CHAPTER 2

LITERATURE REVIEW

2.1. Zucchini

Cucurbita pepo, which has medicinal and nutritional benefits, is generally used worldwide as food and in folk medicine especially in Asia for the treatment of different diseases (Perez, 2016). Many therapeutical and pharmacological studies have confirmed its anticancer, antidiabetic (hypoglycemic), antiulcer, antibacterial, antioxidant, anti-inflammatory, antitumor activity (tumour growth inhibition) as well as antiviral, providing the scientific basis for the use of zucchini in traditional medicine (Adnan et al., 2017). A study reported that zucchini exhibiting important physiological properties as wound healing, hypoglycaemic effects, as well as immunomodulating (Chaturvedi, 2012). Zucchini is also a good supplement of carbohydrates, proteins, lipids and minerals (Adnan et al., 2017). Today's increasing animal food prices have caused people to search for alternative products rich in protein. Zucchini is opulent in nutrients and amount of crude protein (Adnan et al., 2017). Because of these properties of zucchini, it was decided to add green squash as the main component of the product. In addition, the green color of the zucchini contributed greatly to the green color of the chips. While many chips on the market shelves had yellow and orange colors, it was remarkable that these chips had a green color.

2.1.1. Radish(*Raphanus sativus L.*)

Radish is considered a root. It is unique among the cruciferous plants. Because it has glucosinolates, which are the precursor compounds of isothiocyanates (Gamba et al., 2021). Radish has potential use as a source of bioactive compounds. It uses for clinical and health implications in diseases like hypertension, cardiometabolic disorders, and as an antimicrobial and antioxidant agent (Lim, 2012; Manivannan et al., 2019). The

bioactive compounds in *Raphanus sativus* which is a rich source of nutrients and phytochemicals like proteins, use to treat a variety of diseases (M. Gamba et al. , 2021).

Some of the radish compounds are known to have several health benefits, including antioxidant/redox activity, anticancer, cardiovascular and metabolic protective effect, and antimicrobial characteristics (Manivannan et al., 2019). Radish is one of the feedstock for the development of nutraceuticals targeting both infectious and non-communicable diseases (M. Gamba et al. , 2021). The white color of the radish was one of the main components of this study, as it did not adversely affect the color of the snack food, which was designed as a green color. Due to these characteristics of radish, it was decided to add radish to the product developed.

2.1.2. Pumpkin Seed (*Cucurbita Pepo L.*)

Pumpkins are a medicinally and economically important plant group, which have been used frequently as functional food or medicine and belongs to the family Cucurbitaceae, and consists of succulent stem with numerous seeds (Adams et al., 2011; Saganuwan, 2009; Montesano et al., 2018). Pumpkin, a dicotyledonous seed vegetable and pliable succulent stems with trifoliate leaves, belongs to the genus Cucurbita and family Cucurbitaceae (Li et al. ,2021).

Pumpkin seeds may be tiny, but it contains bioactives such as triterpenoids, sesquiterpenoids, carotenoids, tocopherols, polyphenols, numerous phyto-constituents, and also amino acids, unsaturated fatty acids, phenolic compounds, tocopherols, flavonoids, phenolic acids as well as valuable minerals (Dotto and Chacha, 2020). Pumpkin polysaccharides, which have attention in the field of food supplements, could exert good antioxidant activity and also use as a natural immunostimulant for patients (Li et al., 2021). Bioactive compounds in pumpkin seeds exhibit promising activities such as anthelmintic, anti-diabetic, anti-depressant and also anti-oxidant (Dotto and Chacha, 2020).

Polysaccharide is abundant bioactive components in pumpkin (Li et al., 2021). Due to their structures diversity, various health benefits, and no side effects, pumpkin polysaccharides have being exploited in the elds of modern functional food (Li et al., 2021). Pumpkin is rich in pectin, a type of dietary fibre (Fissore et al., 2007), which when

consumed is reported to control glycaemic levels and reduce the need for insulin when fibre-rich foods are consumed by patients with diabetes (Guillon & Champ, 2000). Tryptophan is 0.60 g/100 g in pumpkin seeds (Amin et al., 2019). Depression is a common illness that involves episodes of suppressed psychosocial functioning and diminishes quality of life with such symptoms as disturbed sleep and appetite, reduced concentration, excessive guilt and sometimes suicidal thoughts (Mill, and Petronis, 2007; T. Giraldi, 2017).

2.1.3. Pea flour

The protein of pea has high protein content, so it uses for functionality, sustainability, availability, affordability and hypo-allergenicity (Boukid, Rosell and Castellari, 2021). Pea has phenolic compounds, in recent years, has received special attention due to their antioxidant, anti-inflammatory, anticancer, antimicrobial, and cardioprotective effects (Cosme et al., 2020). Pulses are a rich source of bioactive compounds such as polyphenols and dietary fibers (Millar et al., 2019). Pea can be considered a high-quality protein. It has balanced amino acid ratio, and all essential amino acids, except for methionine. It can fulfil FAO/WHO recommendations (Gorissen et al., 2018). Pea has nutritional benefits, so it uses in food. Pea has peculiar functional benefits like including solubility, foaming capacity, emulsifying, as well as gel forming capacity (Boukid, Rosell and Castellari, 2021). Pea is among the major ingredients, because it has rich in protein so it is used to produce healthier snacks (Arribas et al., 2017; Maskus and Arntfield, 2015). Due to these properties of peas, it was decided to add pea flour to the product developed.

2.1.4. Spice Mixes

This section include spice materials.

2.1.4.1. Onion and Garlic (*Allium sativum L*)

Since the ancient period, onion has been consumed for its medicinal properties (Savitha et al., 2021). Onion has a rich source of flavonoids (flavonols and anthocyanins) and sulfur-containing compounds (Savitha et al., 2021). In a study, Atik and Dıraman (2019) found that components such as quercetin, allicin, kaempferol found in plants such as garlic and onions; it has provided positive results in the treatment of cardiovascular diseases, cholesterol, diabetes and various diseases such as tumor formation.

Garlic is an annual bulbous herb of the Alliaceae family. Garlic is used throughout the world because it has an ingredient of traditional and modern medicine used for cardiovascular health, the prevention of infectious diseases (Rouf et al., 2020; Martins, Petropoulos, and Ferreira, 2016). Garlic is the most richest sources of total phenolic compounds, among the consumed vegetables (Lanzotti et al., 2014; Martins, Petropoulos, and Ferreira, 2016). Garlic is interestingly used due to its therapeutic and medicinal properties, both in traditional and modern medicine (Martins, Petropoulos, and Ferreira, 2016). However, apart from its volatile compounds, garlic is also considered a rich source of other non-volatile phytonutrients, with important medicinal and therapeutic properties, of which a particular emphasis is given to flavonoids, saponins and sapogenins, phenolic compounds, nitrogen oxides, amides and proteins (Lanzotti et al., 2014)

Immunocompromised people including people with cancer, diabetes, malnutrition and certain genetic disorders, are more susceptible to viral infections (Englund et al., 2011). Garlic has been used for centuries as an ethnomedicinal plant to treat infectious diseases (Rouf et al., 2020). Garlic contains vitamins, antioxidants, flavonoids, minerals and bioactive compounds such as organosulfur, phenols and saponins with medicinal properties and biological activities, namely antioxidant, immunomodulatory, hepatoprotective, cardiovascular protective, anti-diabetic, anti-obesity, renal protective, neuroprotective antimicrobia, and antifungal (Tavares, Santos, and Noreña, 2021). Due to these properties of onions and garlic, it was decided to add onions and garlic to the product developed.

2.1.4.2. Matcha Powder

The finely ground powder of leaves of the tea plant (Camellia sinensis) which is grown under specific conditions using about 90% shade is called matcha powder (Kurauchi et al., 2019). In recent years, Matcha became popular worldwide. It is used as a dietary supplement and flavoring ingredient in snacks (Kurauchi et al., 2019). Matcha powder exerts anxiolytic effect because of the activation of the dopaminergic and serotonergic systems (Kurauchi et al., 2019). Matcha tea powder has properties like improve fear and anxiety symptoms. Matcha powder improves mood and mental well-being and ameliorate or prevent various diseases (Kurauchi et al., 2019). Dried tea leaves which especially in the form of ground powder are used as an additive to various foods (Baldi et al., 2020). Matcha tea is used for properties of health-beneficial in food additives (Sugimoto et al., 2021). The interest in the plant-derived healthy foods, functional foods and food supplements are increasing in recent times. Matcha powder properties are used as potential agents in maintenance of health and treatment of diseases (Devkota et al., 2021). In recent years, there is an increased market demand for green tea. There is also consumption of matcha as snack food (Devkota et al., 2021). The biological roles of matcha has in central nervous system. These properties are associated with cognition and memory (Devkota et al., 2021). Matcha could have possible neuroprotective effects. Because it has antioxidant, stress reducing, anxiolytic, therapeutic strategy in dementia and Alzheimer's disease and memory boosting properties (Devkota et al., 2021).

Matcha is rich in taste and bioactive constituents, quality evaluation of matcha is important to ensure flavor and efficacy (Guo et al., 2021). Matcha is added to different food products like ice cream, and dessert to enhance their nutritional as well as commercial value (Guo et al., 2021). Matcha tea ingestion has significant health benefits such as reducing stress, anxiolytic properties, improving mood and cognitive performance (Dietz, Dekker, and Piqueras- Fiszman, 2017). Due to these properties of matcha powder, it was decided to add it to the vegetable snack developed as a spice mix.

2.1.4.3. Avocado oil

The avocado grows in tropical or subtropical climates (Tan, 2019). In recent decades, the consumption of avocado has experienced a sharp increase worldwide. Avocado has nutritional value and beneficial health effects (Del Castillo-Llamosas et al., 2021). Avocado oil contents bioactive compounds potentially beneficial to human health and high levels of monounsaturated fatty acids (Tan,2019). These components allow it to be used as a functional oil. Avocado oil has positive treat effect in hypertension, diabetes as well as fatty liver disease (Tan,2019). Several studies demonstrated that orderly consumption of avocado oil has positive effect for human body. It provide health benefits like in terms of disease prevention (Tan,2019). The lipid content is one of the most important factors in avocado. It has a large amount of oil in comparison to other fruits and is rich in polar lipids (Ranade and Thiagarajan, 2015).

Avocado oil contains high levels of monounsaturated fatty acids, and also a significant quantity of saturated fatty acids compared to other vegetable oils (Duarte et al., 2016). The avocado has high content of potassium and low content of sodium. The avocado oil benefits for persons with low-sodium diets and protect against cardiovascular diseases (Cowan and Wolstenholme, 2016; Zafar and Sidhu, 2011). Avocado has great importance for overall health and wellbeing such as pyridoxine, β-carotene, ascorbic acid, vitamin E, retinol, thiamine, riboflavin, niacin, and folic acid (Alvarez et al., 2012; Duarte et al., 2016). In this study, avocado was added as oil in order to get maximum efficiency since it is an expensive fruit.

2.1.4.4. Ginger

Ginger, known as *Zingiber officinale* Roscoe, is a monocotyledonous. Ginger belongs to the Zingiberaceae family of flowering plants originating from southeast Asia (Menon et al., 2021; Si, Chen, et al*.,* 2018). Ginger uses as a popular food additive and flavoring agent throughout the world (Sahebkar, 2011). Ginger includes over 300 different compounds, but the pharmacological effects of ginger are from its terpene and phenolic compounds (Kiyama, 2020; Mao et al., 2019). Clinically and experimentally, pharmacological benefits of ginger have demonstrated anti-diabetic activities, numerous

therapeutic activities, neuroprotective, anti-migraine, cardiovascular protective, anti-obesity, anti-oxidative, and immune modulatory agents since ancient times (Jafarzadeh, and Nemati, 2018; Menon et al., 2021). Ginger has been used as traditional medicines like multiple symptoms/diseases such as respiratory problems, digestive problems, cardiac complaints, metabolic disorders, neurological disorders and immunological disorders (Remadevi, Surendran, and Ravindran, 2005). Ginger is used to enhance immunity and acts as a chemoprotective and therapeutic for cancer (Menon et al., 2021).

Ginger uses as a spice and dietary supplement as well as a traditional medicine (Jafarzadeh, and Nemati, 2018). Ginger is used as a food, spice, supplement and flavoring agent due to its beneficial such as aroma, nutrients and pharmacological activity (Kiyama, 2020). Since ginger is less preferred in daily consumption due to its bitter taste, it was decided to add it as a component of the product in order to ensure that it is preferred more and more people reach for daily eat habit with nutritional values of ginger. Due to these properties of ginger, ginger powder was used as a spice mix in this study.

2.1.4.5. Mint

*Mentha piperita L. (*peppermint) which belongs to the Lamiaceae family is used in the medical and in industrial and culinary fields (Gholamipourfard, Salehi and Banchio, 2021). The importance of *M. piperita* is mainly due to its essential oils and polyphenols (Gholamipourfard, Salehi & Banchio, 2021). There has been increasing interest in finding naturally occurring antioxidants for applications in medicinal substances, to replace synthetic antioxidants as these may pose a health hazard due to their carcinogenicity (Zheng and Wang, 2001).

The species belonging to the genus Mentha are among the most important plants that have pharmacological, antimicrobial, antifungal, antibacterial, antispasmodic, analgesic, antibloating, antiviral, antioxidant, antidiabetic, antiobesity, dermaprotective, antiulcer and immunomodulatory actions and allelopathic properties (Zekri et al., 2013). In addition to traditional foods flavoring agent, *M. spicata* is well known for its traditional medicinal uses, particularly for the treatment of cold, cough, obesity, as well as digestive problems (Mahendran, Verma, and Rahman, 2021). Spearmint is an ethnobotanical medicinal herb with phytochemical screening, pharmacological action and

pharmacokinetic properties (Mahendran, Verma, and Rahman, 2021). Medicinal plants are considered colossal producers of bioactive therapeutics agents (Eftekhari et al., 2021). The presence of bioactive phytochemicals is the prime reason for the traditional pharmacological activities of Mentha spp (Eftekhari et al., 2021). Due to these properties of mint, dried mint was used as a spice mix in this study.

2.1.4.6. Black seed (Nigella Sativa)

Black seed (Nigella sativa) is an annual flowering plant which is a member of the Ranunculaceae family (Buttercup family), grows widely in many Middle Eastern countries and has a distinctive angular or funnel-shape, with a slightly bitter nutty-peppery taste and strong aroma and colored black and tastes bitter (Shrivastava, Agrawal, and Parveen, 2011; Sharma and Longvah, 2021). The black seed (Nigella sativa) is one of the important medicinal plant with anti-hyperglycemia and anti-hyperlipidemia characteristics in the traditional system of medicine and also is used for treating diabetes, rheumatoid arthritis, inflammatory diseases, and digestive diseases (Ali and Blunden, 2003; Ahmad et al., 2021; Kooti et al., 2016). According to pharmacological studies, Nigella sativa has many biological effects such as neuroprotective, antioxidant, gastroprotective, antidiabetic, antihypertensive, immunomodulatory, spasmolytic, and wound healing activities (Mansi ,2005; Ahmad et al., 2021; Sultan et al., 2014). Due to these properties of black seed, it was decided to add black seed to the product developed.

2.2. Cooking Method

Vegetables and fruits are known for their different nutrients and health benefits (Ajuebor et al., 2017). Drying is a method of food preservation that works by removing water from the food, which prevents the growth of microorganisms (Ajayeoba et al., 2014). Dryers are commonly used as rotary dryers, belt dryers, tray dryers, tunnel dryers, flash dryers, drum dryers, fluidized bed dryers, spray dryers, vacuum dryers and freeze dryers (Ajuebor et al., 2017). The tray dryer is the most extensively used because of its simplicity and economic design (Ajuebor et al., 2017). Tray dryer has basic working principle is; the product to be dried is placed on the tray and dried in the tray at a certain

time and temperature (Cemeroğlu, 2011). Tray dryers are more suitable for granular and sliced products, and they are dried by laying on the shelves (Olgun and Rzayev,2000). The trays are perforated to effectively allow the airflow within the chamber (Ajayeoba et al., 2014). The drying process takes place by the removal of moisture from the product by hot air (Cemeroğlu, 2011).The product feeding system, the hot air heater and the fan, the collector that allows the humid air to be discharged are the main components of the cabinet system (Vega-Mercado et al., 2001). In tray dryers, product feeding is constant throughout drying (Erbay and Küçüköner, 2008). The tray dryers (Figure 1) are easy-to-control, versatile dryers that can be easily adapted to the drying of many different products (Saldamlı, Saldamlı, 2004). The speed and temperature of the air in tray dryers are not at the same level everywhere on the product surface (Erbay and Küçüköner, 2008). The most basic problem in this dryer type is that the product in the area where the hot air first enters dries faster and the items in the other section dries more slowly (Erbay and Küçüköner, 2008).

(a) (b)

Figure 1. (a) exterior view of tray dryer , (b) inside view of tray dryer

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

Pumpkin seed, radish, zucchini, pea powder, matcha powder, onion, garlic were obtained from the market in Gaziemir district of Izmir. n-Hexane (Merck Darmstadt) was used for oil analysis. Antifoaming agent (Antifoam S-3010 (Chem Pure)), catalyzer tablet, EMSURE ISO Sulfuric Acid 95-97% (Merck Darmstadt), NaOH, H_3BO_3 , H_2SO_4, boric acid (3%) and HCl (0.1M) were used for protein analysis. For the DPPH method applied for antioxidant activity analysis, 2,2 diphenyl-1-picryhydrazil (DPPH) (Sigma-Aldrich), ethyl alcohol, distilled water and Tris-HCl buffer solution Tris(hydroxymethyl)-aminomethane (MERCK) were used. Na_2CO_3 (Sodium carbonate Sigma-Aldrich), Folin-Ciocalteu, Gallic acid (3,4,5-Trihydroxybenzoic acid)(Sigma Life Science), and Methanol G CHROMASOLV (Sigma-Aldrich) were used for the analysis for total phenolic content.

3.2. Methods

In the method section of this thesis, the functioning of the scientific study is explained.

3.2.1. Functional Snack Preparation and Baking

The peels of zucchini, radish, and spice materials were removed. They were washed with water. The cleaned raw materials were shredded by the shredder. Spice mix had 10% onion, 10% garlic, 10% ginger, 20% nigella sativa, 15% mint, 20% matcha tea powder and 15% avocado oil and total of 2% spice mix was added to each dough mix. The spice mix ratios were decided as a result of preliminary trials. The dough is obtained by adding pea flour (40%) and mixture of other raw materials. While forming the dough

of the product, 5, 10, 15, 20, 25, 30, 35, 40, and 50% were added. When 30% was added for the first time, the desired consistency began to be obtained in the dough. When 40% was added, the dough had the ideal consistency. While determining the content ratios of the product, it was decided to add pea flour at a fixed rate of 40%. The resulting dough was placed between two parchment baking papers. The dough was rolled out with the help of a rolling pin. Then the dough was rested for 10 minutes. The rested dough is shaped. Baking temperatures and periods for the shaped dough are shown on Table 1. The flow chart of the production of functional snack is shown in Figure 3.

3.2.2. Experimental Design and Analyses

Some statistical analyses were performed by using MINITAB 16.0 (Minitab Inc., State College, PA, USA). Analysis of Variance (ANOVA) and regression models were performed at a 95% confidence interval ($p<0.05$) to define the significant terms of the predictive model. Variance analysis was performed to determine statistically significant effects of the two predictors ($p<0.05$). Multiple comparisons were made by using Tukey's test.

Extreme vertices mixture design (EVMD) method is used for the formulation of mixtures and are shown in Figure 2. The percentage of radish, pumpkin seed, and zucchini in each mix were formulated by EVMD method using MINITAB 16 (State College, PA). A range of 10 to 30 was used for each material during the 13 design points which are shown in Fig 1 and Table 1 for functional snack formulation. All runs were repeated twice. According to the results of these design points, the optimum amount of each material were chosen for the maximum protein content. The mixtures given in Table 1 were cooked in a tray dryer at 50℃, 55℃ and 60℃ with the fan running.

Table 1. Experimental design of three different functional snack mixture

Experiment No.	Zucchini (%)	Pumpkin seed (%)	Radish (%)
1	25	15	20
2	30	12.5	17.5
3	22.5	20	17.5
4	30	10	20
5	27.5	17.5	15
6	25	10	25
7	22.5	12.5	25
8	20	20	20
9	27.5	10	22.5
10	30	15	15
11	25	20	15
12	20	15	25
13	20	17.5	22.5

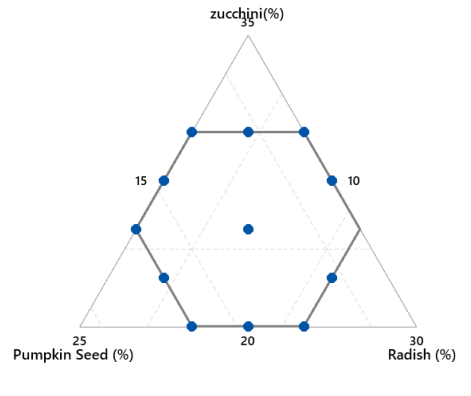


Figure 2. Simplex design plot of amounts for raw materials(%)

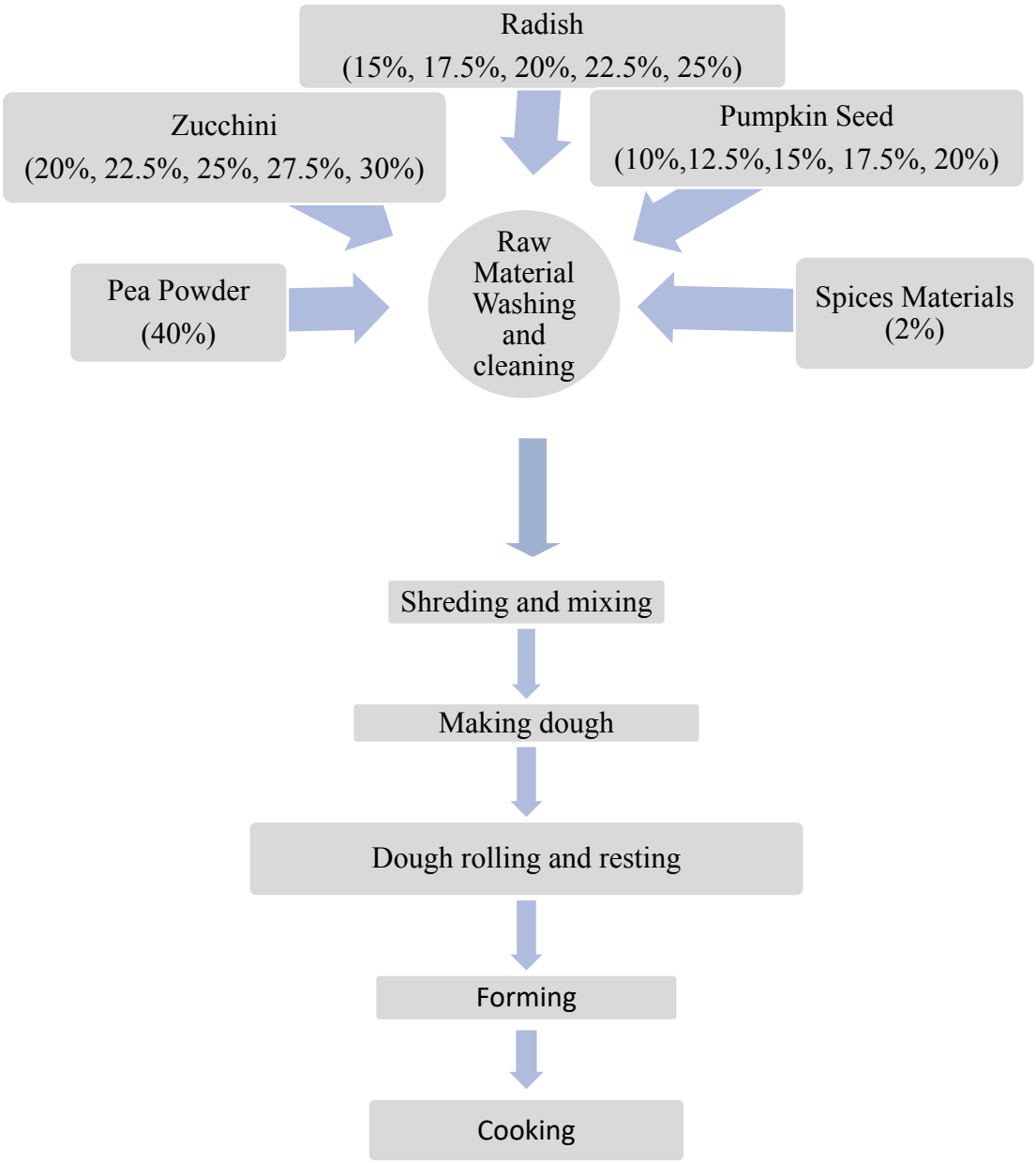
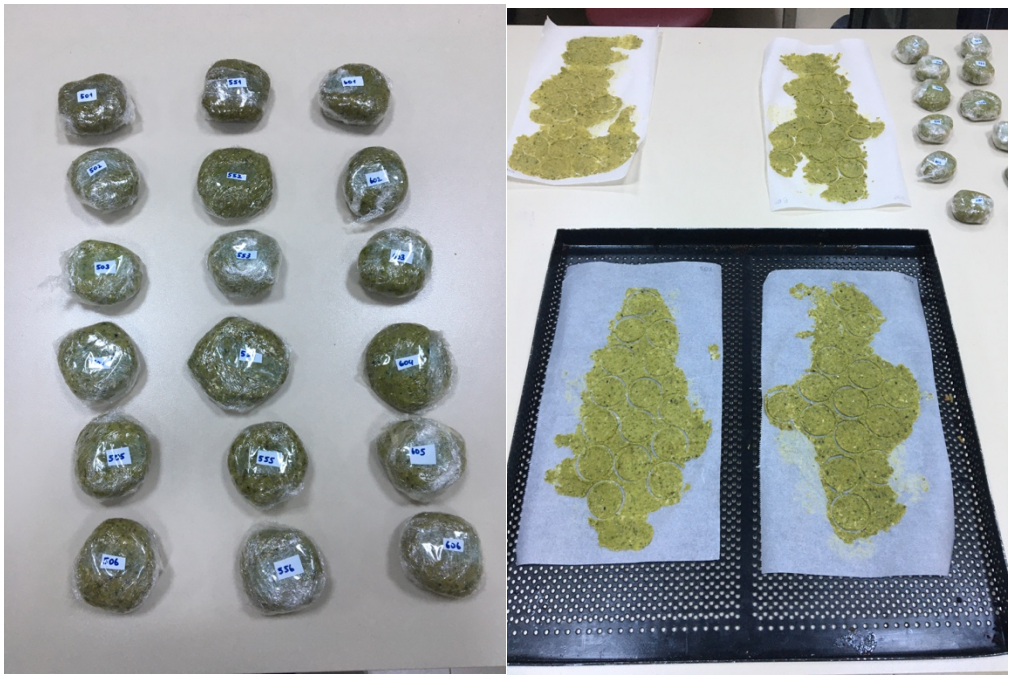


Figure 3. Flow chart of the functional snack production



(a) (b)

Figure 4. (a) the doughs of developed snack, (b) the view of baked product

3.2.3. Proximate Analyses

The proximate analyses were performed on baked samples as well as the raw materials used in dough formulation.

3.2.3.1. Moisture Content Determination

AOAC (2000) method was used for moisture determination. Approximately 5 grams of ground functional snack were weighed into the aluminium sample pans. They were kept in the oven at 105 °C / 3 hours. Afterwards, the aluminium sample pans taken to the desiccator were weighed. Moisture values of snacks were calculated as follows.

$$Moisture\ Content\ (\%) = \left(\frac{((W1 - W2)x100)}{W1}\right) \quad (1)$$

where:

W1= weight (g) of sample before drying

W2= weight (g) of sample after drying

3.2.3.2. Protein Content Determination

Protein content of functional snack was determined using Kjeldahl method based on AOAC (2000) with some modifications. In this procedure, functional snack sample (nearly 1.0 g) was put into Kjeldahl digestion flask. Antifoaming agent (Antifoam S-3010 (Chem Pure)), two catalyzer tablets, and 20 ml of conc. H_2SO_4 were put into per Kjeldahl tubes. A Kjeldahl tube containing all chemicals except the sample was prepared. All the tubes were placed in the Gerhardt Kjeldatherm block digestion unit, incineration was carried out at 450 ℃ for 5 hours. After distillation part, 70 ml of boric acid (3%) was added and HCl (0.1M) in titration process with using Vapodest (Gerhardt GmbH & Co., Germany). 5.83 was used as general factor like other vegetable snack product. The measurements were performed in two parallels for functional snack samples with two replicate, and the results were expressed as percentage of total protein contents were calculated by using the following equations:

$$Protein\ (\%) = \left(\frac{((A-B)xNx1.4007x5.83)}{W}\right) \quad (2)$$

where:

A= volume (ml) of 0.2 N HCl used sample titration

B= volume (ml) of 0.2 N HCl used in blank titration

N= Normality of HCl

W= weight (g) of sample

14.007=atomic weight of nitrogen

3.2.3.3. Fat Content Determination with Soxhlet Extraction

Fat content of functional snack was determined using Soxhlet extraction method. Gerhardt oil beakers were tared. 1 gram of groud functional snack was weighed into the

cartridge. Cartridges were placed inside the Gerhardt oil beaker. 200 ml hexane was added to each beaker. All beakers were placed in the Gerhardt Soxtherm device for extraction. After the completed extraction process, the oil beakers were taken to the vacuum evaporator. Oil beakers were weighed and the oil content of functional snack was calculated as follows.

$$Fat\ (\%) = \left(\frac{((F2 - F0)x100)}{F1}\right) \quad (3)$$

where:

F2-F0= Weight of fat (g)

F1= Weight of sample (g)

3.2.3.4. Ash Content Determination

AOAC, 2000 method was used for ash determination. Approximately 5 grams of ground functional snack were weighed into the tared crucible. The crucibles placed in the muffle furnace. They were heated from room temperature to 105 °C in the muffle furnace. It was kept at 105 °C for 12 minutes. Rapid heating was carried out from 105 °C to 250 °C at 10 °C /min. It was kept at 575 °C for 3 hours. Cooling was carried out from 575 °C to 105 °C. The crucibles were taken to a desiccator to cool. Ash values of chips were calculated as follows.

Calculation for ash (%):

$$Ash\ (\%) = \left(\frac{((A2 - A0)x100)}{A1}\right) \quad (4)$$

where:

A2-A0= Weight of ash (g)

A1= Weight of sample (g)

3.2.4. Chemical Analyses

The methods used in chemical analyses are detailed below.

3.2.4.1. Antioxidant Activity

DPPH method was used for determination of antioxidant reaction with an organic radical. The extraction was carried out by adding 5 mL of methanol to 0.25 g of functional snack powder. This was followed by shaking the mixture for 30 min at room temperature. The methanolic extracts were used for the following analysis (Khajehei et al., 2018). 0.0501 g of 2,2 diphenyl-1-picryhydrazil (DPPH) was weighed into a 250 mL flask and made up to 250 mL with ethyl alcohol to prepare a DPPH solution. The DPPH solution was stored in the dark. 0.752 g of tris (hydroxymethyl) aminomethane was weighed and taken into a beaker and dissolved by adding 40 mL of distilled water. Tris-HCl buffer solution was prepared by adjusting the pH of the solution to 7.4 with 0.1 M HCl and making it up to 50 mL with distilled water in a balloon flask. 0.1 mL of each sample was taken and 0.9 mL of Tris-HCl and 1 mL of DPPH solution were added, mixed in a vortex and kept in the dark for 30 min. The absorbance value of the blank sample and all samples were measured at 517 nm with UV-visible spectrophotometer (UV-1601,Shimadzu, Kyoto, Japan). The % antioxidant activity value was calculated by substituting the measured values in the formula below (Moon and Terao, 1998).

$$DPPH(\%inhibition) = \left(1 - \left(\frac{\text{Abs sample}}{\text{Abs blank}}\right)\right) x100 \qquad (1)$$

3.2.4.2. Total Phenolic Content Determination

Na_2CO_3 (Sigma-Aldrich) 7.5 g was weighed and dissolved in 100 mL of distilled water to prepare sodium carbonate solution. 100 mL of 2 N Folin-Ciocalteu solution was taken, transferred to a 1 L volumetric flask, and 0.2 N Folin-Ciocalteu solution was prepared by completing 1 L with distilled water. The prepared solution was stored at room temperature. Gallic acid solution was prepared by weighing 0.1015 g gallic acid and dissolving it in 100 mL of distilled water. Blank sample was prepared with 1mL of purified water, 5 mL of 0.2 N Folin-Ciocalteu reagent and 4 mL of Na_2CO_3 solution. For the preparation of the gallic acid calibration curve, solutions at concentrations of 0.1, 0.08, 0.06, 0.04, and 0.02 g/L were prepared from the previously prepared 1 g/L gallic

acid stock solution. Then, 1 mL of each of these solutions was taken and 5 mL of 0.2 N Folin-Ciocalteu reagent and 4 mL of Na_2CO_3 solution were added to them, and their absorbance against the blank was measured at 760 nm with UV-visible spectrophotometer (UV-1601,Shimadzu, Kyoto, Japan). The following equation was obtained by drawing the concentration (g/L)-absorbance calibration curve by using the absorbance values obtained. While determining the total phenolic content of the samples, firstly, the samples were diluted 1/10 in order to obtain an absorbance value in the range of 0.2-1.0 at 760 nm wavelength. 1 mL of diluted samples was taken and transferred to glass tubes. First, 5 mL of Folin-Ciocalteu reagent and then 4 mL of sodium carbonate solution were added, and a homogeneous mixture was obtained in the vortex, and the absorbance against the blank was measured at 760 nm.

3.2.5. Physical Analyses

The methods used in physical analyses are detailed below.

3.2.5.1. Measurement of Texture Profile

Texture measurement was performed by using TA.XTplus texture analyser (Stable Micro Systems, UK) and placing the sample on Crisp Fracture Ring (HDP/CFS) using a ¼ inch spherical stainless probe (P/0.25S) and 5 kg load cell (Baltacıoğlu and Esin, 2013). Test type was compression and pre-test speed was 1 mm/s, test speed is 0.5 mm/s, post-test speed is 5 mm/s, target type was distance and the distance was determined as 3 mm. Fracture force or hardness (N) which is maximum force required to break the sample, and fracture work (N.sec) which is calculated as the area under the force-time curve, are calculated. The maximum force indicates the hardness of the product so the greater this value the harder the sample is to fracture.

3.2.5.2. Color Analysis

The Konica Minolta Colorimeter CM-5 device measuring according to L* (brightness), a* (redness) and b* (yellowness) color values was used to determine the color values of the functional snack samples. The color values of functional snacks at room temperature were read with the help of Konica Minolta Colorimeter CM-5 device.

3.2.6. Sensory Analysis

The sample with the mixture determined as a result of the design was cooked in a tray dryer at three different temperatures, 50℃, 55℃, and 60℃, and made ready for sensory analysis. The number of panelists for the analysis was limited to 65 people. As organoleptic analysis, the functional snack samples were evaluated by the panelists in six different parameters: color, appearance, smell, crispness, taste and overall acceptability. Panelists rated each sample between 1 and 5. The score scale was determined as 1 means I did not like at all, 2 means I liked it less, 3 means I neither liked nor disliked, 4 means I liked it a little and 5 means I liked it very much. As a result of the analysis, the panelists were asked to rank the three samples based on their preference liked most to liked least.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1. Proximate Analyses

This section shows how proximate analyses are performed.

4.1.1. Moisture Content

Moisture contents of functional snack samples were measured based on 13 different formulations are shown on Table 2. As the moisture results of this study, at 50℃ and 55℃, Sample 1 (Z:25 PS:20 R:15) had the lowest moisture content which were 2.15±0.12 and 1.94±0.07%, respectively , while Sample 4 (Z:27.5 PS:10 R:22.5) had the highest moisture content which were 2.79±0.14 and 2.80±0.11%, respectively. Although moisture contents at 50℃ and 60℃ were not statistically significant ($p>0.05$), moisture contents at 55℃ was a statistically significant ($p<0.05$). As the moisture results of this study, at 60℃ Sample 2 (Z:20 PS:15 R:25) had the lowest mean(2.16±0.07%), while Sample 4 (Z:27.5 PS:10 R:22.5) had the highest mean(2.74±0.10%). According to Turkish Standards Institution (TSI) 1991, the moisture content in potato chips should be at most 3.5%. According to TSI 1998, moisture content in corn chips should not exceed 3%. It has been stated that if the humidity ratios are above this limit, the product will lose its crunchiness structure. In a study, Uzun et al. (2008) found that the average moisture content of 12 different potato chips and 6 different corn chips from different companies was 2.94±1.17 and 2.07±0.41% in the Turkish market, respectively. In another study, Yuksel, Karaman and Kayacier (2015) reported that the average moisture content of wheat chips samples which enriched with different concentration barley flour was 2.72±1.36%. When based on this all formulation and temperature values were examined, it was observed that there was no significant difference between each other in terms of moisture content.

4.1.2. Protein Content

Protein contents of functional snack samples were measured based on 13 different formulations and are shown on Table 2. As the protein results of this study, at 50℃ and 60℃, Sample 4 (Z:27.5 PS:10 R:22.5) had the lowest mean(19.43±0.03 and 19.20±1.42%, respectively), while Sample 1 (Z:25 PS:20 R:15) had the highest mean(25.58±0.57 and 25.27±0.36%, respectively). As the protein results of this study, at 55℃, Sample 10 (Z:30 PS:10 R:20) had the lowest mean (19.59±0.13%), while Sample 11 (Z:27.5 PS:17.5 R:15) had the highest mean(25.49±0.03%). Protein contents at 50℃ , 55℃ and 60℃ were statistically significant ($p< 0.05$). As a result of baked Sample 1 (Z:25 PS:20 R:15), Sample 7 (Z:20 PS:17.5 R:22.5) and Sample 12 (Z:25 PS:10 R:25) at 50℃, 55℃ and 60℃, the temperature with the highest average protein content was determined as 60℃ and the temperature with the lowest protein content was determined as 50℃. As a result of baked Sample 5 (Z:22.5 PS:20 R:17.5) and Sample 13 (Z:30 PS:12.5 R:17.5) at 50℃, 55℃, and 60℃, and the temperature with the lowest protein content was determined as 55℃. As a result of baked Sample 6 (Z:22.5 PS:12.5 R:25) and Sample 11 (Z:27.5 PS:17.5 R:15) at 50℃, 55℃ and 60℃, the temperature with the lowest protein content was determined as 50℃. As a result of baked other samples at 50℃, 55℃ and 60℃, the temperature with the lowest protein content was determined as 60℃. As a result of baked Sample 2 (Z:20 PS:15 R:25), Sample 4 (Z:27.5 PS:10 R:22.5), Sample 6 (Z:22.5 PS:12.5 R:25), Sample 8 (Z:30 PS:15 R:15) and Sample 11 (Z:27.5 PS:17.5 R:15) at 50℃, 55℃ and 60℃, the temperature with the highest protein content was determined as 55℃. As a result of baked other samples at 50℃, 55℃, and 60℃, the temperature with the highest protein content was determined as 50℃. In a study, Uzun et al. (2008) found that the average protein content of 12 different potato chips and 6 different corn chips from different companies was 8.14±0.70 and 8.40± 0.44% in the Turkish market, respectively. In another study, Yuksel, Karaman and Kayacier (2015) reported that the average protein content of wheat chips samples which enriched with different concentration barley flour was 8.51±0.34%. In the review study by Çetiner and Bilek (2018), it was stated that the protein content of wheat flour, oats, peas, lentils and chickpeas have 10-15, 12-25, 23-31, 21-31 and 20%, respectively. As stated in the method section, 40% of pea flour was added to each mixture in this study. This shows that pea

flour is an important parameter that makes the mixtures rich in protein. It was observed that the protein ratio of the functional snack developed in this study was higher when compared to the protein ratios of chips with different contents in the literature. This result demonstrates that the goal of this thesis of developing a higher-protein containing snack for humans has been successful.

Table 2. The results of moisture (%) and protein (%)

Sample	Moisture (%)			Protein (%)		
	50℃	55℃	60℃	50℃	55℃	60℃
1-Z:25 PS:20 R:15	2.15±0.12^{aA}	1.94±0.07^{cA}	2.34±0.13^{aA}	25.58±0.57^{aA}	25.30±0.17^{aA}	25.27±0.36^{aA}
2-Z:20 PS:15 R:25	2.25±0.08^{aA}	2.36±0.08^{abcA}	2.16±0.07^{aA}	22.86±0.05^{bcdeA}	23.22±0.03^{bcA}	22.50±0.29^{bA}
3-Z:25 PS:15 R:20	2.72±0.15^{aA}	2.62±0.09^{abA}	2.58±0.07^{aA}	21.58±0.45^{defA}	21.54±0.15^{deA}	21.54±0.12^{bcA}
4-Z:27.5 PS:10 R:22.5	2.79±0.14^{aA}	2.80±0.11^{aA}	2.74±0.10^{aA}	19.43±0.03^{gA}	21.43±0.39^{eA}	19.20±1.42^{cA}
5-Z:22.5 PS:20 R:17.5	2.70±0.22^{aA}	2.63±0.08^{abA}	2.67±0.11^{aA}	22.60±0.16^{cdefA}	22.06±0.38^{deB}	22.45±0.13^{bA}
6-Z:22.5 PS:12.5 R:25	2.68±0.09^{aA}	2.66±0.05^{abA}	2.57±0.07^{aA}	21.08±0.35^{efgA}	21.81±0.01^{deA}	21.29±0.02^{bcA}
7-Z:20 PS:17.5 R:22.5	2.51±0.12^{aA}	2.48±0.03^{abA}	2.53±0.10^{aA}	21.25±0.35^{efgA}	22.22±0.48^{cdeA}	22.91±0.24^{bA}
8-Z:30 PS:15 R:15	2.40±0.13^{aA}	2.52±0.09^{abA}	2.43±0.10^{aA}	23.54±0.23^{bcdA}	23.48±0.05^{bA}	22.91±0.53^{abA}
9-Z:20 PS:20 R:20	2.46±0.10^{aA}	2.30±0.17^{bcA}	2.32±0.11^{aA}	22.99±0.42^{bcdefA}	22.47±0.10^{bcdA}	21.73±0.25^{bB}
10-Z:30 PS:10 R:20	2.63±0.10^{aA}	2.48±0.03^{abA}	2.60±0.18^{aA}	20.85±0.22^{efgA}	19.59±0.13^{fB}	18.96±0.27^{cC}
11-Z:27.5 PS:17.5 R:15	2.57±0.16^{aA}	2.42±0.08^{abcA}	2.51±0.11^{aA}	24.12±0.76^{abcA}	25.49±0.03^{aA}	25.28±0.25^{aA}
12-Z:25 PS:10 R:25	2.51±0.10^{aA}	2.46±0.05^{abA}	2.65±0.10^{aA}	21.49±0.67^{fgA}	21.30±0.07^{eA}	21.26±0.12^{bcA}
13-Z:30 PS:12.5 R:17.5	2.65±0.05^{aA}	2.69±0.01^{abA}	2.67±0.06^{aA}	24.64±0.53^{abA}	22.49±0.30^{bcdA}	23.19±0.15^{abA}

Abbreviations: Z(zucchini), PS(pumpkin seed), R (radish)

Data in the same column with the different lowercase superscript letter and the data in the same row with the different uppercase superscript

4.1.3. Fat Content

Fat contents of functional snack samples were measured based on 13 different formulations and are shown on Table 3. As the fat results of this study, at 50℃, 55℃ and 60℃, Sample 2 (Z:20 PS:15 R:25) had the lowest mean(12.51±0.12, 12.48±0.14% and 12.05±0.02%, respectively) , while Sample 6 (Z:22.5 PS:12.5 R:25) had the highest mean(19.77±0.23, 19.87±0.26 and 19.89±0.25%, respectively). Fat contents at 50℃ , 55℃ and 60℃ were statistically significant ($p < 0.05$). As a result of baked Sample 4 (Z:27.5 PS:10 R:22.5), Sample 5 (Z:22.5 PS:20 R:17.5) and Sample 6 (Z:22.5 PS:12.5 R:25) at 50℃, 55℃ and 60℃, the temperature with the highest fat content was determined as 60℃ and the temperature with the lowest fat content was determined as 50℃. As a result of baked Sample 7 (Z:20 PS:17.5 R:22.5) and Sample 13 (Z:30 PS:12.5 R:17.5) at 50℃, 55℃ and 60℃, the temperature with the lowest fat content was determined as 55℃. As a result of baked other samples at 50℃, 55℃ and 60℃, the temperature with the lowest fat content was determined as 60℃. As a result of baked Sample 7 (Z:20 PS:17.5 R:22.5), Sample 11 (Z:27.5 PS:17.5 R:15) and Sample 13 (Z:30 PS:12.5 R:17.5) at 50℃, 55℃ and 60℃, the temperature with the highest fat content was determined as 50℃, 55℃ and 60℃, respectively. As a result of baked other samples at 50℃, 55℃ and 60℃, the temperature with the highest fat content was determined as 50℃. In a study, Uzun et al. (2008) found that the average fat content of 12 different potato chips and 6 different corn chips from different companies was 38.09±2.65 and 27.61±2.12% in the Turkish market, respectively. In another study, Yuksel, Karaman and Kayacier (2015) reported that the average fat content of wheat chips samples which enriched with different concentration barley flour was 22.46±2.10%. It was observed that the fat ratio of the functional snack developed in this study was lower when compared to the fat ratios of chips with different contents in the literature. This result demonstrates that the goal of this thesis of developing a lower-fat snack for humans has been successful.

4.1.4. Ash Content

Ash contents of functional snack samples were measured based on 13 different formulations and are shown on Table 3. As the ash results of this study, at 50℃ and 55℃ Sample 2 (Z:20 PS:15 R:25) had the highest means (3.12±0.29 and 2.95±0.28%, respectively). At 50℃, Sample 6 (Z:22.5 PS:12.5 R:25) had the lowest mean(2.15±0.14), and at 55℃, Sample 1 (Z:25 PS:20 R:15) had the lowest mean(2.24±0.203%). Although ash contents at 60℃ was not statistically significant ($p > 0.05$), ash contents at 50℃ and 55℃ were a statistically significant ($p < 0.05$). As the ash results of this study, at 60℃, Sample 6 (Z:22.5 PS:12.5 R:25) had the lowest mean (2.37±0.05%), while Sample 13 (Z:30 PS:12.5 R:17.5) had the highest mean (2.95±0.27%). In a study, Uzun et al. (2008) found that the average ash contents of 12 different potato chips and 6 different corn chips from different companies were 2.85±0.45 and 2.46±0.19% in the Turkish market, respectively. When all mixture and temperature values were examined, it was observed that there was no significant difference between each other in terms of ash content.

Table 3. The results of fat (%) and ash (%)

Sample	Fat (%)			Ash (%)		
	50℃	55℃	60℃	50℃	55℃	60℃
1-Z:25 PS:20 R:15	17.85 ± 0.02^{bA}	17.23 ± 0.017^{bB}	16.07 ± 0.02^{cC}	2.42 ± 0.22^{bcA}	2.24 ± 0.20^{bA}	2.59 ± 0.21^{aA}
2-Z:20 PS:15 R:25	12.51 ± 0.12^{gA}	12.48 ± 0.14^{gA}	12.05 ± 0.02^{hA}	3.12 ± 0.29^{aA}	2.95 ± 0.28^{aA}	2.85 ± 0.28^{aA}
3-Z:25 PS:15 R:20	17.30 ± 0.26^{bcA}	17.29 ± 0.14^{bA}	17.13 ± 0.17^{bA}	2.77 ± 0.17^{abcA}	2.61 ± 0.04^{abA}	2.76 ± 0.25^{aA}
4-Z:27.5 PS:10 R:22.5	14.08 ± 0.26^{fA}	14.32 ± 0.20^{eA}	14.53 ± 0.15^{eA}	3.07 ± 0.25^{abA}	2.92 ± 0.18^{abA}	3.08 ± 0.22^{aA}
5-Z:22.5 PS:20 R:17.5	15.12 ± 0.25^{eA}	15.52 ± 0.28^{dA}	15.58 ± 0.17^{cdA}	2.89 ± 0.20^{abcA}	2.76 ± 0.21^{abA}	2.74 ± 0.21^{aA}
6-Z:22.5 PS:12.5 R:25	19.77 ± 0.23^{aA}	19.87 ± 0.27^{aA}	19.89 ± 0.25^{aA}	2.15 ± 0.14^{cB}	2.62 ± 0.16^{abA}	2.37 ± 0.05^{aB}
7-Z:20 PS:17.5 R:22.5	13.85 ± 0.26^{fA}	13.17 ± 0.23^{fgA}	13.49 ± 0.12^{fA}	2.91 ± 0.08^{abcA}	2.94 ± 0.18^{aA}	2.83 ± 0.19^{aA}
8-Z:30 PS:15 R:15	17.20 ± 0.24^{bcA}	15.56 ± 0.20^{dB}	12.56 ± 0.22^{ghC}	2.66 ± 0.11^{abcA}	2.72 ± 0.15^{abA}	2.65 ± 0.17^{aA}
9-Z:20 PS:20 R:20	17.46 ± 0.15^{bA}	16.52 ± 0.26^{bcA}	15.48 ± 0.27^{cdB}	2.98 ± 0.22^{abA}	2.88 ± 0.14^{abA}	2.97 ± 0.16^{aA}
10-Z:30 PS:10 R:20	16.48 ± 0.13^{cdA}	15.74 ± 0.16^{cdA}	13.07 ± 0.27^{fgB}	2.59 ± 0.11^{abcA}	2.55 ± 0.20^{abA}	2.62 ± 0.13^{aA}
11-Z:27.5 PS:17.5 R:15	16.31 ± 0.28^{dA}	16.60 ± 0.24^{bA}	16.26 ± 0.20^{cA}	2.61 ± 0.15^{abcA}	2.77 ± 0.12^{abA}	2.50 ± 0.13^{aA}
12-Z:25 PS:10 R:25	15.25 ± 0.22^{eA}	15.17 ± 0.18^{dA}	13.31 ± 0.25^{fgB}	2.52 ± 0.12^{abcA}	2.60 ± 0.16^{abA}	2.47 ± 0.17^{aA}
13-Z:30 PS:12.5 R:17.5	14.02 ± 0.26^{fA}	13.90 ± 0.23^{efA}	14.86 ± 0.23^{deA}	2.74 ± 0.26^{abA}	2.86 ± 0.15^{aA}	2.95 ± 0.27^{aA}

Abbreviations: Z(zucchini), PS(pumpkin seed), R (radish)

Data in the same column with the different lowercase superscript letter and the data in the same row with the different uppercase superscript letter are significantly different ($p<0.05$)

4.2. Total Phenolic Content

Total phenolic contents (mg GAE/g sample d.b.) of functional snack samples were measured based on 13 different formulations and are shown on Table 4. As the total phenolic content results (mg GAE/g sample d.b.) of this study, at 50℃, 55℃ and 60℃ Sample 4 (Z:27.5 PS:10 R:22.5) had the lowest mean (134.27±0.00, 133.15±1.61 and 133.13±1.61 mg GAE/g, respectively). At 50℃, Sample 12 (Z:22.5 PS:12.5 R:25) had the highest mean (307.83±3.22 mg GAE/g), and at 55℃ and 60℃ were Sample 3 (Z:25 PS:15 R:20) had the highest mean (295.31±3.22 and 292.84±1.61 mg GAE/g, respectively). Total phenolic content of samples (mg GAE/g sample d.b.) at 50℃, 55℃ and 60℃ were statistically significant ($p < 0.05$). As a result of baked samples except Sample 11 at 50℃,55℃ and 60℃, the temperature with the highest total phenolic content (mg GAE/g sample d.b.) was determined as 50℃ and the lowest total phenolic content (mg GAE/g sample d.b.) was determined as 60℃. As a result of Sample 11 at 50℃, 55℃, and 60℃, the temperature with the highest total phenolic content (mg GAE/g sample d.b.) was determined as 50℃ and the lowest total phenolic content (mg GAE/g sample d.b.) was determined as 55℃.

Table 4. The results of total phenolic content

Sample	Total Phenolic Content (mg GAE/g sample d.b.)		
	50℃	**55℃**	**60℃**
1-Z:25 PS:20 R:15	274.07 ± 1.61^{eA}	270.70 ± 1.61^{dA}	251.88 ± 1.61^{cB}
2-Z:20 PS:15 R:25	278.78 ± 1.61^{eA}	278.73 ± 1.61^{cA}	275.58 ± 1.61^{bA}
3-Z:25 PS:15 R:20	296.41 ± 3.22^{bA}	295.31 ± 3.22^{aA}	292.84 ± 1.61^{aA}
4-Z:27.5 PS:10 R:22.5	134.27 ± 0.00^{iA}	133.15 ± 1.61^{iA}	133.13 ± 1.61^{gA}
5-Z:22.5 PS:20 R:17.5	281.57 ± 1.61^{deA}	250.16 ± 1.61^{eB}	248.15 ± 1.61^{cB}
6-Z:22.5 PS:12.5 R:25	251.74 ± 3.22^{afA}	201.69 ± 1.61^{ghB}	200.33 ± 1.61^{eB}
7-Z:20 PS:17.5 R:22.5	230.39 ± 1.61^{gA}	226.03 ± 1.61^{fA}	220.77 ± 1.61^{dB}
8-Z:30 PS:15 R:15	204.86 ± 1.61^{hA}	196.03 ± 1.61^{hB}	189.50 ± 1.61^{fB}
9-Z:20 PS:20 R:20	287.87 ± 1.61^{cdA}	293.40 ± 1.61^{aA}	271.64 ± 1.61^{bB}
10-Z:30 PS:10 R:20	206.21 ± 1.61^{hA}	205.48 ± 1.61^{gA}	202.50 ± 1.61^{eA}
11-Z:27.5 PS:17.5 R:15	289.84 ± 1.61^{bcA}	286.14 ± 1.61^{bA}	287.10 ± 3.22^{aA}
12-Z:25 PS:10 R:25	307.83 ± 3.22^{aA}	274.72 ± 1.61^{cdB}	270.15 ± 1.61^{bB}
13-Z:30 PS:12.5 R:17.5	278.42 ± 1.61^{eA}	267.87 ± 1.61^{dB}	254.16 ± 1.61^{cC}

Abbreviations: Z(zucchini), PS(pumpkin seed), R (radish)

Data in the same column with the different lowercase superscript letter and the data in the same row with the different uppercase superscript letter are significantly different ($p<0.05$).

Table 5. The results of raw materials

Response	**Pumpkin Seed**	**Zucchini**	**Radish**	**Pea Flour**
Moisture (%)	1.89±0.06	91.64±0.03	89.02±0.05	1.53±0.04
Protein (%)	36.63±0.25	2.14±0.32	1.64±0.17	32.62±0.35
Fat (%)	40.5±0.25	0.52±0.26	0.25±0.24	0.59±0.22
Ash (%)	3.98±0.32	2.12±0.35	0.65±0.29	4.22±0.25
Total phenolic content (mg GAE/g sample d.b.)	132.5±1.61	162.08±3.23	257.19±3.23	298.4±1.61
DPPH (%inhibition)	5.56±0.08	9.30±0.12	3.28±0.06	7.25±0.09

4.3. Physical Analyses

The results of physical analyses are this section.

4.3.1. Measurement of Texture Profile

Hardness contents of functional snack samples were measured based on 13 different formulations and are shown on Table 5. As the hardness (N) values, at 50℃, Sample 3 (Z:25 PS:15 R:20) had the lowest mean (3.43±0.25N), although at 55℃, Sample 11 (Z:27.5 PS:17.5 R:15) had the lowest mean (4.02±0.25N). At 60℃, Sample 12 (Z:25 PS:10 R:25) had the lowest mean (4.14±0.27N). As the hardness (N) values, at 50℃, Sample 10 (Z:30 PS:10 R:20) had the highest mean (5.28±0.28N), although at

55℃, Sample 13 (Z:30 PS:12.5 R:17.5) had the highest mean (4.96±0.10N). At 60℃, Sample 1 (Z:25 PS:20 R:15) had the highest mean (5.904±0.25N). Hardness (N) at 50℃, 55℃ and 60℃ were a statistically significant ($p< 0.05$).

Table 6. The results of hardness (N)

Sample	Hardness (N)		
	50℃	**55℃**	**60℃**
1-Z:25 PS:20 R:15	3.94 ± 0.08^{cdC}	4.29 ± 0.09^{bcdB}	5.90 ± 0.25^{aA}
2-Z:20 PS:15 R:25	3.96 ± 0.07^{cB}	4.15 ± 0.28^{bcdA}	4.40 ± 0.28^{dAB}
3-Z:25 PS:15 R:20	3.43 ± 0.25^{dB}	5.13 ± 0.27^{aA}	4.83 ± 0.29^{cA}
4-Z:27.5 PS:10 R:22.5	3.84 ± 0.16^{cdA}	4.07 ± 0.13^{deA}	3.96 ± 0.14^{dA}
5-Z:22.5 PS:20 R:17.5	3.63 ± 0.19^{cdB}	4.12 ± 0.21^{cdeA}	3.94 ± 0.13^{dAB}
6-Z:22.5 PS:12.5 R:25	4.04 ± 0.10^{cB}	5.05 ± 0.12^{aA}	4.57 ± 0.25^{cA}
7-Z:20 PS:17.5 R:22.5	3.97 ± 0.24^{cdC}	4.28 ± 0.28^{bcB}	5.08 ± 0.25^{bcA}
8-Z:30 PS:15 R:15	5.38 ± 0.28^{aA}	4.48 ± 0.24^{bcdB}	4.65 ± 0.23^{cAB}
9-Z:20 PS:20 R:20	3.69 ± 0.17^{cdA}	4.36 ± 0.28^{cdeB}	5.16 ± 0.14^{bC}
10-Z:30 PS:10 R:20	5.28 ± 0.28^{aA}	4.82 ± 0.28^{abB}	5.13 ± 0.28^{bcAB}
11-Z:27.5 PS:17.5 R:15	5.17 ± 0.29^{aA}	4.02 ± 0.25^{eB}	5.23 ± 0.26^{bcA}
12-Z:25 PS:10 R:25	4.67 ± 0.29^{bA}	4.88 ± 0.07^{aA}	4.14 ± 0.27^{dB}
13-Z:30 PS:12.5 R:17.5	3.85 ± 0.10^{cdB}	4.96 ± 0.10^{aA}	4.91 ± 0.25^{bcA}

Abbreviations: Z(zucchini), PS(pumpkin seed), R (radish)

Data in the same column with the different lowercase superscript letter and the data in the same row with the different uppercase superscript letter are significantly different ($p<0.05$).

4.3.2. Color Analysis

Impacts of the baking at different temperatures and ingredients on the color change are shown on Table 6. As the *L** value results of this study, at 50℃, 55℃ and 60℃, Sample 1 (Z:25 PS:20 R:15) had the lowest mean (48.68±0.24, 43.75±0.19 and 46.15±0.22, respectively). As the *L** results of this study, at 50℃, 55℃ and 60℃, Sample 6 (Z:22.5 PS:12.5 R:25), Sample 4 (Z:27.5 PS:10 R:22.5), Sample 10 (Z:30 PS:10 R:20) had the highest mean (in order of 56.13±0.17 , 57.59±0.29 and 56.19±0.1, respectively).

As the a* value results of this study, at 50℃, 55℃ and 60℃, Sample 9 (Z:20 PS:20 R:20), Sample 12 (Z:25 PS:10 R:25), Sample 10 (Z:30 PS:10 R:20) had the lowest mean (-2.85±0.13, -1.63±0.07 and, -2.14±0.04, respectively). As the a* value results of this study, at 50℃, 55℃ and 60℃, Sample 12 (Z:25 PS:10 R:25), Sample 3 (Z:25 PS:15 R:20), Sample 1 (Z:25 PS:20 R:15) had the highest mean (0.23±0.14, 1.12±0.06 and, 1.11±0.04, respectively). As the b* value results of this study, at 50℃ and 55℃, Sample 1 (Z:25 PS:20 R:15) had the lowest mean (24.02±0.23, 22.66±0.21 and, 26.14±0.19, respectively). As the b* value results of this study, at 50℃, 55℃ and 60℃, Sample 11 (Z:25 PS:10 R:25), Sample 4 (Z:25 PS:15 R:20), Sample 10 (Z:30 PS:10 R:20) had the highest mean (28.86±0.26, 29.41±0.24 and, 30.41±0.27, respectively). As the b* value results at 60℃ was Sample 9 (Z:25 PS:20 R:15) had the lowest mean (25.72±0.25). For *L**, a* and b* values of this study, at 50℃, 55℃ and 60℃ are statistically significant ($p < 0.05$).

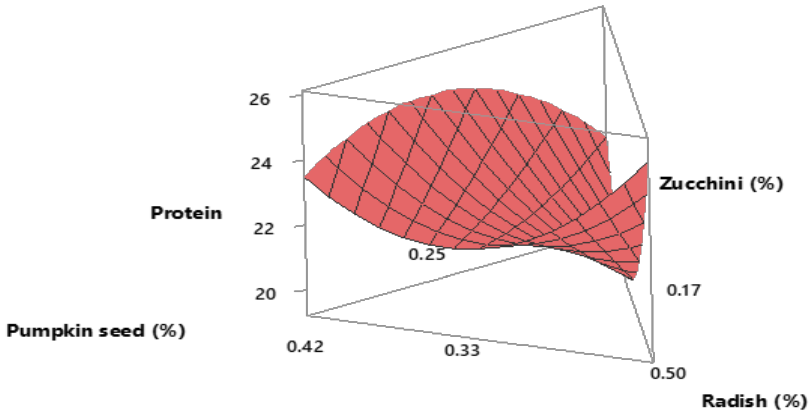
Table 7. Color Measurement Results

Sample	*L**			a*			b*		
	50℃	**55℃**	**60℃**	**50℃**	**55℃**	**60℃**	**50℃**	**55℃**	**60℃**
1	48.68±0.24^{hA}	43.75±0.19^{jC}	46.15±0.22^{iB}	-0.54±0.09^{bB}	0.97±0.09^{aA}	1.11±0.04^{aA}	24.02±0.23^{hB}	22.66±0.21^{gC}	26.14±0.19^{iA}
2	50.19±0.28^{gB}	45.71±0.21^{iC}	51.67±0.2^{eA}	-1.83±0.11^{dB}	-0.38±0.05^{cdA}	-0.41±0.05^{cA}	26.49±0.28^{fB}	24.47±0.27^{fC}	28.96±0.23^{cA}
3	51.53±0.22^{eA}	46.34±0.27^{hC}	48.49±0.25^{hB}	0.21±0.06^{aB}	1.12±0.06^{aA}	0.55±0.14^{bB}	28.85±0.22^{aA}	24.54±0.25^{fC}	27.23±0.11^{gB}
4	53.82±0.24^{cC}	57.59±0.29^{aA}	55.98±0.27^{aB}	-0.55±0.18^{bA}	-0.69±0.04^{deB}	-1.24±0.04^{deC}	28.8±0.26^{abB}	29.41±0.24^{aA}	28.99±0.14^{bcAB}
5	50.82±0.25^{fB}	47.19±0.22^{fgC}	53.81±0.24^{cA}	-0.23±0.15^{abA}	-0.13±0.05^{bcA}	-1.24±0.03^{deB}	27.33±0.25^{eA}	24.62±0.28^{efB}	27.51±0.23^{fgA}
6	56.13±0.17^{aB}	57.8±0.23^{aA}	55.04±0.17^{bC}	-1.30±0.10^{cA}	-1.12±0.04^{fA}	-0.92±0.11^{dA}	28.53±0.22^{bcA}	27.76±0.18^{bB}	27.51±0.27^{efB}
7	52.82±0.26^{dA}	47.54±0.27^{fC}	50.12±0.27^{gB}	-1.56±0.16^{cdB}	-0.79±0.1^{efA}	-1.19±0.18^{dA}	26.7±0.2^{fA}	25.05±0.17^{deB}	26.57±0.16^{hA}
8	53.01±0.18^{dA}	46.9±0.23^{gC}	51.34±0.26^{fB}	-2.71±0.16^{eC}	-1.04±0.12^{efA}	-1.73±0.15^{fgB}	27.89±0.19^{cdA}	25.05±0.18^{deB}	28.2±0.26^{dA}
9	52.56±0.20^{dA}	52.49±0.23^{dA}	52.6±0.26^{dA}	-2.85±0.13^{eB}	-1.56±0.14^{gA}	-1.76±0.19^{gA}	26.99±0.24^{eA}	25.71±0.17^{dB}	25.72±0.25^{jB}
10	52.17±0.14^{eB}	49.08±0.19^{eC}	56.19±0.1^{aA}	-1.68±0.05^{cdB}	-0.38±0.09^{cdA}	-2.14±0.04^{hC}	27.56±0.21^{dB}	27.01±0.23^{cC}	30.41±0.27^{aA}
11	55.07±0.22^{bA}	55±0.26^{bA}	52.39±0.24^{dB}	-1.59±0.12^{cdA}	-1.58±0.14^{gA}	-1.4±0.07^{efA}	28.86±0.26^{aA}	27.81±0.27^{bB}	29.27±0.19^{bA}
12	50.5±0.2^{gB}	53.69±0.24^{cA}	53.64±0.26^{cA}	0.23±0.14^{aA}	-1.63±0.07^{gB}	0.53±0.19^{bA}	25.74±0.24^{gB}	27.69±0.28^{bA}	27.97±0.21^{eA}
13	49.88±0.27^{gB}	44.96±0.27^{iC}	52.76±0.25^{dA}	-0.38±0.06^{bB}	0.2±0.04^{bA}	-1.48±0.09^{efC}	26.28±0.21^{fB}	25.26±0.23^{eC}	29.6±0.26^{bA}

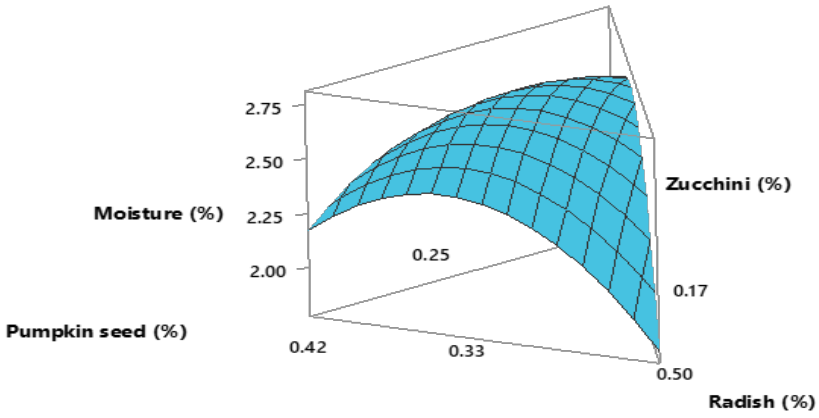
Abbreviations: L* (lightness), a* (red/green value), b* (yellow/blue value)

Data in the same column with the different lowercase superscript letter and the data in the same row with the different uppercase superscript letter are significantly different ($p<0.05$)

4.4. Experimental Design and Optimization



(a)



(b)

Figure 5. Protein (a) and moisture (b) contents with respect to raw materials at 60°C

The EVMD method has 3D response surface plots for the demonstration of the interaction between zucchini, radish, and pumpkin seed. As seen in Figure 4(a), zucchini and pumpkin seed had a significant positive impact on protein content. Conversely, protein content was decreased with zucchini and radish combinations. In Figure 4(b), moisture content increased when zucchini and pumpkin seed were used and decreased with radish replaced with pumpkin seed. The predicted values for the maximum content

of protein and moisture were 21.85 % and 2.5 %, respectively. These results were achieved with 40% pea flour, 22.5% zucchini, 25% radish and 12.5% pumpkin seeds at 60°C. For verification, the predicted proportions were tested, and the content of the protein were found as 21.25±0.38 and and the content of the moisture were found as 2.58±0.03. These experimental values were close to predicted ones therefore it was confirmed that the developed model was successful.

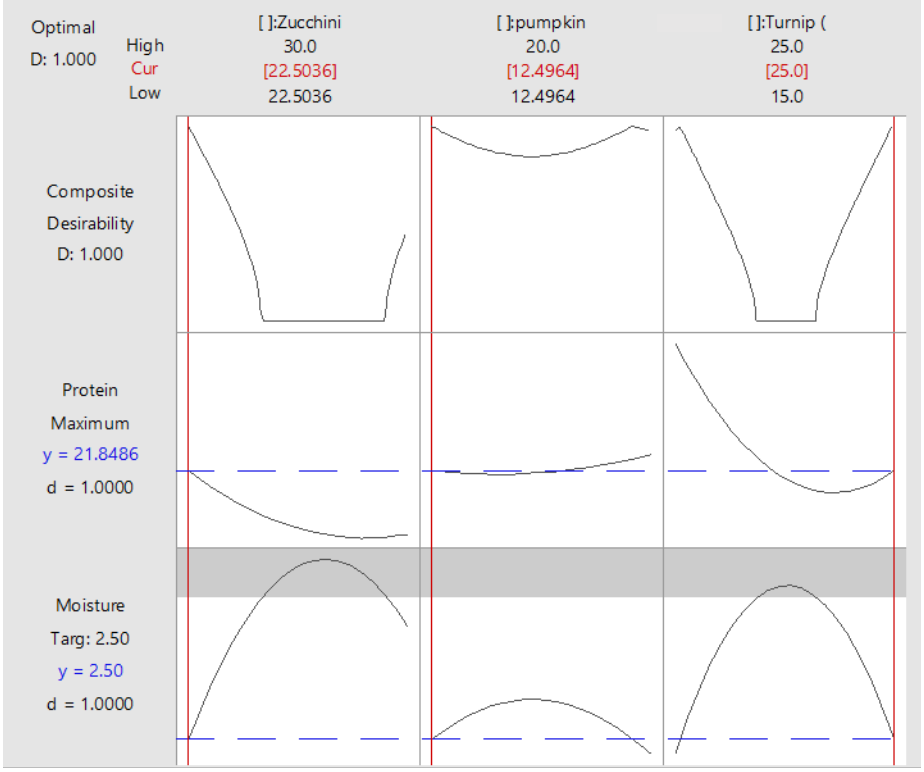


Figure 6. Protein and moisture at 60°C with optimized values

Table 8. Regression model for moisture and protein for developed functional snack

Response	**R^2**	**$R_{(adj)}^2$**	**Experimental value**	**Predicted value**	**Desirability**	**CV(%)**
Moisture(%)	0.63	0.38	2.58±0.03	2.5	1.0	2.22
Protein(%)	0.74	0.57	21.25±0.38	21.85	1.0	1.96

Table 9. The results of optimized samples

	Temperature		
Response	**50℃**	**55℃**	**60℃**
Moisture (%)	2.69±0.06[a]	2.66±0.06[a]	2.58±0.03[a]
Protein (%)	21.16±0.15[a]	21.82±0.08[a]	21.25±0.38[a]
Fat(%)	19.73±0.26[a]	19.8957±0.26[a]	19.84±0.24[a]
Ash (%)	2.27±0.36 [a]	2.5932±0.33[a]	2.46±0.37[a]
Hardness (N)	4.02±0.02[b]	5.01±0.00[a]	4.31±0.15[b]
thickness	0.029±0.00[a]	0.029±0.00[a]	0.02±0.00[a]
*L**	56.14±0.09[b]	57.82±0.22[a]	55.04±0.11[c]
a*	-1.27±0.07[a]	-1.2±0.07 [a]	-0.95±0.09 [a]
b*	28.41±0.13[a]	27.81±0.06[b]	27.58±0.05[b]
DPPH (%inhibition)	9.72±0.06[a]	8.58±0.15[b]	7.28±0.14[c]
Total Phenol Content (mg GAE/g sample d.b.)	250.6± 1.61[a]	202.84±3.23[b]	201.48±3.23[b]

Abbreviations: L* (lightness), a* (red/green value), b* (yellow/blue value)
Differences in superscript letters indicate significant difference ($p<0.05$) in a row.

The moisture content of the optimized samples had at 60℃ with the lowest mean(2.58±0.03%) while at 50℃ with the highest mean (2.69±0.06%). The protein content of the optimized samples had at 50℃ with the lowest mean (21.16±0.15%) while at 55℃ with the highest mean(21.82±0.08%). The fat content of the optimized samples had at 50℃ with the lowest mean (19.73±0.26%) while at 60℃ with the highest mean (19.84±0.24%). The ash content of the optimized samples had at 50℃ with the lowest mean (2.27±0.36%) while at 55℃ with the highest mean (2.59±0.33%). The hardness (N) of the optimized samples had at 50℃ with the lowest mean (4.02±0.02N)while at 55℃ with the highest mean (5.01±0.00N). The thickness of the optimized samples had same mean (0.029±0.00) at 50℃ , 55℃ and 60℃ ($p> 0.05$). The *L** value of the optimized samples had at 60℃ with the lowest mean (55.04±0.11) while at 55℃ with the highest mean(57.82±0.21). The a* value of the optimized samples had at 50℃ with the lowest mean (-1.27±0.07) while at 60℃ with the highest mean (-0.95±0.09). The b* value of the

optimized samples had at 60℃ with the lowest mean (27.58±0.05) while at 50℃ with the highest mean (28.41±0.13). The total phenolic content of the optimized samples had at 60℃ with the lowest mean (201.48±3.23 mg GAE/g sample d.b.) while at 50℃ with the highest mean (250.6± 1.61 mg GAE/g sample d.b.). The DPPH(% inhibition) of the optimized samples had at 60℃ with the lowest mean (7.28±0.14) while at 50℃ with the highest mean (9.72±0.06). For moisture contents (%), protein contents (%), fat contents (%), ash contents (%), thickness and a* value at 50℃ , 55℃ and 60℃ were not statistically significant ($p > 0.05$). For hardness (N) , L^* value, b* value, the total phenolic content, and the DPPH(% inhibition) at 50℃ , 55℃ and 60℃ were statistically significant ($p < 0.05$).

4.5. Sensory Analysis

A total of 65 people participated in the sensory analysis conducted at Izmir Institute of Technology and Manisa Celal Bayar University. Of the participants, 34 were women and 31 were men, and age distribution of the panelists; are given on Table 10. The changes in the sensory scores of the samples determined as the optimum sample among the functional snack samples consisting of 22.5% zucchini, 25% radish, 12.5% pumpkin seeds and 40% pea flour as raw materials are shown in Figure 6. The mixture, which was cooked at 60℃, was the most admired for its appearance, smell, crispness, taste and overall acceptability.

Table 10. Participants in the sensory analysis

GENDER		**AGE**				
Woman	**Man**	**<18**	**18-25**	**26-40**	**41-55**	**>56**
34	31	0	38	25	2	0

In line with these results, according to the tastes of the panelists participating in the sensory panel, the sample that they liked the most was the sample that was cooked at 60℃.

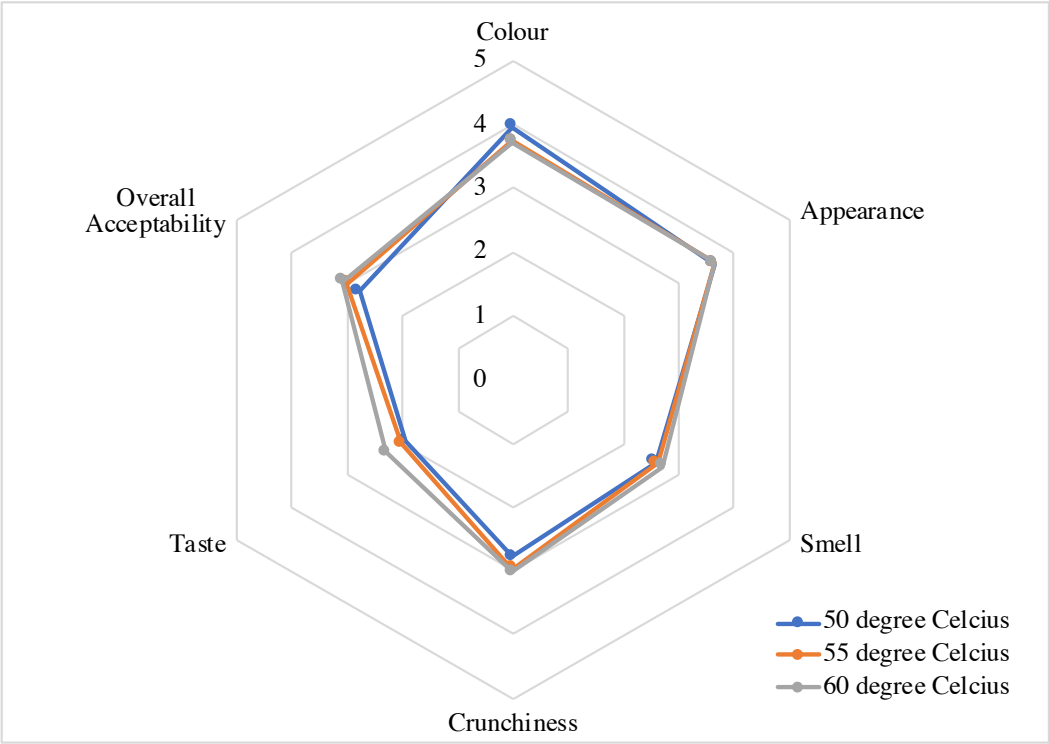


Figure 7. The result of sensory analysis

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

This thesis is about the development of a functional snack from protein-rich vegetables. For the first time in the literature, mixing different protein-rich vegetables and drying them in a tray dryer was investigated. The major conclusions reached by this thesis is as follows; (1) temperature change did not affect the protein loss of the food much, (2) zucchini and pumpkin seeds were observed rich in protein, (3) people were seen willing to try new foods. The color of the product is green and people who have the opportunity to try the product felt that they consume a healthier and more nutritious snack. This study showed that there is a need to focus on the development new products to meet people's daily dietary needs.

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APPENDICES

APPENDIX A.

THE TOTAL PHENOLIC CONTENT

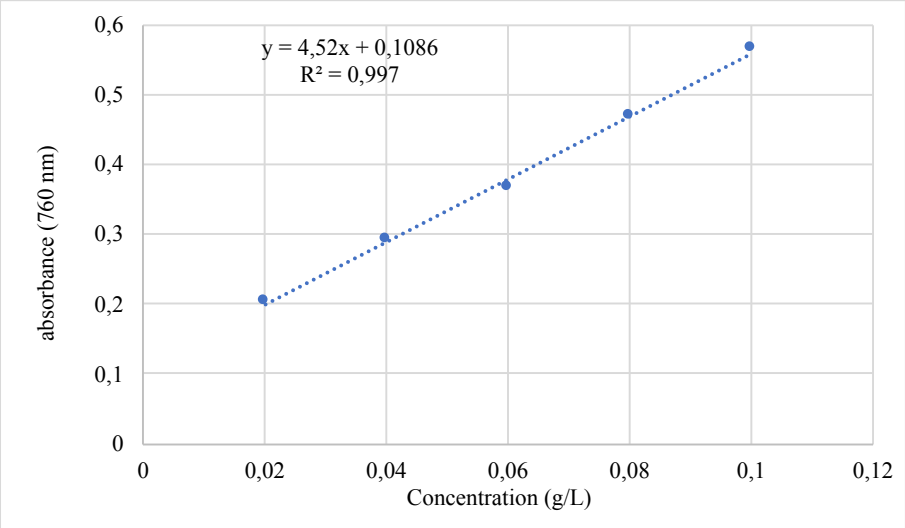

Figure A.1. Gallic acid calibration curve for the total phenolic content

APPENDIX B.

SENSORY ANALYSIS PANEL FORM

FUNCTIONAL SNACK PRODUCT SENSORY PANEL FORM

Gender: ( ) Female ( ) Male Date:

Age: ( ) <18 years old ( ) 18-25 years old ( ) 26-40 years old ( ) 41-55 ( ) >56 years old

Different functional snack products offered to you consist of zucchini, radish, pumpkin seeds and pea flour. Taste the products offered to you with three different codes, from left to right, and write one of the scores on the scale for each quality parameter in the form below, according to your liking.

Score Scale
1: I did not like it at all
2: I like it less
3:neither I like nor dislike
4: I like it a little
5: I like it very much

		1	2	3	4	5
Sample Code:684	Color					
	Appearance					
	Smell					
	Crunchiness					
	Taste					
	Overall Acceptability					
		1	2	3	4	5
Sample Code:349	Color					
	Appearance					
	Smell					
	Crunchiness					
	Taste					
	Overall Acceptability					
		1	2	3	4	5
Sample Code:572	Color					
	Appearance					
	Smell					
	Crunchiness					
	Taste					
	Overall Acceptability					

Please write the code of the product you like the most out of the three products you have just tasted, in the first part, the code of the other product you like in the second part and the code of the least favourite product in the third part.

1.	2.	3.

Thank you very much for joining our sensory panel.
Zeynep Tuğba MAŞA (zeynepmasa@iyte.edu.tr)