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Will the metaverse simulate everything?

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Effects of Disodium Octaborate Tetrahydrate (DOT) on Seed Germination and Development in Rocket (*Eruca sativa* Mill.) and Cress (*Lepidium sativum* L.)

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Abstract: Rocket (Eruca sativa Mill.) is cultivated throughout the year. Thanks to the rich metabolites of its leaves, it has a wide usage area in pharmacy. Cress (Lepidium sativum) is in the group of annual vegetables and has a herbaceous structure. Due to its fragrant and slightly spicy structure, it is a vegetable that is used as an appetizer. Its seeds and green parts are very beneficial for health. Rocket is a short-day plant whose leaves are considered to be rich in many minerals and vitamins. The present research was conducted to investigate the effects of doses of Disodium octaborate tetrahydrate (DOT) (0 (control), 15, 30, 45, 60 mg L^{-1} on the germination and development of seeds of rocket and cress plants grown in pot experiments. In order to determine the effect of DOT on the development and yield of rocket and cress plants, the percentage of germinated seeds, cotyledon length and dry matter amounts were determined. ANOVA test was used to analyze the data obtained in the present study. Tukey test was used to determine which groups were in significant differences between the groups. Overall, it was seen that there were significant growth differences between the doses used statistically, the particularly 45 mg L^{-1} application DOT positively affected the germination and dry matter content of rocket and cress seeds.

Keywords: Rocket, Cress, Disodium octaborate tetrahydrate (DOT), seed germination.

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1. INTRODUCTION

In recent years, vegetable consumption has increased considerably with its beneficial effects on human health and nutritive contents (Chang et al. 2013). Rocket (*Eruca sativa* Mill.) and cress (*Lepidium sativum* L.) both cruciferous plants have rich sulforaphane ingredients that provide to induce NRF2-HO-1 antioxidant pathway (Bell et al. 2015). In usual, it is beneficial to eat brassicaceous greens that reduce cancer, cardiovascular issues and diabetes (Podsędek 2007; Tounsi et al. 2019).

Rocket is classified into Brassicaceae family. In general, rocket are harvested from nature as well as cultivated edible plants. Leaves are highly nutritive in terms of vitamins and minerals (Moussa 2006). Rocket is a plant rich in nutrients and has been reported to contain 5.13% K, 4.32% N, 0.25% P, 0.58% Mg, 2.95% Ca, 799.88 mg kg^{-1} Na, 64.86 mg kg^{-1} Zn, 5.36 mg kg^{-1} Cu, 350 mg kg^{-1} Fe, and 40.58 mg kg^{-1} Mn (Barlas et al. 2011). Rocket has health promoting beneficial properties (Guijarro-Real et al. 2020). It is known that rocket cultivation dates back to ancient times as a source of food, oilseeds crop and medicinal plants (Padulosi and Pignone 1996; Hall et al. 2012). Rocket is used as a salad vegetable because of low-calorie content, it is as well beneficial for health-promoting nutraceutical and anticancer properties (Higdon et al. 2007; Bell and Wagstaff 2014). Rocket essential oil is an important precursor for triazoles synthesis which creates a-glucosidase inhibitors. Therefore could be an important oppress for postprandial hyperglycemia in diabetic patients (Hichri et al. 2019). Seeds of rocket have been used in folk medicine since ancient times for their diuretic, antimicrobial, lactagogue, aphrodisiac, and many other effects (Hussain et al. 2020). It was determined that rocket was used as a garden crop and spice in studies dating back to the middle ages, and special attention was paid to its biological diversity (Yaniv et al. 1998). Cruciferous plants have some phytochemicals protective against DNA damage such as sulforaphane erusine and erysoline. Rocket diet could increase hepatic ABC transporters' expression which reduces the risk of toxic compounds (Roma et al. 2019). Rocket is widely spread all over the world (Barillari et al. 2005).

Cress belongs to the Brassicaceae family has been cultivated in Europe, the US and India. The seeds are edible and beneficial with medicinal properties (Gokavi et al. 2004; Mali et al. 2007; Karazhiyan et al. 2009; Diwakar et al. 2010). Cress is a fast-growing herb with a tangy flavour and aroma (Manohar et al. 2012). Cress could grow up to 50 cm as annual plants and rich in some minerals also vitamins A and C (Ajdanian et al. 2019). Besides, it is included an important amount of folic acid, iron and calcium. (Sharma and Agarwal, 2011). Previous studies have shown that cress seeds are used for nutritional food or dietary purposes (Gokavi et al. 2004; Karazhiyan et al. 2009). In addition, seeds were traditionally used for diet breastfeeding woman's milk secretion (Diwakar et al. 2008; Datta et al. 2011). In addition, leaves have antibacterial properties, also helpful in cure hepatopathy and scurvy (Karazhiyan et al. 2011a). The performed studies on cress hepatoprotective effect were indicated that CCl_4 (carbon tetrachloride) liver damage avoided in rats (Wadhwa et al. 2012). Cress seed has the potential to be used as a thickener in the food industry due to its hydrocolloid property (Karazhiyan et al. 2009; Karazhiyan et al. 2011 b; Behrouzian et al. 2014). Another study conducted by Naji et al. (2012) was stated that cress seed gum stands higher thermal treatments increase viscosity as desirable. In oppositely refrigeration conditions, cress seed gum was also functional with increased viscosity (Naji and Razavi, 2014). Leaves are generally used for salad, sandwiches, garnish and animal forage (Mali et al. 2007; Karazhiyan et al. 2009). Nehdi et al. (2012) was stated that cress seed oil has potential for biodiesel production due to be atomized readily and finer droplets.

Plants are important to drug research even though desired substances can be obtained as synthetic molecules (Eddouks et al. 2005). One of the medicinal plants is cress. Cress seed mucilage was used as a natural ingredient in pharmaceuticals (Behrouzian et al. 2014). Studies conducted by Paranjape and Mehta (2006) signify that cress was highly effective against bronchial asthma, 4 weeks treatment cress seed powder to 30 patients without any other cure medication. There were important beneficial improve clinical symptoms and asthmatic attacks. Another study performed by Maghrani et al. (2005) stated that aqueous extract of cress demonstrated antihypertensive and diuretic activities. Furthermore, cress seed oil was beneficial in albino rats due to the dietary source of alpha-linolenic acid (Diwakar et al. 2008). Cress leaves and seeds were suggested to rheumatism and muscular pain. Moreover, it has aperient, aphrodisiac properties (Sharma and Agarwal 2011; Doke and Guha 2014; Hadi and Hameed 2017). Aqueous extract of cress seed stimulates apoptosis and necrosis in human breast cancer cell line MCF-7 (Mahassni and Al-Reemi 2013).

The main fatty acids in cress oil were linoleic and oleic acid. In addition to its high viscosity, cress oil has excellent lubricating properties (Moser et al. 2009). It was stated that cress seed oil is highly steady at cold temperatures. Cress seed oil is contained carotenoids and tocopherol as natural antioxidants. Therefore it maintains oil quality; cress seed oil is very helpful for the treatment of skin disease, leprosy, lumbago, dysentery (Kirthikar 1952; Diwakar et al. 2010). Investigation performed by Kasabe et al. (2012) expressed that cress seed has medicinal and nutritional properties also antioxidant activities. Attia et al. (2019) reported that cress methanol extract was anti-diabetic and anti-oxidant properties. It was stated that cress has fracture healing properties in rabbits (bin Abdullah 2007). Chemo protective effects of cress investigation were performed by Kassie et al. (2002) signify that cress provides an important decrease to DNA damage in colon and liver cells. Conducted investigation along 14 weeks by Datta et al. (2011) emphasizes that cress seeds were none-toxic and trustworthy for feeding to rats in both genders. Cress seed has plenty of rich protein content and fat (Sharma and Agarwal 2011; Doke and Guha 2014).

Macro and micronutrients are very important in the agricultural ecosystem, but the insufficient knowledge of the use of micronutrients in agricultural lands and their limited availability in the soil cause microelement deficiency in agricultural plants. Boron is a micronutrient element that is rapidly depleted in the soil (Tahir et al. 2009). Boron is an essential element required for optimum growth and development in advanced plants (Marschner, 1995). It is known that boron plays an important role in nutrient transport by plant membranes (Tanada, 1983). Studies show that boron can affect the accumulation and utilization of other plant nutrients as a regulator or inhibitor (Alvarez- Tinaut et al., 1979). In plant cell walls, boron is an important component to crosslink pectic polysaccharides rhamnogalacturonan-II (RG-II) complex (Onuh and Miwa 2021).

We have done extensive research on the benefits and different uses purpose of both herbs. Cress and rocket are used in the treatment of various diseases. It is also rich in vitamins and minerals and especially consumed as a salad. Therefore it is important to rapid growth to meet the demand. In this research, we investigated germination percentage (%), cotyledon length (mm), Dry weight per plant (mg) under boron fertilizer in the 4th, 8th, 12th days.

2. MATERIAL AND METHODS

2.1. Materials and Treatments

Standard seeds of rocket (Rota variety) and cress (Helen variety) were used as plant material in the study. Pot soil with properties of pH 5.5-6.8, EC (μs cm^{-1}) 220, organic matter 54-60%, humidity 53.43%, water holding capacity 575.03, and purity 95% was used as the growing medium. Seeds were planted in 1.5 L (15x15 cm) pots with 40 seeds each. The study was set up according to a randomized plot trial pattern with three replicates and 3 pots for each repeat. The seeds were grown in pots at 22±1°C in a growth chamber under controlled conditions, 12 hours dark and 12 hours light (400 μmol m^{-2} s^{-1}) photo-cycle. The seeds in the pot were irrigated with different doses of sodium borohydride dissolved in distilled water at regular intervals during the application. Sodium borohydride ($Na_2B_8O_{13}.4H_2O$ disodium octaborate tetrahydrate) (DOT) (Brand name: ETIDOT-67, water soluble boron 20.8%) administration doses were determined as 0 (control), 15, 30, 45, 60 mg L^{-1}. The harvest time was determined according

to the preliminary studies we carried out before. Accordingly, the cotyledon samples were harvested on the 4th, 8th, and 12th days. Generally, after the seeds of arugula and cress are planted, they begin to absorb water into their bodies. On the fourth day after planting, the cotyledon grows upward while its roots move through the soil. These properties were effective in the selection of the first harvest time.

2.1. Methods

Determination of the germination percentage

While determining the germination percentage; The seeds germinated on the 4th, 8th, and 12th days of pots in each application were counted and calculated according to the formula below.

Germination (%)= (Number of seeds that germinated / Number of seeds on the pot) x 100

Determination of average cotyledon length

In determining the average cotyledon length; On days 4, 8, and 12, the above-soil part of five plants randomly selected from each pot was measured with a digital caliper and their average was calculated in mm**.**

Determination of cotyledons dry weight per plant

On the harvest days, the parts above the root node of 5 plants randomly selected from each pot were dried in the oven at 45 ° C until they reached constant weight. It was then weighed on an analytical balance and divided by the number of plants and expressed in mg.

Data analysis

Experiments were conducted with 3 replicates per analysis. The importance of the implementation effect was determined at the 5 % prospect level by utilizing the Tukey test of one-way ANOVA with the assistance of SPSS 15 (Statistical Package for Social Sciences, SPSS Inc., IL, USA).

3. RESULTS AND DISCUSSION

The increasing polycyclic aromatic hydrocarbons were decreased to *L. sativum* germination rate in polluted artificial soil (Maila and Cloete 2002). Pavel et al. (2013) stated that metal ions were inhibiting to seed germination of *L. sativum.* Another investigation indicated that Myrigalone A was impeded seed germination of *L. sativum.* (Oracz et al. 2012). The imidazolium ionic liquids were decreased to seed and root germination (Studzińska and Buszewski 2009). Research clarifies that volatile organic compounds (VOC) affected seed germination. It was expressed that high-VOC biochar causes total inhibition (Buss and Masek 2014). Another study revealed that microplastics can cause a delay of germination and root growth to *L. sativum* (Bosker et al. 2019). Therefore it is important to understand negative factors to prevent seed germination. As a result of our research, it has been determined that DOT promotes seed germination of both two plants (Table 1). We applied plants to DOT and harvested to 4th, 8th,12th days of growth.

Results indicated that treatments of 0 mg L^{-1}, 15 mg L^{-1}, 30 mg L^{-1}, 45 mg L^{-1}, 60 mg L^{-1} DOT affect seed germination in cress. It is seen that the germination percentage is quite low in control plants that have never been applied DOT by examined in table 1. Control (0 mg L^{-1}) plants germination percentage 4th days 30%, 8th days 42.5%, and 43.3% in the end of 12th days of growth in cress (Table 1). The 45 mg L^{-1} DOT treatments have the best results in seed germination. In the cress, treatments of 45 mg L^{-1} sodium borahydride demonstrated germination rate in a row 4th days 50%, 8th days 75% and 76.66% at the end of 12th day of growth. According to other concentrations, 45 mg L^{-1} DOT treatments are showed the fastest germination rate at the end of the 8th and 12th day in cress (Table 1). In similar to our research, the effect of 0, 0.1, 0.2, 0.4 and 0.8 kg da-1 DOT doses in vetch (Vicia ervilia (l.) willd), Kılıç (2019) was found that 0.4 kg da-1 DOT application increased seed yield compared to all applications.

Rocket is normally a fast-growing plant in nature. However, many factors can affect germination and growth (Garg and Sharma 2014; Shariatinia et al. 2021). Rocket germination results are similar to cress plants, control plants (0 mg L^{-1}) have minimum germination 70.83 %, the best germination rate 92.5 % in 45 mg L^{-1} DOT treatments.
Germination rate in both cress and rocket, 60 mg L^{-1} DOT treatment shows similarly less germination than 45 mg L^{-1}. Therefore we suggest 45 mg L^{-1} DOT fertilizer in both cress and rocket (Table 1).
.

Table 1. Cress and rocket germination percentage (%)

Days (cress seed germination percentage)		4. day	8. day	12. day
Treatments (DOT mg L^{-1})	0 mg L^{-1}	30.00±1.44^{d}	42.50±1.44^{c}	43.33±0.83^{d}
	15 mg L^{-1}	37.50±1.44^{c}	45.00±0.00^{bc}	45.00±1.44^{d}
	30 mg L^{-1}	37.50±0.00^{c}	50.00±1.44^{b}	55.00±1.44^{c}
	45 mg L^{-1}	50.00±0.00^{b}	75.00±1.44^{a}	76.66±0.83^{a}
	60 mg L^{-1}	62.50±2.88^{a}	68.33±2.20^{a}	68.33±2.20^{b}
Days (rocket seed germination percentage)		**4. day**	**8. day**	**12. day**
Treatments (DOT mg L^{-1})	0 mg L^{-1}	62.50±1.44^{c}	70.00±2.88^{c}	70.83±2.20^{d}
	15 mg L^{-1}	74.16±0.83^{b}	78.33±1.66^{bc}	78.33±1.66^{cd}
	30 mg L^{-1}	82.50±1.44^{a}	82.50±1.44^{b}	82.50±1.44^{bc}
	45 mg L^{-1}	87.33±2.33^{a}	92.50±1.44^{a}	92.50±1.44^{a}
	60 mg L^{-1}	83.33±0.83^{a}	86.66±1.66^{ab}	86.66±1.66^{ab}

$^{a, b, c, d}$ The values designated by different letters on the same column are significantly different on 5 % significance level.

Note: (***) means 99.9% confidence level, (**) means 99% confidence level, (*) means 95% confidence level (ns) is statistically insignificant and (a, b, c, d, e) means homogeneous groups

Pandey (2012) investigated the effects of different concentrations of boron (0, 0,33, 3.3, 33, 330 mM) applications on flaxseed (Linum usitatissimum L. var R552) on seed germination and cotyledon length. It was stated that low doses of boron applications affected the cotyledon length positively, but with the increase of boron concentration, seed germination and vitality index decreased and percent phytotoxicity increased. Cress and rocket DOT treatment is importantly effective to cotyledon length (Table 2). On the one hand, cress control treatments maximum cotyledon length 20.05 mm, on the other hand, 45 mg L^{-1} boron fertilizer were positively increased to 50.42 mm at the end of the 12th growth. Rocket control plants cotyledon length 26.07 mm in 12th growth. However, this results up to 49.64 mm when the 45 mg L^{-1} boron fertilizer was applied to plants (Table 2). Both two plants growing regularly, according to our observation 45 mg L^{-1} boron fertilizer quite effective over cotyledon length.

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Table 2 Cress and rocket cotyledon length (mm)

Days (Cress cotyledon length mm)		4.day	8.day	12.day
Treatments (DOT mg L^{-1})	0 mg L^{-1}	6.54±0.55^{b}	8.86±0.22^{c}	20.05±1.12^{d}
	15 mg L^{-1}	7.46±0.91^{b}	9.80±0.224^{c}	30.41±0.97^{c}
	30 mg L^{-1}	29.73±0.21^{a}	36.30±1.47^{b}	39.91±1.89^{b}
	45 mg L^{-1}	30.40±0.34^{a}	40.19±1.07^{a}	50.42±0.83^{a}
	60 mg L^{-1}	28.15±0.94^{a}	38.68±0.17^{ab}	40.07±2.03^{b}
Days (Rocket cotyledon length mm)		**4.day**	**8.day**	**12.day**
Treatments (DOT mg L^{-1})	0 mg L^{-1}	5.29±0.28^{d}	21.94±1.30^{b}	26.07±1.75^{c}
	15 mg L^{-1}	20.19±0.72^{c}	38.12±0.81^{a}	40.31±0.61^{b}
	30 mg L^{-1}	24.43±0.76^{b}	38.04±0.54^{a}	40.80±1.17^{b}
	45 mg L^{-1}	29.90±0.31^{a}	39.97±1.81^{a}	49.64±1.02^{a}
	60 mg L^{-1}	26.24±0.18^{b}	38.11±0.53^{a}	42.47±0.24^{b}

$^{a, b, c, d}$ The values designated by different letters on the same column are significantly different on 5 % significance level.

Although boron is generally considered immobile, plays an important role in the transport of sugars. It also helps carbohydrate, RNA (ribonucleic acid) and IAA (indoleacetic acid) metabolisms (Kacar et al. 2020).

Cress and Rocket dry weight per plant were analyzed in presence of various concentrations of boron fertilizer (Table 3). There were no negative effects of DOT fertilizers. All other concentrations were higher than control treatments. In the parallel of our investigation, Besheit et al. (1992) applied 40 ppm doses of B, Zn, Mn and Fe separately to sugar beet seeds before planting. In the same study, they reported that the dry weight was increased by 40 ppm Boron application compared to the control and other applications.

Cress and Rocket's dry weight per plant (mg) were more raised at 45 mg L^{-1} DOT fertilizers (Table 3). In the 4th day of growth dry weight per plant was 2.23 fold and 1.42 fold more at cress and rocket respectively in the 45 mg L^{-1} DOT treatments when compared to control plants.

In the parallel of our investigation, Besheit et al. (1992) applied 40 ppm doses of B, Zn, Mn and Fe separately to sugar beet seeds before planting. In the same study, they reported that the dry weight was increased by 40 ppm Boron application compared to the control and other applications.

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Table 3 Cress and rocket dry weight per plant (mg)

Days (Cress Dry weight per plant mg)		4.day	8.day	12.day
Treatments (DOT mg L^{-1})	0 mg L^{-1}	0.59±0.04^{c}	0.78±0.07^{c}	0.99±0.08^{b}
	15 mg L^{-1}	0.92±0.04^{b}	1.08±0.05^{b}	1.16±0.04^{b}
	30 mg L^{-1}	1.21±0.03^{a}	1.45±0.03^{a}	1.48±0.05^{a}
	45 mg L^{-1}	1.32±0.06^{a}	1.58±0.03^{a}	1.67±0.04^{a}
	60 mg L^{-1}	1.28±0.02^{a}	1.48±0.04^{a}	1.54±0.05^{a}
Days (Rocket Dry weight per plant mg)		**4.day**	**8.day**	**12.day**
Treatments (DOT mg L^{-1})	0 mg L^{-1}	1.01±0.01^{b}	1.16±0.02^{b}	1.20±0.01^{d}
	15 mg L^{-1}	1.13±0.00^{b}	1.60±0.03^{a}	1.64±0.02^{c}
	30 mg L^{-1}	1.48±0.02^{a}	1.63±0.07^{a}	1.85±0.02^{ab}
	45 mg L^{-1}	1.44±0.03^{a}	1.69±0.04^{a}	1.92±0.02^{a}
	60 mg L^{-1}	1.41±0.06^{a}	1.56±0.02^{a}	1.77±0.01^{b}

a, b, c, d The values designated by different letters on the same column are significantly different on 5 % significance level.

4. CONCLUSION

It is important that DOT fertilizer has not been studied before to stimulate the germination of cress and rocket seeds and subsequent growth. Cress, at the end of the 12th day 45 mg L^{-1} DOT treatments 1.76 fold more germination rate than control plants. Rocket, at the end of the 12th day 45 mg L^{-1} DOT treatments 1.30 fold more germination rate than control plants. Cress and rocket, boron fertilizer was increased to cotyledon length according to control treatments. In both two plants, 45 mg L^{-1} boron fertilizer was the highest cotyledon length. When comparing control plants to 45 mg L^{-1} boron fertilizer, cress 2.51 fold rocket 1.90 fold have more cotyledon length. Cress and rocket dry weight per plant 45 mg L^{-1} boron fertilizer were 1.68 fold and 1.60 fold more than control plants respectively. Overall, we proposed that 45 mg L^{-1} DOT treatments have a quite good improvement at germination %, cotyledon length, dry weight per plant both cress and rocket. Therefore it shortens the period to deliver products from field to market. We think that the determination of the appropriate dose with our research will shed light on future studies and guide the growers in terms of product yield.

Ethics Committee Approval
N/A

Peer-review
Externally peer-reviewed.

Author Contributions
All authors have read and agreed to the published version of manuscript.

Conflict of Interest
The authors have no conflicts of interest to declare.

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Effect of Altitude on Polysaccharide and Lignin Contents of Brutian pine (*Pinus brutia* Ten.) Wood and Kraft Pulp

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Abstract: This study investigated the chemical properties of Brutian pine (*Pinus brutia* Ten.) wood samples collected at altitudes of 800 m, 900 m, and 1000 m and the behaviors of the chemical components of the same materials during kraft pulping. Chemical component analysis indicated that the wood and pulp sample from 1000 m contained higher holocellulose and α-cellulose contents, and a lower lignin content. For both wood and pulp samples, a continuous increase in holocellulose and α-cellulose contents and a continuous decrease in lignin content were observed with increasing altitude. Gas chromatography analysis of monosaccharides determined that the highest cellulose and hemicellulose contents were obtained from the wood and pulp sample from 1000 m. Quantity of these components increased with increasing altitude. Lastly, kraft pulping degraded 14.10-15.70% of cellulose, 56.49-61.35% of galactoglucomannan, 65.75-69.61% of arabinoglucoronoxylan, and 92.42-93.48% of lignin in the samples.

Keywords: Brutian pine, chemical composition, kraft pulping, altitude.

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1. INTRODUCTION

The substantial reliance on wood, wood products, and paper in spite of a shortage of cellulosic and forestry resources has prompted wood and paper researchers to study in more detail the appropriate use of resources (Nemati and Samariha, 2011).

Cellulose, hemicelluloses, and lignin are the main chemical components of wood. Cellulose and hemicelluloses are the polysaccharides of the plant world; cellulose can comprise between 40% and 50% of wood, with hemicellulose making up another 15%-35%. Cellulose is constituted of glucose units that offer a linear and homopolymeric structure. In contrast, hemicelluloses present a branched heteropolymeric structure; in wood, they arise from pentoses, hexoses, and uronic acids. In softwood species, O-acetylgalactoglucomannan is the dominant hemicellulose polymer, comprising between 12% and 18%, depending on species. The main molecular chain of O-acetylgalactoglucomannan consists of β–D-glucopyranosyl and β–D-mannopyranosyl units that are linked linearly with one another by β-(1,4) bonds, with acetyl groups bonded to the -OH groups of the C2 and C3 atoms of β–D-mannopyranosyl. This main chain is then branched with α–D-galactopyranose units linked by α-(1,6) bonds. Another common softwood hemicellulose is arabino-4-O-methylglucoronoxylan, which makes up between 8% and 10%, depending on species. The main molecular chain of arabino-4-O-methylglucoronoxylan is constituted of β–D-xylopyranose units that are linked linearly with one another by β-(1,4) bonds; to this chain, α–L-arabinofuranose and 4-O-methyl-α-D-glucopyranosyluronic acid units are bonded by α-(1,3) and α-(1,2) bonds. The third major chemical component of wood, lignin, is a natural biopolymer complex constituted of phenylpropane units. Softwood lignins appear to consist primarily of guaiacyl units with small quantities of syringyl and p-hydroxyphenylpropane, while hardwood lignins appear to consist of guaiacyl and syringyl units with a small portion of p-hydroxyphenylpropane. After cellulose, lignin is the second most abundant biopolymer in the plant world, comprising between 20% and 32% of wood content (Fengel and Wegener, 1984; Krassig, 1993; Sjoström, 1993; Teleman et al., 2009; Kapu and Trajano, 2014; Persson and Jönsson, 2017).

In addition to being the main component in the production of paper, rayon, or cellulose derivatives, cellulose is an important raw material when converted into glucose units

(Fengel and Wegener, 1984; Sjoström, 1993). Like cellulose, hemicelluloses have attracted interest in recent years as raw materials for the production of derivatives, with hydrolysis of different hemicelluloses yielding different monosaccharide units (Sun et al., 1999, 2000, 2004; Yaşar, 2018; Yaşar and Kılınç, 2018). Most recently, a partial pre-extraction of hemicelluloses has been proposed that can be carried out without affecting the quality or yield of pulp produced for use in the pulp and paper industry (Van Heiningen, 2006). This provides a source of hemicelluloses for diverse industrial processes. For wood monosaccharides, including glucose, mannose, xylose, galactose, arabinose, rhamnose, and uronic acids, the main utilization is to produce other industrial chemicals. The third main component of wood, lignin, can be utilized in a wide range of applications, from fuels to advanced chemicals and materials. However, about 85% of the world's total lignin production consists of kraft lignin, which is a kind of industrial lignin recovered from kraft pulp. About 630,000 tons of kraft lignin is produced annually, and is primarily used for heat recovery in the form of combustion, which is a low-value use (Wegener, 1982a, 1982b; Fengel and Wegener, 1984; Sjoström, 1993; Yoon and Van Heiningen, 2008; Vila et al., 2011; Hongzhang, 2015).

Brutian pine (*Pinus brutia* Ten.) is distributed extensively throughout the Mediterranean, Aegean, and Marmara regions, and also in some localities of the western Black Sea region. In Turkey, the total growing area of Brutian pine is approximately 5.8 million hectares, which is 25.11% of the nation's forests (OGM, 2015). Brutian pine is the leading species for Turkish wood production, with 5140007 m^3 being used as industrial wood and 1055335 m^3 as fuelwood (OGM, 2016). The wood is used in the production of construction materials, packing cases, agricultural tools, telegraph poles, mine poles, palings, and watercraft (Bozkurt, 1971, 1982). In addition, it is a useful source for paper production using the kraft (sulfate) process (Erten and Taşkın, 1985; Öktem, 1987). Moreover, Brutian pine wood is one of the most important sources of raw material for use in the production of particle board and fiberboard (Özdemir and Uçar, 2016). Finally, Brutian pine bark can be used in tannin production (Erten and Taşkın, 1985; Öktem and Sözen, 1995).

Recently, an integrated bio-refinery that can extract a portion of hemicellulose content while retaining cellulose for the production of pulp has come into prominence (Van Heiningen, 2006). As kraft lignin obtained from kraft pulp comprises 85% of the total lignin production of the world (Hongzhang, 2015), this study investigated the chemical composition of Brutian pine woods harvested from different altitudes and of kraft pulps produced from the same woods. Moreover, the effect of harvesting altitude on the chemical composition of wood and pulp was evaluated.

2. MATERIAL AND METHODS

2.1. Material

Wood samples were obtained from Ağlasun-Burdur in Turkey. Three trees were harvested, one each from 800 m, 900 m, and 1000 m above sea level. A disk with a thickness of 50 cm was taken from a height of 1.30 m on the trunk of each tree. The disks were separately debarked, chipped, and dried naturally in an air-conditioned room for a month.

2.2. Method

Kraft pulping

Kraft pulping was performed to cook the Brutian pine wood chips. Each trial used 500 g Brutian pine chips (on an oven-dry basis). Pulping was carried out automatically in a controlled way in the laboratory with a 15 L stainless steel reactor rotating at four rotations per minute and heated by electricity. The conditions of kraft pulping were as follows (Gülsoy et al., 2015): The active alkali concentration of the pulping liquor was 20%, with equal sulfidity of 25%. Cooking was carried out for 75 minutes at a maximum temperature of 170 ℃. The ratio of liquor to wood was 4:1. After cooking, the treated chips were washed with water to remove the black liquor, and then disintegrated. To determine the effect of kraft cooking on the entire sample, the pulp was used directly for chemical analysis, without any separation of rejected or screened pulp. For this reason, the total yield of each sample was used in the study, expressed as a percentage of oven-dried material weight.

Chemical analyses

For chemical analyses, wood chips were milled in a Retsch SK-1 mill so as to pass through 40 to 100 mesh. To remove extractives, the milled wood and pulp samples were extracted with cyclohexane:ethanol (2:1 v/v) and then ethanol in a Buchi Extraction System B-811. To determine holocellulose and α-cellulose contents in extract-free wood and pulp samples, the acid chlorite method developed by Browning (1967) and the ASTM D 1103 method (1980) were applied. Holocellulose and α-cellulose contents were calculated as percentages of oven-dried material weight. The lignin contents of the wood and pulp samples were determined on a Perkin Elmer Lambda 20 UV/visible Spectrometer using the acetyl bromide procedure introduced by Liyama and Wallis (1990). Lignin content was reported as a percentage of oven-dried material weight. Extract-free wood and pulp samples were hydrolyzed using the acid hydrolysis method developed by Dill et al. (1984) with a slight modification (Yaşar et al., 2010). The monosaccharides in the acid hydrolysates of the extract-free wood and pulp samples were analyzed using a Perkin Elmer Autosystem XL gas chromatograph according to the method developed by Cao et al. (1997). Monosaccharide contents were calculated as percentages of oven-dried material weight. Finally, uronic acid content was analyzed on a Perkin Elmer Lambda 20 UV/visible Spectrometer using the method described by Filisetti-Cozzi and Carpita (1991) and expressed as a percentage of oven-dried material weight.

Statistical analysis

Statistical analyses were done using Minitab 16 software. If a significant difference between the mean values of the

samples was found by ANOVA, then Duncan's test was performed to determine the different groups.

3. RESULTS AND DISCUSSION

The main chemical components of Brutian pine wood and pulp samples are listed in Table 1. The holocellulose contents of wood samples were in agreement with the values of 70.8%, 72.57%, 72.07%, 72.64%, and 72.09% reported for Brutian pine wood by Kırcı (1991), Kılıç et al. (2010), Güler and Yaşar (2018), Beram and Yasar (2020), and Güler and Yaşar (2020). Similarly, the α-cellulose contents of wood samples were consistent with the values of 48.56%, 46.5%, 48.16%, 48.23%, and 48.24% observed by Tutuş et al. (2012), Taş (2017), Güler and Yaşar (2018), Beram and Yasar (2020), and Güler and Yaşar (2020). Lignin contents of wood samples in this study were higher than the previously reported klason lignin contents of 27.4%, 27.34%, 27.60%, 27.16%, 27.98%, and 27.33% demonstrated by Kırcı (1991), Kılıç et al. (2010), Tutuş et al. (2012), Güler and Yaşar (2018), Beram and Yasar (2020), and Güler and Yaşar (2020). However, klason lignin content reflects only the acid-insoluble part of the total lignin content of wood (Rodrigues et al., 1999). For Brutian pine pulp, Taş (2017) reported holocellulose and klason lignin contents of 94.86% and 5.77%, respectively. The holocellulose contents of pulp samples in this study were consistent with the result of Taş (2017), while the lignin contents were higher; as with the wood samples, this is because klason lignin in the pulp represents the acid-insoluble part of total lignin content (Koljonen et al., 2004; Shin et al., 2004).

Table 1. Main chemical components of Brutian pine wood and pulp samples.

Sample	Holocellulose (%)	α-cellulose (%)	Lignin (%)
Wood-800 m	71.82 (0.09)[1] a[2]	47.36 (0.08) a	30.83 (0.08) a
Wood-900 m	72.04 (0.16) a	47.91 (0.09) b	30.02 (0.07) b
Wood-1000 m	72.82 (0.18) b	48.33 (0.11) c	29.75 (0.09) b
Pulp-800 m	94.41 (0.02) a	84.01 (0.03) a	5.34 (0.04) a
Pulp-900 m	94.42 (0.02) a	84.07 (0.01) a	5.13 (0.08) b
Pulp-1000 m	94.67 (0.10) b	84.34 (0.23) b	4.27 (0.03) c

[1] Standard deviation, [2] Homogenous groups by Duncan test: $p < 0.001$ for wood holocellulose, wood α-cellulose, and wood lignin; $p < 0.01$ for pulp holocellulose; $p < 0.05$ for pulp α-cellulose; and $p < 0.001$ for pulp lignin.

The holocellulose and α-cellulose contents of pulp samples produced from Brutian pine wood samples using kraft pulping increased slightly with increasing altitude, whereas lignin contents were reduced (Table 1). The same trends were observed in wood samples. Generally, the wood sample findings here are consistent with observations by Dönmez et al. (2013) in Scots pine wood samples, for which a continuous increase in altitude from 822 m to 1031 m led to continuous increase in holocellulose and α-cellulose contents and decrease in klason lignin contents. However, Musule et al. (2016) reported continuous increase in cellulose and klason lignin contents of oyamel fir wood samples with increasing altitude from 3000 m to 3500 m.

After kraft pulping, the total yield of pulp obtained was 43.77%, 43.99%, and 44.01% for samples of Brutian pine harvested from 800 m, 900 m, and 1000 m altitudes, respectively. This corresponded to 218.85 g, 219.95 g, and 220.05 g of pulp (on an oven-dry basis) being obtained from 500 g of dry chips (the starting material for cooking).

In the starting materials, holocellulose contents were 359.10 g, 360.20 g, and 364.10 g for samples from 800 m, 900 m, and 1000 m, respectively. After kraft pulping, 206.62 g, 207.68 g, and 208.32 g holocellulose was recovered in the pulp samples (Figure 1). Thus, kraft cooking reduced the holocellulose content by 42.46%, 42.34%, and 42.79% respectively for samples harvested from 800 m, 900 m, and 1000 m. The decrease in holocellulose content is explained by polysaccharide degradation during the cooking process due to peeling reactions and alkaline hydrolysis (Fengel and Wegener, 1984; Krassig, 1993; Sjoström, 1993).

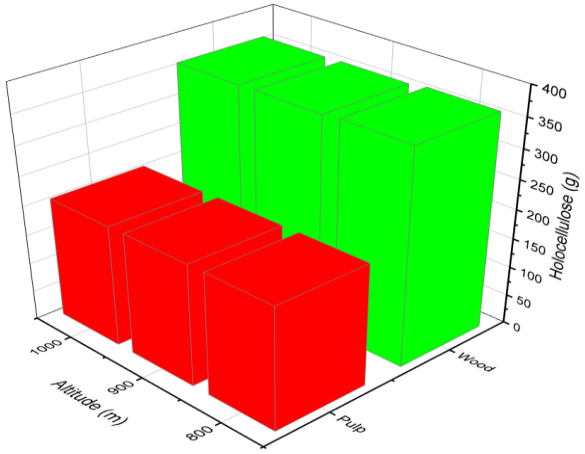

Figure 1. Change in Brutian pine holocellulose content during kraft pulping

Brutian pine wood (500 g dry chips) had α-cellulose contents of 236.80 g, 239.55 g, and 241.65 g for samples collected at 800 m, 900 m, and 1000 m, respectively. In the pulps produced by cooking, the corresponding contents were 183.86 g, 184.91 g, and 185.59 g (Figure 2), representing decreases of 22.36%, 22.81%, and 23.20%, respectively.

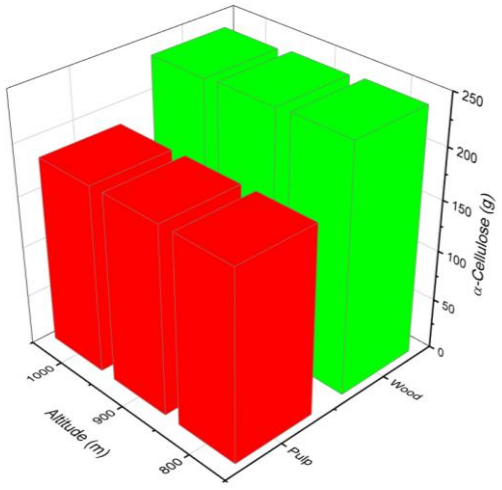

Figure 2. Change in Brutian pine α-cellulose content during kraft pulping

Meanwhile, lignin contents of the starting materials were 154.15 g, 150.10 g, and 148.75 g respectively for samples collected at 800 m, 900 m, and 1000 m. In pulp samples, the cooking process decreased lignin content to 11.69 g, 11.28 g, and 9.37 g (Figure 3), corresponding to losses of 92.42%, 92.48%, and 93.68% during cooking. The findings for lignin content are in agreement with Huang et al. (2015), who reported that 93.9%-95.5% of lignin could be removed from wood with the use of 22% effective alkali and 20% sulfidity and cooking at a temperature of 160 ℃ for 70 minutes. Similarly, Yuan et al. (2017) removed 97.54% of lignin from wood chips during kraft pulping (170 °C, effective alkali 22%, sulfidity 25%).

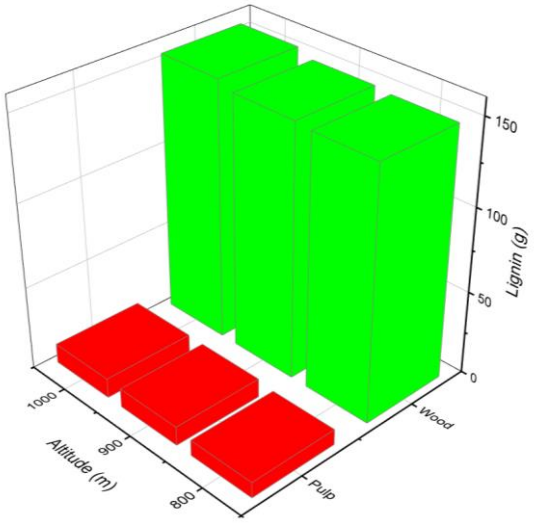

Figure 3. Change in Brutian pine lignin content during kraft pulping

The monosaccharide units that constituted Brutian pine polysaccharides are presented in Figure 4, and consisted of glucose, galactose, mannose, xylose, and arabinose in both wood and pulp.

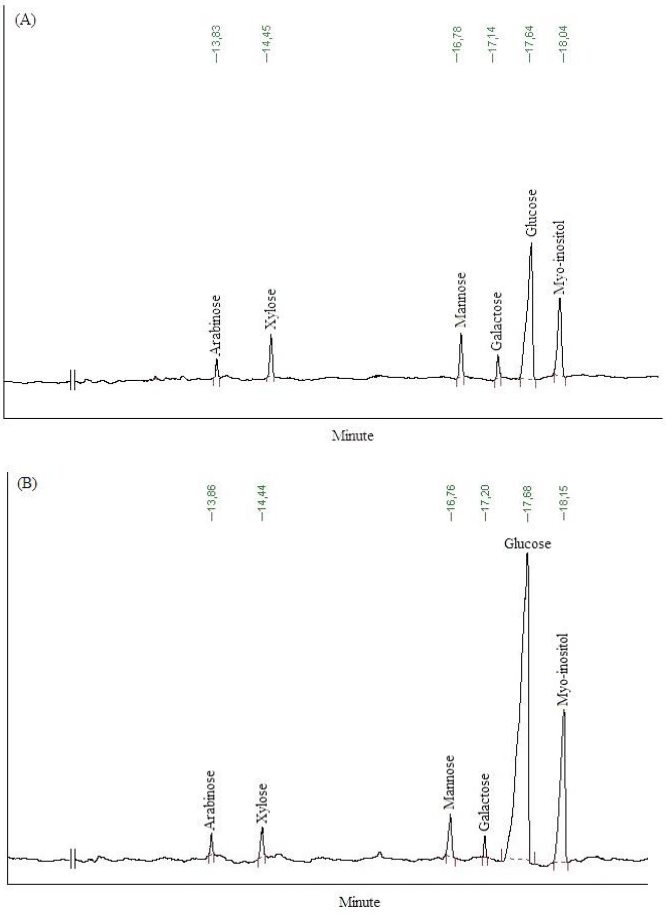

Figure 4. GC chromatograms of Brutian pine wood (A) and pulp (B) samples

Monosaccharide and uronic acid contents of Brutian pine wood and pulp samples are indicated in Table 2. The monosaccharide composition of Brutian pine wood samples was in agreement with observations by Yaşar (2014), Yaşar and Güler (2018), and Güler and Yaşar (2020). Glucuronic acid was not found in the pulp samples, as that this unit is more prone to decomposition in kraft pulping than other monosaccharide units (Fengel and Wegener, 1984).
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Table 2. Monosaccharide and uronic acid composition of Brutian wood and pulp samples.

Sample	Glu (%)	Man (%)	Xyl (%)	Gal (%)	Ara (%)	UA (%)
Wood-800 m	47.61 (0.03)[1]	10.78 (0.01)	7.82 (0.02)	2.13 (0.01)	1.07 (0.01)	1.56 (0.01)
Wood-900 m	48.16 (0.02)	11.02 (0.02)	7.99 (0.01)	2.18 (0.02)	1.09 (0.01)	1.60 (0.01)
Wood-1000 m	48.89 (0.04)	11.24 (0.02)	8.15 (0.02)	2.22 (0.01)	1.12 (0.01)	1.63 (0.01)
Pulp-800 m	87.85 (0.01)	4.40 (0.01)	2.70 (0.01)	0.51 (0.01)	0.48 (0.01)	-
Pulp-900 m	87.91 (0.03)	4.53 (0.01)	2.90 (0.01)	0.55 (0.01)	0.52 (0.01)	-
Pulp-1000 m	88.20 (0.02)	5.17 (0.01)	3.17 (0.01)	0.60 (0.01)	0.56 (0.01)	-

Glu: Glucose, Man: Mannose, Xyl: Xylose, Gal: Galactose, Ara: Arabinose, UA: Uronic acid, 1: Standard deviation.

The determined glucose contents were related to the cellulose and O-acetylgalactoglucomannan contents of Brutian pine wood. For softwoods, the reported mannose:glucose ratio in O-acetylgalactoglucomannan is 3:1 (Timell and Mian, 1960; Fengel and Wegener, 1984). Using this ratio, the glucose content of each wood and pulp sample was calculated and then combined with mannose and galactose contents to indicate the galactoglucomannan content. The remainder of the determined glucose content was attributed to cellulose and used to calculate the cellulose content of each sample. Pekgözlü et al. (2020) similarly determined the rhamnose and galacturonic acid

contents of Brutian pine samples, which compose the pectin in wood, but rhamnose was not found in the samples in this study.

In addition, the xylose:methylglucuronic acid:arabinose ratio of arabino-4-O-methylglucoronoxylan in softwoods was reported to be 8:1.6:1 (Fengel and Wegener, 1984). Here, the determined ratios for Brutian wood samples were 7.82:1.56:1.07, 7.99:1.60:1,09, and 8.15:1.63:1.12, which are consistent with observations by Fengel and Wegener (1984). This is meaningful as the uronic acids determined in the present study can be ascribed to glucuronic acids. Xylose, uronic acid, and arabinose contents were therefore used to calculate the arabinoglucoronoxylan content of each sample. However, when composing the polymeric structure of polysaccharides in wood, the monosaccharide and uronic acid units lose 1 mol H_2O (Fengel and Wegener, 1984; Krassig, 1993; Sjoström, 1993). Therefore, we used a conversion factor of 0.9 when determining the cellulose and hemicellulose (galactoglucomannan + arabinoglucoronoxylan) contents of each sample (Table 3).

Table 3. Polysaccharide composition of Brutian wood and pulp samples.

Sample	Cellulose (%)	Galactoglucomannan (%)	Arabinoglucoronoxylan (%)	Hemicellulose (%)
Wood-800 m	39.62	14.85	9.41	24.26
Wood-900 m	40.04	15.19	9.61	24.80
Wood-1000 m	40.63	15.49	9.81	25.30
Pulp-800 m	77.75	5.74	2.86	8.60
Pulp-900 m	77.76	5.93	3.08	9.01
Pulp-1000 m	77.83	6.74	3.36	10.10

Brutian pine wood (500 g of chips) had cellulose contents of 198.1 g, 200.20 g, and 203.15 g for samples collected at 800 m, 900 m, and 1000 m, respectively. In the produced pulp samples, the corresponding contents were 170.16 g, 171.03 g, and 171.26 g (Figure 5), representing decreases of 14.10%, 14.57%, and 15.70% due to the kraft cooking. The findings here are supported by Huang et al. (2015) and Yuan et al. (2017). Huang et al. (2015) indicated that over 80% of glucan could be retained in pulped wood with milder sulfidity conditions (i.e. 14 to 24%). Similarly, Yuan et al. (2017) reported the extraction of 20.80% of glucan from original wood chips during kraft pulping (170 °C, effective alkali 22%, sulfidity 25%).

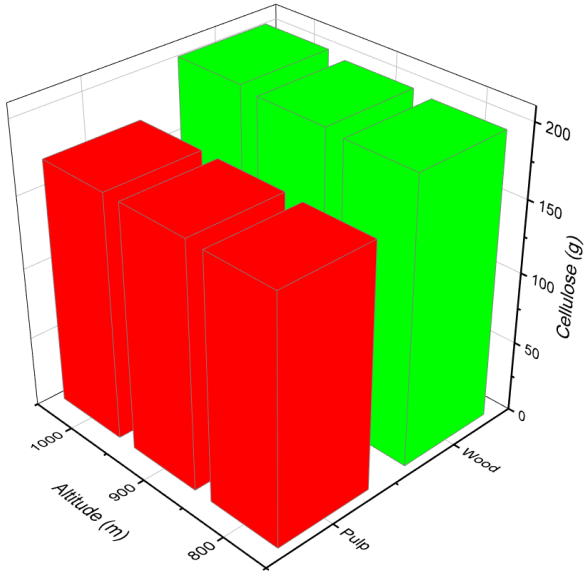

Figure 5. Change in Brutian pine cellulose content during kraft pulping

The hemicellulose contents of the wood samples were 121.30 g, 124.00 g, and 126.50 g respectively when harvested at 800 m, 900 m, and 100 m altitude. Meanwhile, the corresponding pulp contents were 18.82 g, 19.82 g, and 22.23 g (Figure 6), indicating respective degradation of 84.48%, 84.02%, and 82.43% of hemicelluloses during kraft pulping. The findings here are compatible with observations by Yuan et al. (2017), who reported that over 75% of hemicelluloses were extracted from original wood chips during kraft pulping (170 °C, effective alkali 22%, sulfidity 25%).

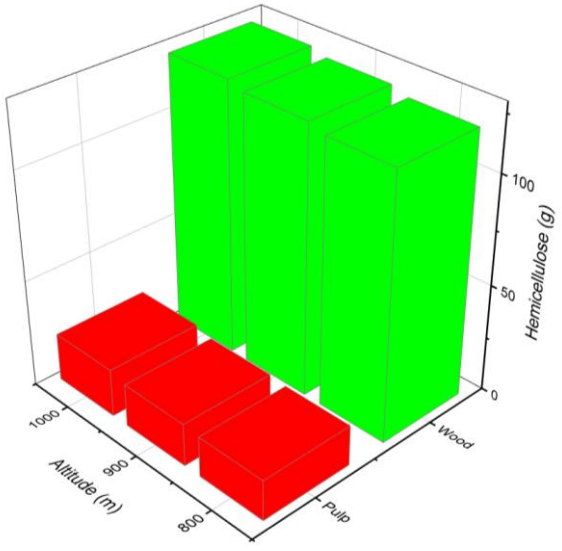

Figure 6. Change in Brutian pine hemicellulose content during kraft pulping

Taken together, the wood sample collected at 1000 m offered more cellulose and hemicellulose contents and less lignin content than did the wood samples collected at 800 m and 900 m. Similarly, the pulp produced from the wood

sample collected at 1000 m yielded the highest cellulose and hemicellulose contents, but the lowest lignin content.

4. CONLUSION

In this study, the chemical properties of Brutian pine wood samples, which are used for pulp production by kraft pulping, were investigated in the context of harvesting altitude (800 m, 900 m, and 1000 m). Moreover, the chemical composition of produced pulps was determined. Proximate analysis showed that increasing the harvest altitude of Brutian pine wood could raise the holocellulose and α-cellulose contents of wood and pulp, while decreasing the lignin content. GC analysis indicated a continuous increase in cellulose and hemicellulose contents with increasing altitude. Kraft pulping was determined to degrade 14.10-15.70% of cellulose, 56.49-61.35% of galactoglucomannan, 65.75-69.61% of arabinoglucoronoxylan, and 92.42-93.48% of lignin. After pulping, a considerable quantity of extracted polysaccharide was obtained that could be used in the food, pharmacology, and bio-refinery industries. The lignin content, which is generally considered an energy resource for heat recovery in the form of combustion, was remarkably degraded during kraft pulping.

Ethics Committee Approval
N/A

Peer-review
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Author Contributions
All authors have read and agreed to the published version of manuscript.

Conflict of Interest
The authors have no conflicts of interest to declare.

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Investigation of Microstructural and Intrinsic Defect States of Facile Synthesized WO_3 Film

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Abstract: In this study, WO_3 films were simply deposited onto In:SnO_2 (ITO) substrates by chemical bath in acidic medium. Structural, mophological and optical properties of the synthesized WO_3 film were investigated by using x-ray diffraction (XRD), scanning electron microscope (SEM), atomic force microscope (AFM), photoluminescence (PL), Uv-Vis and Raman spectrophotometer. From x-ray patterns, the tungsten oxide coating exhibits a monoclinic phase structure. Relative homogeneous particle distribution of nanorod/nano-tooth mixed forms have been observed on the surface and also surface roughness is less compared to similar studies in the literature. Surface defect emission peaks esepecially oxygen vacancies are determined from PL spectrum. Green emission is attributed to heterogeneous film growth process. Raman spectra of the films is proof WO_3 formation. From these results, the aggregation–deposition mechanism is responsible to WO_3 film growth process.

Keywords: WO_3 film, chemical bath, structural, optical

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1. INTRODUCTION

Metal oxide nanomaterials are known to exhibit improved optical, electrical and chemical reactivity properties compared to bulk counterparts (Pavia-Santos vd., 2021). Amog them, as an anode or cathode material (tungsten trioxide) WO_3 film is so preferable layer in electrochromic device applications due to its high coloration efficiency, transparent in oxidation state, deep blue in the reduction state and has a large number of coloration (Pooyodying vd., 2021). It exhibits a colour change according to this reaction;

$$WO_3 + xLi^+ + xe^- \rightarrow Li_xWO_3 \quad (1)$$

where WO_3 and Li_xWO_3 are transparent and absorbing, respectively. However WO_3 can not meet the color neutrality required for many smart window applications due to its blue color in the spectrum (Lagier vd., 2021). Therefore, controlled growth is targeted so that the optical properties of WO_3 films are improved to achieve high performance from them. At the same time, fast and efficient production is targeted

There are a lot of study including WO_3 films and their optical properties with using different techniques such as sol-gel, electrospinning and magnetron sputtering etc. (Hariharan vd., 2019). Recently, chemical bath deposition is very interesting among chemical film production methods because it is simple, allows large area application, inexpensive and does not need vacuum pump.

In this work, our goal is to investigate the growth process as well as to determine the intrinsic defect states of WO_3 films with using cost-effective chemical bath.

2. MATERIAL AND METHOD

ITO substrates were cleaned by detergent, alcohol and n-hexzane in ultrasonic bath along one hour. All chemicals were purchased from Sigma-Aldrich without further purification. Sodium tungsten dihydrate ($Na_2WO_4.2H_2O$)(≥% 99) was used as a tungsten source and HCl was used as a complex agent to adjust pH of the solution. In 100 ml. distilled water, 0.1 M W-source was dissolved and mixed on the magnetic stirrer. ITO substrates were immersed in aqueous solution and WO_3 films were grown into acidic bath

solution (pH=2). Along the film synthesis process, working temperature and immersion time were 70±5 °C and 15 min. Finally, WO_3 film annealed at 500 ˚C for 2 h in the furnace. The crystal structure and measured structural parameters of the sample were determined by X-ray powder diffraction (XRD; Rigaku Smart Lab x-ray diffractometer; CuK_α radiation; 45 kV; 40 mA; step size 0.013˚) with powder method. Nanostructural investigation was done using scanning electron microscopy (SEM; JEOL JSM- 7100F; Au-Pd (80-20 %) coating) and atomic force microscopy (AFM;Witec Alpha 300 RA; non-contact mode). Surface elemental composition was determined by using energy-dispersive x-ray spectrometer (EDX; OXFORD Instruments X-Max) which was attached to SEM. UV-Vis spectra of the samples was investigated between 300-900 nm range. Optical parameters (T and A) was investigated by Analytic Jena Uv-Vis spectroscopy in the 300-900 nm. range. Photoluminescence emission measurements were realized by ANDOR SR500i-BL with excitation wavelength of 349 nm. Raman spectrophotometer (Thermo DXR) was used to confirm the degree of microstructural disorder.

3. RESULTS

Figure 1 shows the x-ray patterns of the produced film. The sharp peaks indicate that the as-synthesized products are well-crystallized. ITO-based peak and W-related peak are observed in the 2θ= 65˚-70˚ and 2θ= 45˚-50˚ range, respectively due to less crystal nuclei at pH value of 2.0 (Lu vd., 2014). According to JCPDS Card No: 43-1035, characteristic three monoclinic WO_3 phase peaks of (001), (020) and (200) are detected between 2θ= 25˚-30˚ and (220) peak is observed in the 2θ= 30˚-35˚ range. From these results, retention level of WO_3 film onto ITO substrate is high.

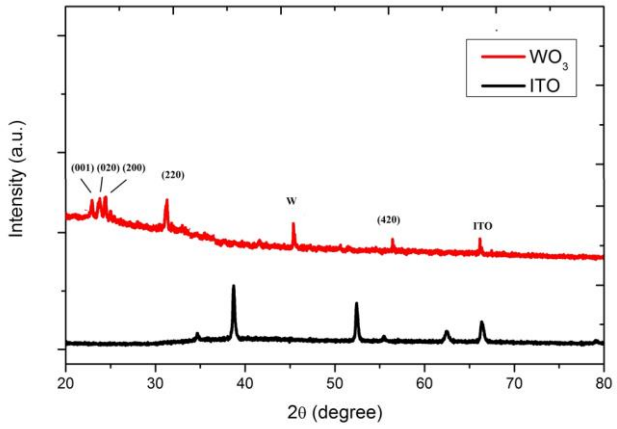

Figure 1. X-ray patterns of WO_3 film in the 2θ= 20˚-80˚ range

In Figure 2., surface image of the WO_3 film is shown. Relatively homogeneous and smooth surface is observed with mixed nanorod/nano-tooth forms due to some particles are growing fast, but others are growing slowly. Nanorod forms are associated with the crystal planes are parallel to the c axis WO_3 crystal nucleus. Observed nano-tooth forms can provide porosity on the surface, thus increasing the surface/volume ratio. It may be useful due to functionality of material is strongly depends on surface morphology.

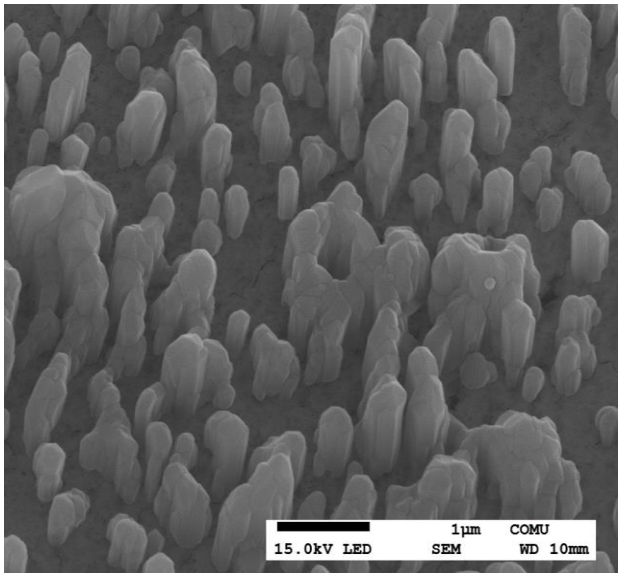

Figure 2. Surface morphology of WO_3 films

Elemental analysis results are given as below in Figure 3. It is proof that tungsten and oxygen peaks are recorded. Sodium and sulphur peaks are shown due to low solubility of W-source aqueous solution whereas annealing process is applied to remove surface impurities.

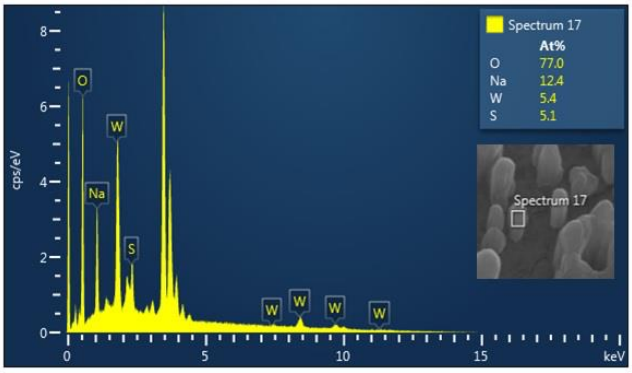

Figure 3. Elemental analysis results WO_3 films

Figure 4 shows that two dimension (2D) and three dimension (3D) AFM images of WO_3 film. It is kown that surface roughness is so important for optical studies (Ramkumar vd., 2016). Surface roughness values of SA and SQ are 16.02 nm and 19.29 nm, respectively. From this result, surface roughness of the synthesized sample is lower than compared to literatüre (Babu, 2016). When looking at both 2D and 3D AFM images, the particle distribution is relatively uniform and agglomeration of atoms on the surface can cause the formation of large grains which correlated to SEM images.

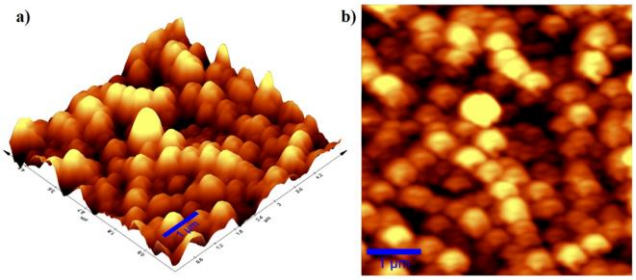

Figure 4. 3D (a) and 2D (b) surface topography of WO_3 films

Figure 5 exhibit the PL emission spectra of WO_3 film which measuring between 350 to 800 nm with 349 nm excitation wavelength of Nd–YLFQ laser. A series of peak are detected at 432, 522, 574 and 700 nm which may be attributed to surface defect related peaks. Emission peaks of surface defects are often associated with oxygen vacancies and tungsten interstitial sites, as generalized in metal oxides.

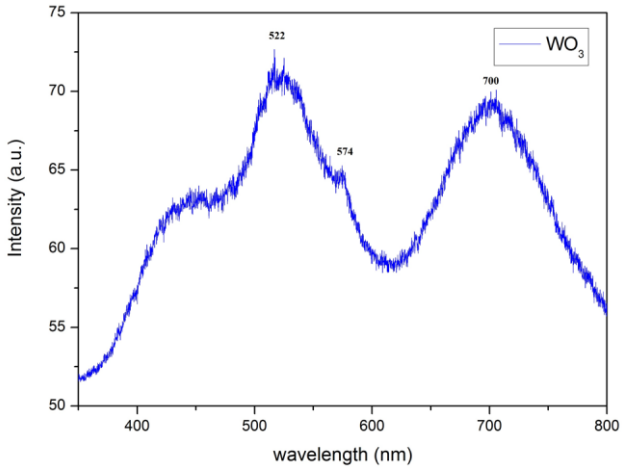

Figure 5. PL spectrum of WO_3 films

In Figure 6, Raman spectra of WO_3 films, which is sited at range 200-1200 cm^{-1}. Symmetric streching mode of W=O is observed in the 950-1050 cm^{-1} range (Díaz-Reyes, 2008). Crystalline WO_3 phase is determined in 806 cm^{-1} which corresponds to stretching vibrations of the bridging oxygen (Tagtstrom, 1999). δ(O–W–O) bending modes are determined in the 200-400 nm range (Ou, 2011). Impurity based peaks are determined in in the 400-700 cm^{-1} range.

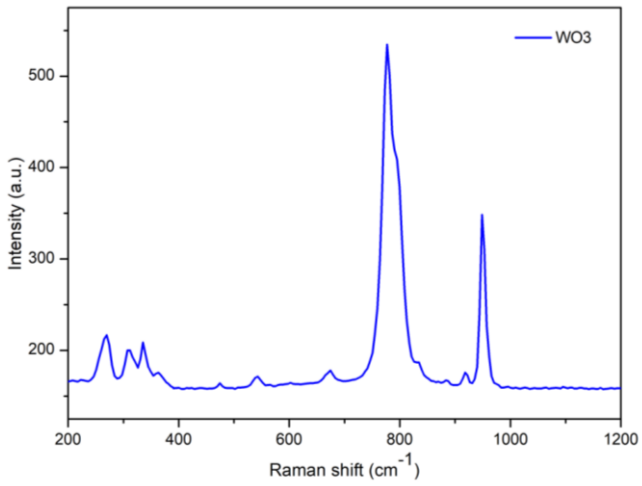

Figure 6. Raman spectrum of WO_3 films

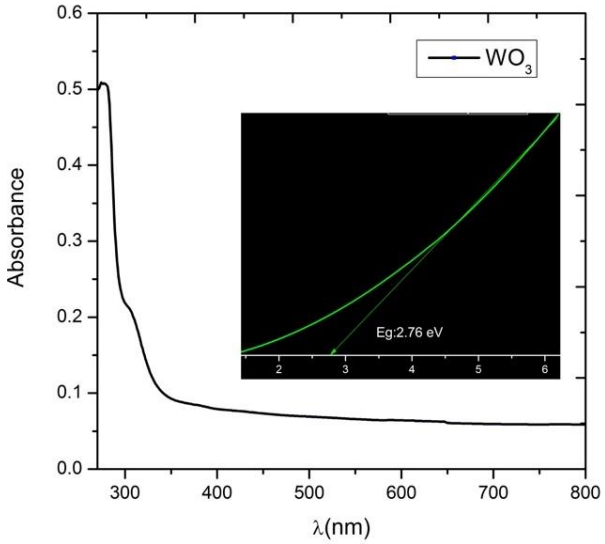

Figure 7. Uv-Vis spectrum and optical band of WO_3 film

In Figure 7, Uv-Vis spectra in the 300-900 nm range and opitcal band gap of WO_3 film is shown. The wide optical absorption band in the optical transmittance spectra of the deposited WO_3 film is attributed to the presence of tungsten ions with the W^{5+} states (Zou, 2014). Low optical band gap value of 2.7 eV is proper for opto-electronic device applications.

4. DISCUSSION AND CONCLUSIONS

Herein, WO_3 film was deposited by chemical bath deposition and structural, morphological and optical properties of the WO_3 film were investigated in detail. Monoclinic WO_3 crystal phase from XRD, W and O indexed peaks from elemental analysis and optical phonon modes of W-O from Raman spectrum are evidence of the WO_3 film production. Nanorod and nano-tooth forms are exhibited whn investigated surface images. Oxygen vacancy correlated defect energy states are determined at 522, 574 and 700 700 nm. According to the obtained results, the aggregation–deposition mechanism can be explain the WO_3 film growth process under film deposition parameters (complex agent was HCl, W-source was sodium tungsten dihydrate, pH=2, T=70 °C, t=15 min.)

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Tübitak 2237-A Doğa Bilimlerinde İstatistiksel Modelleme Yöntemleri ve Uygulamaları Eğitiminin Değerlendirilmesi

Gamze Özel [1], Semra Türkan[1] , Ceren Ünal[1*]

Özet: Günümüzde teknolojik ve bilimsel gelişmeler sonucunda yığınlar halindeki ham veri ve bilgi trafiği nedeniyle meydana gelen karmaşa verilerin analizi ve yorumlanmasını daha da önemli hale getirmiştir. Bu yüzden, öğretim programlarında istatistiksel yöntem ve araçlara daha çok ihtiyaç duyulmaktadır. Ekolojik çalışmalarda hedef türlerin yetişme ortamına uygunluğunun, tür çeşitliliğinin/ verimliliğinin ve jeolojik araştırmalarda heyelan, deprem ve taşkın gibi doğa olaylarının modellenmesinde genel olarak lojistik ve çoklu regresyon analizleri gibi klasik olan yöntemlerden yararlanılmaktadır. Aynı zamanda doğa bilimlerindeki çalışmalarda ampirik, kural tabanlı ve olasılıksal temellere dayanan yöntemlerin kullanılmasında özellikle son yıllarda hızlı bir artış görülmektedir. Bu çalışmada, TÜBİTAK 2237-A Bilimsel Eğitim Etkinliklerini Destekleme Programı kapsamında gerçekleştirilen "Doğa Bilimlerinde İstatistiksel Modelleme Teknikleri ve Uygulamaları" projesinin doğa bilimlerinde istatistiksel modelleme teknikleri ve yazılımların sağladığı ve sağlayacağı faydalar üzerinde durulmuştur.

Araştırmanın örneklemini etkinliklere katılan lisansüstü programlara kayıtlı 90 lisansüstü öğrenci oluşturmaktadır. Proje kapsamında, istatistiğin doğa bilimlerindeki uygulama örneklerinin istatistiksel yazılımlar ile aktarıldığı etkinlikler gerçekleştirilmiştir. Bu etkinliklerin katılımcılar üzerindeki etkisini ölçmek için proje araştırmacıları tarafından oluşturulan etkinlik değerlendirme formları kullanılmıştır. Projedeki RStudio ve Python temelli doğa bilimlerinden uygulama örnekleri ile istatistiksel modelleme analizlerinin aktarılmasının katılımcıları pozitif yönde etkilediği, öğrenme ve araştırma isteklerini arttırdığı gözlenmiştir.

Anahtar Kelimeler: Doğa bilimleri, istatistik kaygısı, istatistiksel modelleme, RStudio, Python.

Tübitak 2237-A Evaluation of Statistical Modeling Methods and Applications Training In Natural Sciences

Abstract: Today, the complexity of raw data and information traffic as a result of technological and scientific developments has made the analysis and interpretation of data even more important. Therefore, statistical methods and tools are needed more in curricula. Classical methods such as multiple and logistic regression analysis are generally used in modeling the suitability of target species to the habitat, productivity/species diversity in ecological studies, and natural events such as earthquakes, landslides, and floods in geological studies. However, especially in recent years, there has been a rapid increase in the use of rule-based, empirical, and probabilistic-based methods in studies in natural sciences. In this study, the benefits of statistical modeling techniques and software in natural sciences and the benefits of the "Statistical Modeling Techniques and Applications in Natural Sciences" project carried out within the scope of TÜBİTAK 2237-A Scientific Education Activities Support Program are emphasized.

The sample of the research consists of 90 graduate students enrolled in graduate programs participating in the activities. Within the scope of the project, activities in which the application examples of statistics in natural sciences were transferred with statistical software were carried out. Activity evaluation forms created by the project researchers were used to measure the impact of these activities on the participating students. It was observed that the transfer of statistical modeling analyzes and application examples from RStudio and Python-

based natural sciences in the project affected the participants positively and increased their desire for learning and research.

Keywords: Natural sciences, statistical anxiety, statistical modelling, RStudio, Python..

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1. GİRİŞ

Geçmişi anlamak, bugünü yönetmek ve geleceği planlamakta istatistik ile birlikte son yıllarda popüler hale gelen veri bilimi anahtar olmaktadır. Bu sebeple, istatistik ile veri biliminden sosyal bilimler ve doğa bilimlerine, fen bilimlerinden mühendisliğe kadar pek çok alanda faydalanılmaktadır. Büyük veri çağı olarak da adlandırılan günümüzde, doğa bilimlerinde de istatistiksel yöntemlere ve istatistiksel yazılımlar yardımıyla yapılan analizlere olan ihtiyaç gün geçtikçe daha da artmaktadır.

Belirli bir sistemdeki değişime dair süreçleri yakalamakta olan doğa bilimciler, ileri sürdükleri hipotezlerini kanıtlamak için deneyler ile gözlemlerle verilerini toplamaktadırlar. Diğer birçok bilimlerde olduğu gibi veri doğa bilimlerinde de oldukça önemlidir. Hatta bazı çevre politikalarının uygulanmasında bilimsel olarak kritik bir kanıttır. Günümüzde hemen hemen her alanda veri ve bilgi patlaması, doğa bilimlerinde de istatistiksel modelleme yöntemlerinin kullanımına olan ihtiyacı daha da artırmıştır. Bunun sonucunda da istatistik ve veri ile alakalı dersler pek çok üniversitenin öğretim programlarında yer almaya başlamıştır. Fakat çarpık veya basık, sıfır-yığmalı, eksik veri gibi özel durumları içeren doğa bilimlerine ait verilerin analizinde üniversitelerde istatistik öğretimi yetersiz kalmaktadır. Bu durum, lisansüstü öğrencilerin tez hazırlama süreçlerinde zorlanmasına ve çözümsel olarak düşünme becerisinde güçlü olmayan temele sahip olmalarına neden olmaktadır.

TÜBİTAK, kamu çalışanlarından öğrencilere kadar geniş bir hedef kitlenin bilime dokunmaları amacıyla BIDEB 2237A-Bilimsel Eğitim Etkinlikleri Desteği isimli programını uygulamaya koymuştur. Bu program dâhilinde yurt içinde çevrimiçi olarak yapılacak olanlar da dâhil olmak üzere seminer ve kurs gibi uygulamalı/teorik bilimsel eğitim etkinliklerinin düzenlenmesi desteklenmektedir. Ön lisans, lisans ve lisansüstü öğrenciler ile araştırmacılara kendi alanlarında çalışma yapmaya teşvik ederek yönlendirmek ve bilimsel olarak gelişmelerine katkı amaçlarıyla düzenlenecek olan bilimsel eğitim etkinliklerini içermektedir. TÜBİTAK 2237A- Bilimsel Eğitim Etkinlikleri Desteği kapsamındaki projelerin sonuçlarına dair yapılan çalışmalar incelendiğinde, yetersiz sayıda değerlendirme çalışması olduğu sonucuna ulaşılmıştır. Kırıkkale Üniversitesi'nde 16-21 Eylül 2019 tarihleri arasında TÜBİTAK BİDEB 2237A Bilimsel Eğitim Etkinlikleri Destek Programı kapsamında öğretmenlere yönelik olarak gerçekleştirilen "STEM Eğitimi ve Arduino ile Fiziksel Programlama" etkinliğinin değerlendirilmesini içeren Sarı ve Yazıcı (2020) tarafından yapılmış çalışma haricinde herhangi bir çalışma bulunmamaktadır. Bu nedenle, bu çalışmanın bir amacı da TÜBİTAK 2237A-Bilimsel Eğitim Etkinlikleri Desteği programının katılımcılar üzerindeki etkilerinin analiz edilmesi ve değerlendirilmesidir.

TÜBİTAK 2237-A kapsamında 2019 yılından 2021 yılına dek dört kez düzenlenen, "Doğa Bilimlerinde İstatistiksel Modelleme Teknikleri ve Uygulamaları" adlı bu eğitim kapsamında katılımcılara doğa bilimlerinde önemli bir yeri olan regresyon modellerinden lojistik, koşullu lojistik, negatif binom, Poisson, sıfır yığmalı negatif binom ve sıfır yığmalı Poisson regresyon modelleri; kural tabanlı yöntemlerden sınıflama ve regresyon ağaçları (SRAT); ampirik yöntemlerden ise yapay sinir ağları (YSA) ve olasılıksal yöntemlerden istatistiksel dağılımlar, Poisson süreçleri ve Markov zinciri katılımcılara anlatılmaktadır. Bu belirtilen yöntemlerin doğa bilimlerinde yer alan uygulamaları gerçek veriler kullanılarak sınırlı süreli ve açık kodlu yazılımlardan yararlanılarak gösterilmekte ve devamında elde edilen sonuçların yorumlamaları alanında uzman eğitmenler tarafından gerçekleştirilmektedir.

Literatür incelendiğinde doğa bilimlerinde istatistiksel yöntemlerin öneminin üzerinde durulduğu herhangi bir bilimsel çalışmaya ulaşılmamıştır. Ancak TÜBİTAK 4004 kapsamında önerilen "İstatistiği Doğada Öğren" adlı etkinlik incelenmiş ve bu eğitime katılmak isteyen öğretmen sayısının oldukça fazla olduğu görülmüştür (TÜBİTAK, 2021). Ancak bu eğitim öğretmenlere doğa eğitimi tabanlı olarak uygulanmıştır. Ayrıca TÜBİTAK 2237A kapsamında "Analitik Doğa: Kümeleme ve Ordinasyon Teknikleri", "Arazi Çeşitliliğinin Entropi Temelli Algoritmalar ile Hesaplanması ve Haritalanması", "Biyolojik Çeşitliliğin Tür, Taksonomik, Fonksiyonel ve Yapısal Özelliklere Dayalı Tespiti", "Doğal Ekosistemler için CBS ve Uydu Görüntüleri Kullanarak Çevresel Altlıkların Hazırlanması" adlı dört farklı projede istatistik ve doğa temelli içeriği ile eğitim etkinliklerine temel oluşturmaktadır.

Bu çalışmanın amacı TÜBİTAK 2237-A kapsamlı "Doğa Bilimlerinde İstatistiksel Modelleme Teknikleri ve Uygulamaları" isimli projenin sonuçlarını değerlendirmek ve lisansüstü öğrenciler için açık kodlu ve sınırlı süreli

yazılımlar ile istatistiksel modelleme tekniklerinin aktarılmasının faydalarına yer vermektir. Bölüm 2'de istatistik kaygısı, lisansüstü eğitimde istatistik derslerinin yeri üzerinde durulmuştur. Bölüm 3'te "İstatistiği Doğada Öğren" projesinde yer alan etkinliklere, katılımcılara uygulanan proje değerlendirme anketi sonuçlarına, röportajlara, kurgusal ve uygulamalı eğitim içeriklerine yer verilmiştir. Bölüm 4'te ise çalışmaların sonuç ve tartışmaları üzerinde durulmuştur.

2. İSTATİSTİK BİLİMİNİN LİSANSÜSTÜ EĞİTİMDEKİ YERİ

İstatistik tüm bilim dallarına yardımcı olduğundan lisansüstü öğretimdeki tüm branşlarda faydalanılabilecek bir bilim dalıdır. Bilimsel araştırmaların önemli aşamalarından biri, toplanan verilerin istatistik biliminden faydalanarak uygun olan yöntemler ile analizinin yapılmasıdır. Analizde kullanılacak olan yöntem, yanıt aranan probleme ve verinin elde edilme biçimine göre farklılık göstermektedir. İstatistik bilgisi, literatür takibi ve yapılan çalışmaların anlaşılmasının yanı sıra kendi verilerini de analiz edebilmesidir. Bu sebeple, istatistik bilimsel araştırmalarda bütünleyici bir araç olmakta (Sutarso, 1992) ve araştırmacıların istatistiksel teknikler hakkında da yetişmiş olması beklenir. Dolayısıyla, eğitim, doğa bilimleri, sosyal ve sağlık vb. alanlarda yer alan lisansüstü programlarında en az bir istatistik dersine yer verilmektedir. Fakat lisansüstü eğitimde olan öğrenciler için bu dersi almak kimi zaman olumsuz deneyim olmaktadır (Collins ve Onwuegbuzie, 2007). İstatistik kaygısı sebebiyle öğrencilerin bu derse karşı olumsuz davranışlar sergilemektedirler.

İstatistik dersi sırasında ya da verileri toplama, analiz ve yorumlama gibi istatistiksel işlemler sırasında ortaya çıkabilen kaygı istatistik kaygısı olarak ifade edilir (Onwuegbuzie vd. 1997).

Lisanüstü eğitim öğrencilerinin yaklaşık olarak %80'inin bu kaygıyı yaşadığı Onwuegbuzie (2004) tarafından yapılan çalışmada rapor edilmiştir. İstatistiğe karşı taşıdığı kaygı, makaleleri anlama, verileri analiz edip yorumlama ve dolayısıyla da istatistik ve araştırma yöntemleri derslerindeki başarısını ve uzun vadede kayıtlı olduğu programdan mezun olup olmamasını ve tez aşamasındaki lisansüstü öğrencilerin tezlerini tamamlamasını dahi etkileyebilmektedir (Rodarte Luna ve Sherry, 2008; Onwuegbuzie, 1997; Onwuegbuzie ve Seaman, 1995; Lalonde ve Gardner, 1993; Fitzgerald vd., 1996; Onwuegbuzie vd., 2000). Literatürde istatistiğe karşı olan kaygı ile alakalı birçok çalışma yapılmıştır. Bu çalışmalardan matematiksel alt yapısının zayıf ve eğitiminin sınırlı olan öğrencilerin kaygısının daha yoğun olduğu görülmektedir (Baloğlu, 2004; Primi, Donati ve Chiesi, 2018; Baloğlu ve Zelhart, 2004). Aynı zamanda, lisansüstü ödevlerinin ertelenmesi ve kaygı arasında pozitif ilişki olduğuna ulaşılmıştır (Onwuegbuzie, 2004). Lisansüstü öğrencileri, akademik kariyer yapmada istatistik bilimini bir engel olarak görmektedir (Rodarte-Luna ve Sherry, 2008; Collins ve Onwuegbuzie, 2007). Yaş, cinsiyet gibi demografik değişkenlerin istatistik kaygısı üzerindeki etkisini belirlemeye yönelik araştırmalarda farklı sonuçlar elde edilmiştir. Bu çalışmalardan, Baloğlu (2004), Benson (1989) ile Rodarte-Luna ve Sherry (2008) kadınların erkeklere kıyasla kargılarının anlamlı derecede daha yüksek olduğu; Sutarso'nun (1992) çalışmasında ise anlamlı bir fark olmadığı görülmüştür. Beurze vd. (2013) tarafından yapılan çalışmada, yaş değişkenine göre kaygının farklılaşmadığı; Baloğlu (2004) çalışmasında ise yaşla birlikte istatistik kaygısında artış olduğu sonucuna ulaşılmıştır. Genel olarak, ülkemizde de istatistik öğretimi üzerine yapılan çalışmalar, lisansüstü öğrencilerin çoğunlukla istatistik kaygısına sahip olduğunu kanıtlamaktadır (Akkoç ve Yeşildere, 2015). Bu sonuçlar göz önünde bulundurulduğunda, istatistik öğretiminin sağlanmasında kullanılabilecek geleneksel sınıf içi öğretim yöntemlerinden farklı teknik ve yazılımların kullanılarak gerçek veriler ile yapılan istatistiksel modelleme çalışmaları büyük önem arz etmektedir.

İstatistik eğitimi, veri büyüklüğü ve hacminin artması ve kullanımı nedeniyle lisansüstü eğitimde her geçen gün değer kazanmaktadır. Bu nedenle, istatistik öğretiminin kalitesinin de yüksek olmasını gerekmektedir (Özdemir, 2014). Ancak, birçok lisansüstü öğrenci tarafından istatistik halen öğrenilmesi zor ve sevilmeyen bir alan olarak görülmektedir (Garfield ve Ben-Zvi, 2008). İstatistik biliminde yer alan temel kavramlar, grafik ve tabloların okunması ve aynı zamanda yorumlanması, betimsel istatistiklerin, basit korelasyon katsayılarının hesaplanması ile birlikte yorumlanması gibi konular lisans düzeyinde ele alınmaktadır. Lisansüstü düzeyde ise, bilimsel bir araştırma planlayarak istatistiksel süreci başından sonuna kadar mezunun kendisi tarafından beklenmekte ve kapsam genişlemektedir. Diğer bir ifadeyle ise, birey lisansüstü eğitimi sonunda artık ilgili alanında uzman kabul edilmektedir. Bu nedenle, lisansüstü eğitim öğrencilerine yönelik olan istatistik eğitimi tez ve yayın kalitesinin arttırılması amacıyla tüm branşlarda çalışmalar yapan lisansüstü öğrenciler için önemlidir.

TÜBİTAK 2237-A kapsamında önerilen ve dört kez gerçekleştirilen "Doğa Bilimlerinde İstatistiksel Modelleme Teknikleri ve Uygulamaları" adlı proje kapsamında doğa bilimleri alanında çalışan lisansüstü öğrencilerin bilgisayar ve doğa ve yer bilimlerinden elde edilen gerçek verilerin ele alındığı uygulamalı bir istatistik öğretiminin aktarılması ve lisansüstü öğrencilerin istatistik kaygısının azaltılması amaçlanmıştır. Bu etkinler ile bilgilendirme, bilinçlendirme, geliştirme, uyarma, dengeleme, koruma vb. süreçler sağlanarak katılımcı 90 katılımcıda bu yönde olumlu davranışların geliştirilmesi hedeflenmiştir.

3. YÖNTEM

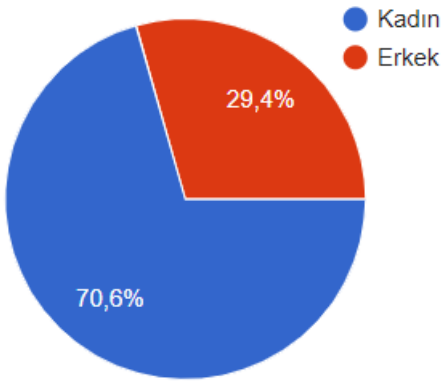
3.1. Etkinliğin Uygulama Biçimi

Bu çalışmada, katılımcılar bir hafta süresince toplamda 51 ders saati boyunca istatistiksel modelleme eğitimi ve bu eğitimin bir parçası olarak RStudio, Python, sınırlı süreli SPSS ve WEKA ile fiziksel programlama kapsamında 16 adet farklı etkinlik gerçekleştirmişlerdir. Katılımcılar etkinlikleri genel olarak bireysel olarak gerçekleştirmektedir. Uygulama sürecine dair dört etkinlikten görüntüler Şekil 1'de verilmiştir.

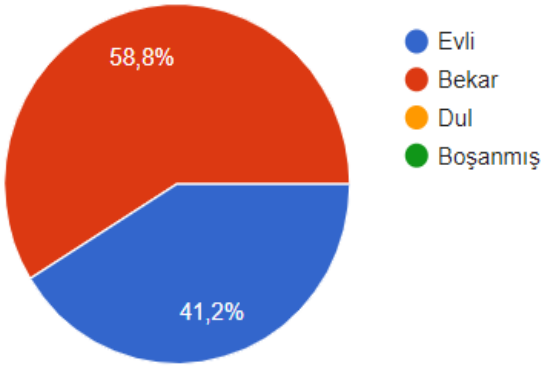
Şekil 1. Dört Etkinlik döneminden uygulama sürecine ait görüntüler

3.2. Örneklem

Çalışmanın örneklemini, TÜBİTAK 2237A Bilimsel Eğitim Etkinliklerini Destekleme Programı kapsamında TÜBİTAK tarafından dört kez desteklenen "Doğa Bilimlerinde İstatistiksel Modelleme Teknikleri ve Uygulamaları" projesine katılan, farklı il ve üniversitelerde öğrenim gören 90 katılımcı oluşturmaktadır. 1250 başvuru arasından projeye ilgi, başvuruların ve cinsiyetin ağırlıklı olduğu gruplar dikkate alınarak her eğitim döneminde 22 kişinin seçimi gerçekleştirilmiştir. Katılımcılara ait cinsiyet ve medeni durum dağılımları sırasıyla Şekil 2.a ve 2.b'de gösterilmiştir. Şekil 2.a'ya göre katılımcıların %70.6'sının kadın ve %29.4'ünün erkek, Şekil 2.b'ye göre %58.8'inin bekar ve %41.2'sinin evli olduğu görülmektedir. Katılımcılar arasında medeni durum olarak boşanmış ya da dul bulunmamaktadır.

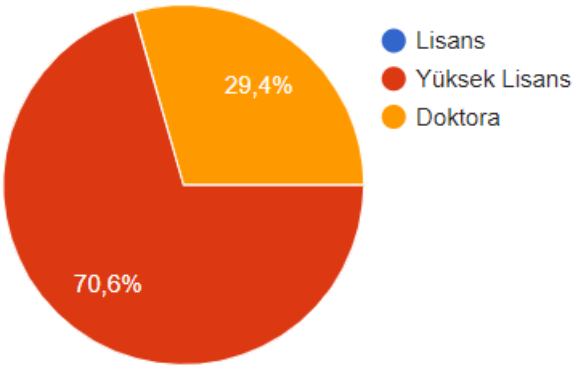


Şekil 2.a. Katılımcıların cinsiyet dağılımları



Şekil 2.b. Katılımcıların medeni durum dağılımları

Eğitime beslenme ve diyetetik uzmanı, biyoloji, orman mühendisi, gıda mühendisi, coğrafya mezunu, ziraat mühendisi, peyzaj mühendisi, jeoloji mühendisi, bilgisayar mühendisi vb. meslek gruplarından katılım sağlanmıştır. Eğitime katılanların %59'u daha önce TÜBİTAK 2237A türü etkinliğe katılmadığını, %35'i 1 kez katıldığını ve %6'sı 2 kez katıldığını belirtmiştir. Şekil 2.c'de öğretmenlerin eğitim düzeylerinin dağılımları gösterilmiştir. Buna göre, öğretmenlerin %70.6'sı yüksek lisans eğitimi ve %29.4'ü doktora eğitimi görmektedir. Lisans eğitimi gören öğretmen bulunmamaktadır.



Şekil 2.c. Katılımcıların eğitim düzeylerinin dağılımları

3.3. Ön Test ve Son Test Değerlendirme Anketlerine ait Sonuçlar

Ekim 2019, 3-9 Mart 2020, 10-16 Mart 2021, 27 Ekim- 2 Kasım 2021 eğitim dönemlerine katılan ve gönüllü katılımcılara uygulanan isimsiz değerlendirme anketinden elde edilen görüşler analiz edilmiştir. Katılımcıların eğitmenler, eğitim içeriği ve eğitimin katkısı ile ilgili sorulara ilişkin verilen cevapların 5'li likert ölçeği üzerinden puan ortalaması Tablo 1'deki gibi elde edilmiştir.

Genel olarak eğitim ile ilgili sorulara verilen cevapların ortalamaları incelendiğinde, eğitim ile ilgili sorulara verilen cevapların genel ortalaması 4,91 olarak bulunmuştur. Buna göre, katılımcıların eğitim ile ilgili tüm olumlu görüşlere kesinlikle katıldıkları görülmektedir.
Anket çalışmasında ayrıca katılımcılara eğitim başlamadan önce eğitimde yer alan konu başlıkları ile ilgili bilgi düzeyleri ve eğitim bittikten sonra belirtilen konu başlıkları

hakkında eğitim sonu bilgi düzeylerinin ne olduğu tekrar sorulmuştur.

Katılımcıların konulara ilişkin eğitim öncesi ve eğitim sonrası bilgi düzeylerine ilişkin ortalama değerleri arasında fark olup olmadığını araştırmadan önce normallik testi yapılmıştır. Test sonucu Tablo 2'deki gibi elde edilmiştir.

Tablo 2 incelendiğinde, verilerin normal dağılıma uyduğu görülmektedir. Verilerin dağılımı normal dağılıma uygun olduğundan eğitim öncesi ve sonraki katılımcıların eğitimde verilen konular hakkındaki bilgi düzeyleri arasında fark olup olmadığı bağımlı örneklem t testi ile araştırılmış ve Tablo 4'deki sonuçlar elde edilmiştir.

Tablo 4 incelendiğinde eğitim öncesi ve sonrasında eğitimde anlatılan konular hakkındaki bilgi düzeyleri arasında istatistiksel olarak anlamlı bir farklılığın bulunduğu görülmektedir. Sorulara verilen puanlar incelendiğinde eğitim sonrası puanların daha yüksek olduğu dolayısıyla eğitime katılan katılımcıların eğitimde anlatılan konular hakkında bilgi düzeylerini arttığı görülmektedir Sorulara verilen cevapların ortalama değerleri incelendiğinde eğitim öncesinde anlatılacak konular hakkında bilgileri olmadığını ifade eden katılımcıların eğitim sonrasında anlatılan konular hakkında orta düzeyde bilgi sahibi oldukları görülmektedir. Bu nedenle TÜBİTAK 2237A etkinliklerinin katılımcılar üzerinde olumlu etkiye sahip olduğu, eğitimlerden elde ettikleri kazanımlarının fazla olduğu sonucuna ulaşılmıştır.

Tablo 1. Etkinliğe ait verilen cevapların puan ortalamaları

	Eğitmenler İle İlgili Sorular	Ortalama
1	Eğitmenlerin konu ile ilgili bilgisi yeterliydi.	5.00
2	Eğitmenlerin konu ile ilgili tecrübeleri yeterliydi.	5.00
3	Eğitmenlerin kullandığı dil açık ve anlaşılırdı.	5.00
4	Eğitmenler eğitimin içeriğini katılımcıların görev alanı ile ilişkilendirdi.	4.71
5	Eğitmenler zamanı etkin ve verimli kullandı.	4.94
6	Eğitmenler konu ile ilgili kaynaklar konusunda bilgiler verdi..	4.82
		4.91
	Eğitim İçeriği İle İlgili Sorular	
7	Dokümanlar ve etkinlikler yeterliydi.	4.88
8	İçerik konuya ilişkin temel kavramları kapsıyordu.	4.94
9	Kullanılan materyaller öğrenmeyi kolaylaştırdı.	4.88
10	Eğitimin içeriği açık ve anlaşılırdı.	4.88
11	Eğitim amacına uygun olarak tasarlanmıştı.	5.00
		4.92
	Eğitimin Katkısı İle İlgili Sorular	
12	Eğitim mesleki gelişimime olumlu katkı sağladı.	4.94
13	Eğitim kişisel gelişimime olumlu katkı sağladı.	4.88
14	Eğitim istatistiğe ve doğaya olan ilgimi artırdı.	4.71
15	Eğitimi diğer çalışma arkadaşlarıma da öneririm.	5.00
16	Eğitimden hedeflerime ulaşmış bir şekilde ayrılıyorum.	4.88
17	Eğitim yeni bilgi ve beceriler kazandırdı.	4.94
18	Eğitim meslektaşlarımla paylaşabileceğim yeni mesleki bilgi ve beceriler kazandırdı.	5.00
	Ortalama Etkinlik Puanı	4.91

Tablo 2. Normallik testi sonuçları

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	İstatistik	sd	p-değeri	İstatistik	sd	p-değeri
Ön test- Son test	0.133	14	0.200*	0.942	14	0.451
	0.207	14	0.105	0.927	14	0.276

Tablo 3. Ankete verilen cevapların ön ve son test puanlarına ait ortalama ve standart sapma değerleri

		Ortalama	n	Standart Sapma	Ortalama Standart Hata
	Ön test	2.12643	14	0.600777	0.160564
	Son test	3.20500	14	0.770781	0.206000

Tablo 4. Bağımlı örneklem t-testi sonuçları

	Bağımlı örneklem Farklar					t	sd	İki yanlı p-değeri
	Ortalama	Standart Sapma	Ortalama Standart Hata	% 95 Güven Sınırları				
				Alt Sınır	Üst Sınır			
Ön test – Son test	-1.078571	0.880112	0.235220	-1.586733	-0.570410	-4.585	13	0.001

3.4. Katılımcı Görüş Yazıları

TÜBİTAK 2237A "Doğa Bilimlerinde İstatistiksel Modelleme Teknikleri ve Uygulamaları" etkinliğine katılan katılımcıların etkinlik bitiminde uygulanan anket formunda ayrıca kişisel görüş ve önerilerini dile getirmeleri, en beğenilen etkinlikleri belirtmeleri istenmiştir. Çalışmada etik açıdan katılımcıların isimlerini kullanmak yerine K1, K2, K3… şeklinde isimlendirmeler kullanılmıştır.

25-31 Ekim 2019, 3-9 Mart 2020, 10-16 Mart 2021, 27 Ekim-2 Kasım 2021 eğitim dönemlerinde katılımcıların etkinlikler hakkındaki bazı görüşleri aşağıda özetlenmiştir:

- **K1:** Öncelikle böyle bir etkinlik düzenlediğiniz için çok teşekkür ederim. Okuduğum ve görev aldığım üniversitenin bulunduğu şehirde imkanlarımız çok sınırlı olduğu için ve bu tarz eğitimlerin gerçekleşme sıklığı çok az olduğu için kendi adıma çok faydalı bir eğitim oldu. Eğitimde bir çok programın temel bilgileri verildiği için yorumlama kısmı daha çok tekrar edilmiş oldu. Eğitime gelmeden önce özellikle yorum kısmında eksiklerim vardı. Şu an o kadar büyük eksiklik hissetmiyorum. Tüm hocalarımıza emeklerinden ötürü ve vakit ayırıp bu organizasyonu planladıkları için çok teşekkür ederim.

- **K2:** Eğitime katkısı olan herkese çok teşekkür ederim. Eğitim bana hangi konuları detaylı araştırmam gerektiği konusunda çok yardımcı oldu. Emeği geçen herkese çok teşekkür ederim.

- **K3:** Multi-disipliner konuların entegre edilmesi konusunda ufuk açıcı bir eğitim. İlginiz ve eğitiminiz için çok teşekkür ederim. Sizleri tanıdığıma çok memnun oldum. Sevgi ve saygılarımla,

- **K4:** Eğitimden önce sadece SPSS programı hakkında bilgim vardı ancak sınırlı alanda. Bu eğitimden üzerine yeni yöntem ve programlar ekleyerek ayrılıyorum.

- **K5:** Gelirken tedirgin olduğum ölçüde giderken üzgün ayrılıyorum. Projem için harika bir fikrim var.

- **K6:** Kurs beklediğimden daha faydalı oldu. Güler yüzünüz ve sabrınız için teşekkür ederim. Ayrıca dağılımların da anlatılacağı bir kurs düzenlenirse mutlu olurum. Sevgi ve saygılarımla,

- **K7:** Bu eğitimin devamı olan bir eğitim daha düzenlenirse bizlere çok faydalı olacaktır. Emeklerinize sağlık

- **K8:** Bu projenin devamı niteliğinde ileri eğitimleri verilmeli, projenin diğer etaplarının oluşturulması ve desteklenmesinin bilim dilinin ortak kullanılmasını katkı sağlayacağına inancım büyük

- **K9:** Hocalarıma vermiş olduğu emek ve çabaları için çok teşekkür ederim. Çalışma alanlarımız ilgili verimli bir eğitim oldu.

- **K10:** Gerçekten doğa bilimlerinde uzmanlaşmak isteyenlerin alması gereken bir etkinlik olduğunu belirtmek isterim.

- **K11:** Eğitimin genel içeriği ve hocalarımızın katkılarından çok memnunum. Teşekkürler, emeğinize sağlık.

- **K12:** Fenni pozitif bilimler üzerine çalışan ve genelde ekolojik verileri inceleyen araştırmacılar için Spss haricinde programların da var olduğu ve bu programlarla daha amaca uygun analizlerin gerçekleştirilebileceği bilgisi oldukça önemliydi. Gerçekten çok çok faydalı bir eğitim oldu. Eğitmenler olağanüstü ilgilendiler ve gerektiğinde tek tek bireysel olarak adeta hizmet ettiler. RStudio, Weka ve Python gibi programların çalışma prensipleri ve birbirinden farklı olduğu konular vurgulandı. Temel düzeyde kodlar aracılığıyla bol örnekli bol verili bir analiz haftası geçti. Emeği geçenlere çok çok teşekkür eder bu tip projelerin yaygın hale gelerek her bir araştırmacının faydalanmasını tavsiye ederim.

- **K13:** Eğitimde öğretilen programlardan sadece SPSS bilen biri olarak diğer programlar hakkında bilgi edinmiş olduk. Ancak bütün programlarda aynı işlemleri yapabileceğimizi gördük ve edindiğim izlenimle RStudio programının diğerlerine nazaran daha kapsamlı olduğunu düşünmekteyim.

3.5. Etkinliklerin Yaygın Etkilerinin İncelenmesi

25-31 Ekim 2019, 3-9 Mart 2020, 10-16 Mart 2021, 27 Ekim- 2 Kasım 2021 etkinlik dönemlerinde katılımcıların etkinlik sonrası bu projelerden yararlanarak gerçekleştirdiği yayın çalışmalarında TÜBİTAK 2237A "Doğa Bilimlerinde İstatistiksel Modelleme Teknikleri ve Uygulamaları" adlı

etkinliğe yer verdikleri yayınlarında bulunan atıf ve teşekkürler aşağıda listelenmiştir:

Atıf ve Teşekkürler

- Turgut, S. S., Feyissa, A. H., Küçüköner, E., Karacabey, E. (2021). Uncertainty and sensitivity analysis by Monte Carlo simulation: Recovery of trans-resveratrol from grape cane by pressurised low polarity water system. Journal of Food Engineering, 292, 110366. (SCI kapsamında)

- Cekim, H. O., Güney, C. O., Şentürk, Ö., Özel, G.,Özkan, K. (2021). A novel approach for predicting burned forest area. Natural Hazards, 105(2), 2187-2201. (SCI kapsamında)

- Yesil, P., Güzel, M. (2021). Giresun Kent Merkezi'nde Konut Fiyatlarına Etki Eden Yapısal ve Çevresel Etkenlerin Belirlenmesi, Akademik Ziraat Dergisi, 10(2), 305-316.

- Unal, C., Kadilar, C. (2021). Improved Estimators using Exponential Function for the Population Mean in Simple and Stratified Random Samplings. Pakistan Journal of Statistics and Operation Research, 17(2), 333-342. (ESCI kapsamında)

- Cekim, H. O., Kadilar, C. (2020). ln-Type Variance Estimators in Simple Random Sampling. Pakistan Journal of Statistics and Operation Research, 16(4), 689-696. (ESCI kapsamında)

- Özel, G., Unal, C., Düz, Y. N., Özkan, K. (2021). İstatistik Öğretiminde Yeni Bir Yaklaşım: Doğada Uygulamalı İstatistik. Bilge International Journal of Science and Technology Research, 5(2), 124-138. (Google Scholar kapsamında)

Projelerde Yer Verilen Atıf ve Teşekkürler

25-31 Ekim 2019, 3-9 Mart 2020, 10-16 Mart 2021, 27 Ekim- 2 Kasım 2021 etkinlik dönemlerinde katılımcıların etkinlik sonrası bu projelerden yararlanarak gerçekleştirdiği yayın çalışmalarında TÜBİTAK 2237A "Doğa Bilimlerinde İstatistiksel Modelleme Teknikleri ve Uygulamaları" adlı etkinliklerde projede yer alan eğitmenler veya katılımcıları arasında işbirlikleri de geliştirilmiştir. Bu kapsamda önerilen ve TÜBİTAK tarafından 2020 ve 2021 yıllarında desteklenen diğer TÜBİTAK projeleri aşağıdaki gibidir:

- TÜBİTAK 4004 "İstatistiği Doğada Öğren 1", "İstatistiği Doğada Öğren 2"
- TÜBİTAK 4005 "Eğitimde Yenilikçi Bir Yaklaşım: Veri Görselleştirme Teknikleri ve Uygulamaları"
- TÜBİTAK 2204C "Antartika'nın Geleceği İçin Ekolojik Niş Modelleme"- Lise Öğrencileri Kutup Araştırma Projeleri Yarışması Birincisi
- TÜBİTAK 1001 Deprem Özel Çağrısı "Yapay Zeka ve Olasılıksal Model Tabanlı Deprem Tehlike Haritası"

4. TARTIŞMA VE SONUÇLAR

Günümüzde, veriye ulaşma kısmının kolay olmasına karşılık doğru veriyi seçme ile toplama ve yorumlama kısımlarının daha zor ve önemli olduğu İstatistik eğitiminin lisansüstü eğitimdeki yeri ve önemi giderek artmaktadır. Bu nedenle lisansüstü eğitimde İstatistik öğretiminde kavramların anlamları, veriden yararlanarak tahmin ve çıkarımlar elde etme, öneri, tartışma ve yorum kısımları, hesaplamalar, matematiksel beceriler ve grafik çizimleri kısımlarına göre daha çok üzerinde durulmalıdır.

Doğa bilimleri alanında istatistik öğretiminde RStudio ve Python gibi açık kodlu yazılımlardan yararlanılmalı ve öğretim aracı olarak kullanılmalıdır. Günlük hayattan karşımıza çıkan durumlar örneklenerek istatistikle gerçek yaşam ortaklığından bahsedilmelidir. Aynı zamanda teknoloji sayesinde, öğrenciler hesaplamalara harcayacakları zamanları çıkarsama, yorum ve tartışma kısımlarında kullanmalıdır.

Lisansüstü öğrencilerin tamamı proje etkinliklerinde yaşadıkları deneyimler doğrultusunda istatistiksel modelleme tekniklerine gelecekteki meslek hayatlarında istatistik kaygısı olmadan kullanacağını belirtmiştir. TÜBİTAK 2237-A tarzı uygulamalı eğitimler ilgi ve merak uyandırıcı olması ile birlikte öğrencilerde kariyer bilinci oluşturarak kalıcı öğrenme sağlaması, problem çözme becerisini geliştirmesi ile tez çalışmalarında yaratıcılığı ortaya çıkarması, öğrencide özgüven sağlaması ve günlük yaşamla ilişkilendirmesi gibi sağladığı getirileri açısından önemlilik taşımaktadır. Ayrıca bu etkinlikler yardımıyla proje eğitmen ve yürütücüleri arasında oluşturulan akademik işbirlikleri ile birçok proje, yayın çalışması gerçekleştirilmiştir. Bu bağlamda genel olarak öncelikle lisansüstü öğrencilere bu tarz uygulamalara yönelik bilginin yanı sıra deneyim ve beceri kazanmalarına imkân sağlayacak eğitimlere uygulamalı bir şekilde yer verilmesi ve bu tarz eğitimlere katılımları hakkında teşvik edilmelidir.

TEŞEKKÜR

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Conflict of Interest
The authors have no conflicts of interest to declare.

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Characterization of Chickpea (*Cicer arietinum*) Stalk Pulp and Evaluation in Paper Production

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Abstract: In this study, the evaluability of chickpea stalks generated after harvest in chickpea production was investigated in pulp and paper production. Also, paper production was carried out by blending the chickpea stalk fibers with primary and secondary fibers in certain proportions and the effects of chickpea stalk fibers on the paper properties were determined. The modified kraft method was used in the pulping of chickpea stalks and anthraquinone (AQ) was added to the cooking liquor as a catalyst. Some chemical, mechanical and optical properties of the pulps produced with the addition of different AQ charges were compared and the 0.7% AQ added cooking experiment gave the best results. The yield and viscosity values of the chickpea stalk pulps increased by 12.6% and 34.2%, respectively and the kappa number decreased by 46.7% with the addition of 0.7% AQ to cooking liquor. Paper production was carried out by blending the fibers obtained from an optimum cooking condition with primary and secondary fibers at certain rates and the effects of chickpea stalk fibers on the paper properties were examined. Depending on the amount of chickpea stalk fibers, the mechanical properties of the papers produced with secondary fibers and the optical properties of the papers produced with primary fibers improved. The strength losses that occur during the recycling of waste paper could be reduced by blending the chickpea stalk fibers with secondary fibers and it is possible to produce various paper types such as writing-printing paper by blending the short fiber chickpea stalk fibers and long fibers.

Keywords: Chickpea stalk, pulp, paper, anthraquinone, primary fiber, secondary fiber.

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1. INTRODUCTION

Cellulose is one of the most available polymers on the planet. Wood, cotton, and other lignocellulosic materials like agricultural waste and annual plants contain cellulose (Akgül et al., 2018; Gündüz et al., 2014; Moon et al., 2011). Natural cellulose fibers retain their strength even when wet. It is the combination of these properties, as well as strength and flexibility, which distinguishes cellulose as a valuable material for paper manufacturing. By far, the most common source of papermaking fiber is wood (Yaşar et al., 2017). Wood is primarily made up of fibers with only a few nonfibrous elements such as pith and parenchyma cells. However, due to forest resource protection laws, difficulties in obtaining wood raw materials have arisen. Many organizations advocate the agricultural wastes and residuals used as cellulose sources in the paper industry, owing to rising environmental concerns (Ateş et al., 2014; Camarero et al., 2004; Güler and Beram, 2018).

Agricultural waste is unwanted or non-sellable material that is entirely produced from agricultural activities which are directly related to the cultivation or breeding of animals with the main purpose of generating profits or livelihoods (Dai et al., 2018; Viets, 1975). Agricultural wastes, whose annual production amounts are quite high, are one of the most abundant renewable resources in the world (Bian et al., 2019; Jordan et al., 2021; Mancera et al., 2012). The reuse of agricultural wastes has gained great importance in terms of both environmental and commercial aspects. In terms of the environment, leaving agricultural wastes to rot or burned on the land causes soil, water, and air pollution. It is economically important to transform agricultural wastes into products with high added value such as pulp, paper, fiberboard, particleboard and composites. It is important to note that in most cases, non-wood raw materials have a low density, a porous structure, and a low lignin content, which requires less energy and chemicals for fiber separation during the pulping process. Due to the restrictions on non-reusable resources, the demand for renewable materials,

especially cellulose-based, has been increasing (Hapani et al., 2020; Mohanty et al., 2005).

Chickpea is the world's second most significant legume and is cultivated in at least 33 countries in South Asia, East Africa, West Asia, North Africa, Australia and Southern Europe (Singh, 1997). In 2019, approximately 14.2 million tons of chickpeas were produced in the world (FAO, 2022). *Cicer arietinum* L. is one of the earliest legumes to be produced around the globe, and it is thought to have originated in an area near Syria in present-day Turkey. Chickpea is in a tribe of its own, *Cicereae Alef* and 43 species have been reported: 33 perennial, 9 annuals (including the cultivated one), and one unspecified (Van der Maesen, 1987). As a result of the interviews with the farmers producing chickpea, it was determined that 1 kg of chickpea stalk is obtained from one kilogram of chickpea production. In line with these data, approximately 14.2 million tons of chickpea stalks are produced annually in the world. These stalks are either used in animal feed production or left in the soil and rotted. It has become extremely important to obtain products with high added value from lignocellulosic material, which is found in such a large amount.

Wood, annual plants and waste paper are used as raw materials in the production of paper and pulp in the world. Considering some laws and regulations, it has become difficult to obtain wood raw materials as mentioned before, and papermakers have focused on using waste paper and annual plants as raw materials. The most widely used pulp production method in the world is chemical pulping. Chemical pulping gives higher quality fibers than mechanical and semi-chemical pulping methods. The sulfate (kraft) method, one of the chemical pulping methods, gives the strongest fibers and is one of the most widely used chemical pulping methods in the world. Pulping methods are modified to improve some optical, chemical and mechanical pulp and paper properties. These modifications, it is aimed to slow down or stop the peeling reactions that occur in alkaline cooking processes. Therefore, some chemical additives such as anthraquinone (AQ) and boron compounds are added to the cooking liquor. These additives protect the shortening of cellulose chains by reducing end-groups of hemicellulose and cellulose so it modifies keto and the aldehyde groups to the hydroxyl group by reducing easily (Çiçekler & Tutuş, 2021; Saraçbaşı et al., 2016; Tutuş et al., 2015). Anthraquinone (AQ), a redox catalyst, oxidizes the reducing ends of cellulose and hemicelluloses in pulp and protects against alkaline degradation such as peeling reaction (Samp, 2008; Tutuş et al., 2016).

In this study, pulps were produced from chickpea stalks by the kraft-AQ cooking method, and some chemical, mechanical and optical properties of these pulps were characterized, then their usability with primary and secondary fibers was investigated.

2. MATERIAL AND METHODS

2.1. Material

The chickpea stalks used in the study were obtained from the experimental fields of Kahramanmaraş Sütçü İmam University, Faculty of Agriculture (Turkey). The chemical components and fiber dimensions and parameters of the chickpea stalks determined by (Özdemir et al., 2020) were given in Table 1.

Table 1. The chickpea stalks chemical components and fiber dimensions

Chemical Components		Fiber Dimensions and Parameters	
Holocellulose content (%)	78.8	Fiber length (mm)	0.89
Cellulose content (%)	52.6	Fiber width (μ)	18.3
Alpha cellulose content (%)	47.8	Lumen diameter (μ)	11.7
Lignin content (%)	17.1	Cell wall thickness (μ)	3.27
Ash content (%)	4.70	Felting rate	50.2
Extractive (%)	4.40	Elasticity coefficient	62.6
1% NaOH solubility (%)	39.7	Runkel ratio	0.68
Cold water solubility (%)	16.0	Rigidity coefficient	18.7
Hot water solubility (%)	12.8	F factor	272.5

Chemicals used in cooking processes were purchased from Merck Inc. (Germany). In the study, fibers obtained from red pine chips by the kraft method were used as primary fibers and fibers recycled from old corrugated cardboard (OCC) were used as secondary fibers.

2.2 Pulp Production

Chickpea stalks were chipped in 6-8 cm dimensions after being purified from their impurities and non-lignocellulosic structures. To be used in each cooking, 500 g oven-dried samples, whose water content was determined, were kept in polyethylene bags and made ready for cooking processes. Four different cooking trials were applied to chickpea stalks, using the kraft-AQ pulping method and the cooking conditions were presented in Table 2.

Table 2. Cooking conditions applied to the chickpea stalks

Conditions	Value
Active alkali (%)	20
Sulfidity (%)	25
AQ charge (%)	0, 0.3, 0.5, 0.7
Cooking temperature (°C)	160
Time to max. temp. (min)	40
Time at max. temp. (min)	100
Liquor to stalk ratio (l/kg)	5/1

Cooking processes were carried out in an electrically-heated rotary digester resistant to high temperature (350 °C) and pressure (25 bar), and the digester rotates 4 revolutions per minute. The pulps obtained after the cooking processes were washed with plenty of tap water on a 200-mesh screen to remove the black liquor. Then, the pulps were transposed to

a slotted (0.15 mm) screen in order to separate the uncooked parts (screen rejects). After the screening processes were completed, pulp yields (screened and screen rejects) were determined according to the amount of raw material used at the beginning of the cooking process. The viscosity values and kappa numbers as chemical properties of pulps were determined according to ISO 5351 and ISO 302 standards, respectively.

2.3 Paper Production

The pulp that gave optimum properties under the conditions given in Table 2 was used in the paper production. Test papers were produced by blending chickpea stalk fibers, primary (virgin) and secondary (recycled) fibers. The fibers were gradually beaten to 35±2 SR° freeness level in a laboratory-type Hollander device before paper production. According to ISO 5269-2 standard, ten test papers with 80±3 ($g.m^{-2}$) grammages were manufactured from each blend by using Rapid Kothen (RK-21) semi-automatic paper machine. Then, the papers were conditioned in a conditioning room for 24 hours at 23±1 °C and 50±2% relative humidity in accordance with the ISO 187 standard.

2.4. Analysis of Mechanical Properties

Printability and runnability depend on the mechanical properties of papers. Breaking length, burst and tear indices, which are main indicators of the mechanical properties of papers, were determined according to ISO 1924-2 (breaking length-50 mm/min), ISO 2759 (burst index) and ISO 1974 (tear index) standards. Fig. 1 indicates where the breaking length, burst and tear test samples were taken from the papers.

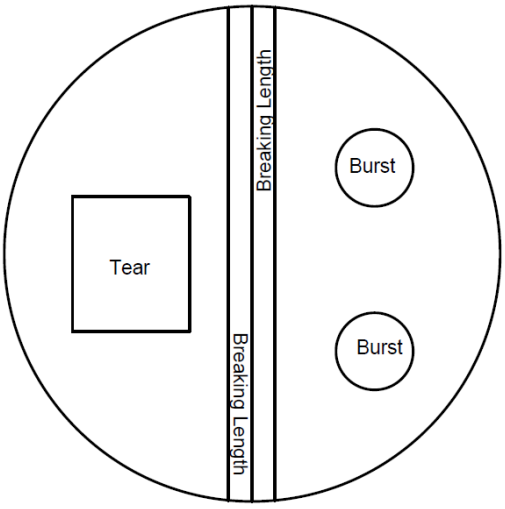

Figure 1. Parts used in determining the mechanical properties of papers.

The mentioned mechanical tests were replicated 10 times on the papers obtained from each fiber blend.

2.5. Analysis of Optical Properties

The optical properties of the papers are a kind of indicator of the printing quality of the paper. Whiteness, brightness and yellowness values of papers are the prominent parameters in optical properties. These values were measured by Datacolor Elrepho 450x spectrometer according to ISO 2470 (whiteness), ISO 11475 (brightness) and ISO 17223 (yellowness).

2.6. Statistical Analysis

One-way analysis of variance (SPSS for Windows, Version 16.0) was used to test whether there is a statistically significant difference between the means of independent groups. Then, the Duncan test which indicates the effects of chickpea stalk fiber content on the mechanical and optical properties of papers was applied.

3. RESULTS AND DISCUSSION

3.1 Chickpea Stalk Pulp Properties and Effects of Anthraquinone

The yield and some chemical properties of the pulps obtained from chickpea stalks by the modified kraft method were given in Table 3.

Table 3. Yield and chemical properties of chickpea stalk pulps produced by the kraft-AQ cooking method

AQ Charge (%)	Screened Yield (%)	Screen Reject (%)	Total Yield (%)	Kappa No	Viscosity ($ml.g^{-1}$)	DP
0	37.9^{d}	1.07^{d}	39.0^{d}	53.5^{d}	421^{d}	577^{d}
0.3	40.4^{c}	0.17^{c}	40.1^{c}	37.5^{c}	446^{c}	615^{c}
0.5	40.7^{b}	0.12^{b}	40.9^{b}	34.5^{b}	511^{b}	715^{b}
0.7	43.9^{a}	0.07^{a}	43.9^{a}	28.5^{a}	565^{a}	800^{a}
Sig	.000	.000	.000	.000	.000	.000

* According to Duncan's mean separation test, mean values with the same lower-case letters are not significantly different at the 95 % level of confidence.

AQ addition in cooking liquor enhanced the pulp yield, viscosity and degree of polymerization (DP) values of the chickpea stalk pulps. AQ oxidizes the reducing end of the polysaccharide in the pulp, preserving it from alkaline deterioration (Liu et al., 2017; Masrol et al., 2018; Shao et al., 2017). Peeling reactions that cause yield loss are reduced and pulp yields increase (Fig. 2).

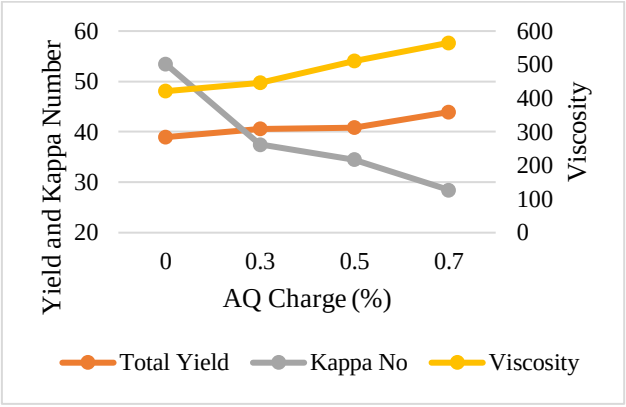

Figure 2. Effects of AQ on yield, kappa number and viscosity of the chickpea stalk pulps

Besides, the viscosity and DP values of the pulps were also increased by the addition of AQ into the cooking liquor. According to statistical analyzes, 0.7% AQ added cooking experiment gave the best results in pulp yield and chemical properties. With the addition of 0.7% AQ, the total pulp yield, viscosity and DP values of the chickpea stalk pulps were increased by about 12.6%, 34.2% and 38.6%, respectively. Kappa numbers, which are indicators of the remaining lignin content in the pulp, decreased by about 47% with the addition of 0.7% AQ (Fig. 2). AQ is an important catalyst that has been used widely in alkaline wood pulping for its effectiveness in an acceleration of delignification (Fišerová et al., 2006; Masrol et al., 2018). Table 4 shows some mechanical and optical properties of chickpea stalk pulps.

Table 4. Mechanical and optical properties of chickpea stalk pulps produced by kraft-AQ cooking method

AQ Charge (%)	Breaking Length (km)	Burst Index ($kPa.m^2.g^{-1}$)	Tear Index ($mN.m^2.g^{-1}$)	Whiteness (ISO%)	Brightness (ISO%)	Yellowness
0	4.10^{c}	1.84^{b}	2.91^{a}	34.4^{c}	26.3^{c}	34.8^{b}
0.3	4.26^{d}	1.89^{c}	2.94^{a}	36.1^{b}	27.7^{b}	33.8^{a}
0.5	4.44^{b}	1.97^{a}	2.93^{a}	36.6^{b}	28.0^{b}	34.4^{ab}
0.7	4.62^{a}	1.94^{a}	2.95^{a}	39.8^{a}	29.5^{a}	36.9^{c}
Sig	.000	.000	.154	.000	.000	.000

According to Table 4, AQ addition to the cooking liquor had a positive effect on both mechanical and optical properties of the pulps. The breaking length and burst index of the pulp obtained from the 0.7% AQ-added cooking trial increased by approximately 12.7% and 5.4% compared to the AQ-free cooking trial (Fig. 3).

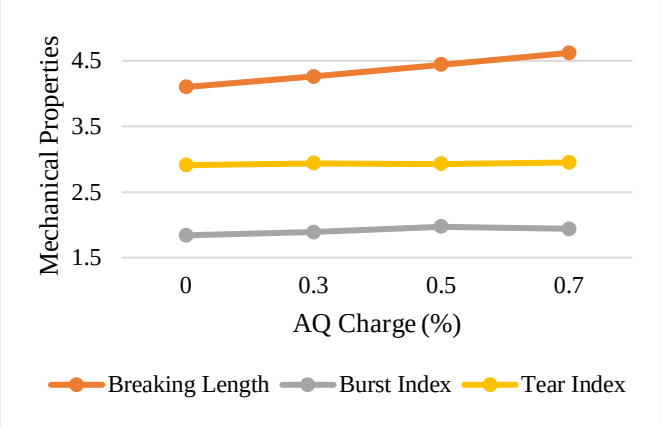

Figure 3. Effects of AQ on the mechanical properties of chickpea stalk pulps

It was observed that AQ had no impact on the tear strength of the pulps. Since AQ slows down or stops the peeling reactions occurring in the cellulose chains, pulps with a high degree of polymerization (DP) are obtained. Thus, the strength properties of pulps with high DP are better (Hassan et al., 2013; Khristova et al., 2006; Sarwar Jahan et al., 2012; Tutuş et al., 2016). Whiteness and brightness values of the AQ-added pulps were found to be higher than AQ-free pulps. On the contrary, the yellowness values of the pulps were negatively affected by using AQ (Fig. 4).

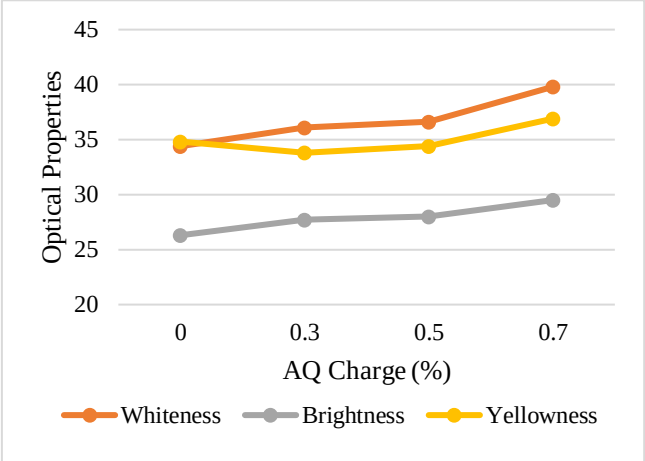

Figure 4. Effects of AQ on the optical properties of chickpea stalk pulps

As mentioned before, due to the effectiveness of AQ in accelerating delignification, the amount of lignin that adversely affects the optical properties of the paper is less. In addition, when the kappa numbers of the pulps in Table 4 are examined, it is clearly understood that AQ reduces the amount of lignin remaining in the pulps (Fišerová et al., 2006; Masrol et al., 2018).

As a result of the analysis of the data in Tables 3 and 4, optimum conditions for pulp production from chickpea stalks were obtained with the addition of 0.7% AQ. Fibers obtained from 0.7% AQ-added cooking trial were used in paper production with primary and secondary fibers.

3.2 Properties of Papers Produced with Chickpea Stalk and Primary Fibers Blends

Table 5 indicates some mechanical and optical characteristics of papers made with chickpea stalk (CF) and primary (PF) fiber blends.

Table 5. Some mechanical and optical characteristics of the papers made with CF and PF blends

Blending ratios (%)	Breaking Length (km)	Burst Index ($kPa.m^2.g^{-1}$)	Tear Index ($mN.m^2.g^{-1}$)	Whiteness (ISO%)	Brightness (ISO%)	Yellowness
100^{PF}	6.35^{a}	3.26^{a}	9.69^{a}	28.53^{g}	20.46^{g}	43.41^{f}
$50^{CF}+50^{PF}$	5.43^{b}	2.64^{b}	5.78^{b}	32.92^{f}	23.99^{f}	39.85^{e}
$60^{CF}+40^{PF}$	5.38^{c}	2.66^{b}	5.43^{c}	34.18^{e}	24.95^{e}	39.16^{d}
$70^{CF}+30^{PF}$	5.13^{d}	2.73^{cd}	4.85^{d}	35.61^{d}	26.30^{d}	37.65^{c}
$80^{CF}+20^{PF}$	4.95^{e}	2.76^{d}	4.25^{e}	36.80^{c}	27.25^{c}	37.43^{bc}
$90^{CF}+10^{PF}$	4.89^{f}	2.81^{e}	3.93^{f}	38.69^{b}	29.04^{b}	37.11^{ab}
100^{CF}	4.61^{g}	2.94^{f}	2.94^{g}	39.78^{a}	29.51^{a}	36.96^{a}
Sig.	.000	.000	.000	.000	.000	.000

As it is known, fibers produced from softwood by the kraft method are quite strong compared to annual plant and hardwood fibers. One of the main reasons for this is that softwood fibers are longer than others (Madakadze et al., 1999; Przybysz Buzała et al., 2018; Schönberg et al., 2001; Wan Rosli et al., 2009). Many paper qualities are influenced by fiber dimensions, especially strength, and fiber dimensions contribute to the optical and surface properties of paper (Hiltunen & Paulapuro, 2011; Larsson et al., 2018).

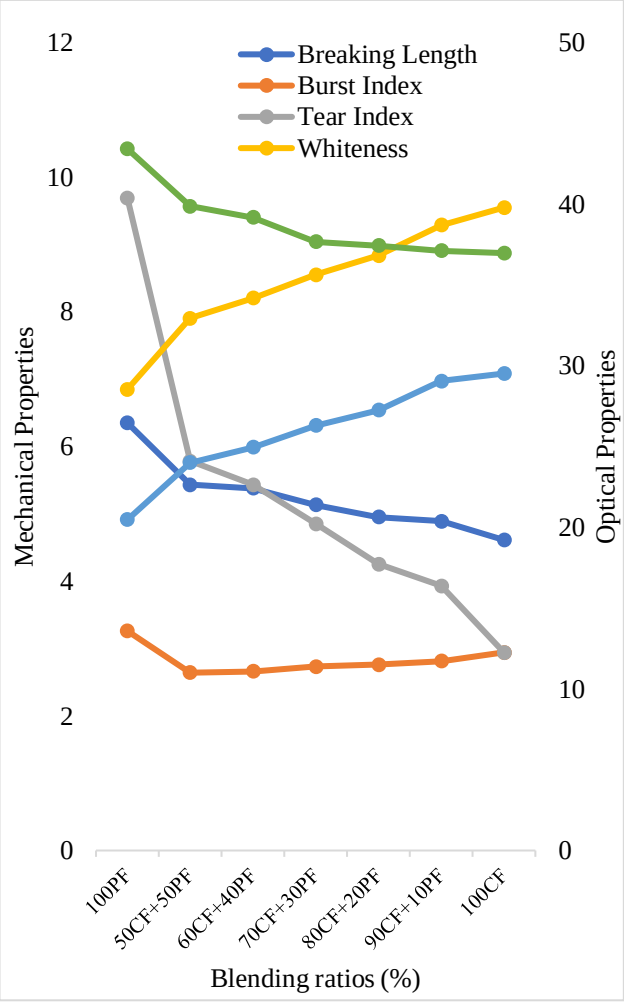

Figure 5. The mechanical and optical properties of the papers produced with CF and PF blends

The effect of fiber properties on paper strength led to the common consensus that paper with the desirable mechanical properties could only be produced from long fiber (Hiltunen & Paulapuro, 2011; Li & Kim, 2018; Watson & Dadswell, 2017). While the long fibers generally improve the mechanical of the paper, the short fibers mostly increase the surface and printing properties (Özdemir et al., 2020; Wan Rosli et al., 2009). As can be seen from Fig. 5, decreases in mechanical properties occurred in parallel with

the addition rate of CF to PF. As indicated in Table 1, the fibers obtained from chickpea stalks were classified as short fiber (0.89 mm). Accordingly, the strength properties of the papers produced by blending with CF decrease.

However, when the optical properties were examined, whiteness, brightness and yellowness values were positively affected by the addition of CF. As can be seen from Figure 4, the whiteness and brightness values increased with the addition of AQ to the cooking liquor. Besides, since chickpea stalk fibers have thin-walled (3.27 micron) fibers, they reflect light better than thick-walled fibers (Hubbe et al., 2008; Scallan & Borch, 1974). For these reasons, the optical properties improve as the CF ratio increases in papers produced from CF and PF blends.

3.3 Properties of Papers Produced with Chickpea Stalk and Secondary Fibers Blends

In Table 6, some mechanical and optical characteristics of papers manufactured with CF and secondary fibers (SF) blends were presented.

Table 6. Some mechanical and optical characteristics of papers manufactured with CF and SF blends

Blending ratios (%)	Breaking Length (km)	Burst Index ($kPa.m^2.g^{-1}$)	Tear Index ($mN.m^2.g^{-1}$)	Whiteness (ISO%)	Brightness (ISO%)	Yellowness
100^{SF}	3.80^{e}	2.39^{e}	5.46^{a}	35.25^{f}	26.47^{f}	37.15^{c}
$50^{CF}+50^{SF}$	4.11^{d}	2.41^{d}	4.73^{c}	36.82^{e}	27.84^{e}	36.51^{ab}
$60^{CF}+40^{SF}$	4.26^{cd}	2.45^{d}	4.97^{b}	37.10^{d}	28.05^{d}	36.53^{ab}
$70^{CF}+30^{SF}$	4.33^{c}	2.76^{bc}	3.97^{d}	37.80^{c}	28.64^{c}	36.25^{a}
$80^{CF}+20^{SF}$	$4.39b^{c}$	2.81^{b}	3.97^{d}	38.13^{b}	28.84^{b}	36.26^{a}
$90^{CF}+10^{SF}$	4.45^{b}	2.89^{ab}	3.39^{e}	38.19^{b}	28.95^{b}	36.82^{bc}
100^{CF}	4.61^{a}	2.94^{a}	2.94^{f}	39.78^{a}	29.51^{a}	36.96^{c}
Sig.	.000	.000	.000	.000	.000	.000

Corrugated cardboard papers production in the world is generally carried out by using the pulp obtained from the recycling of waste papers (Barbash & Yashchenko, 2020; Pereira et al., 2020). Fibers recovered from waste paper are called secondary fibers in the literature, and typically the hornified fibers shorten in length and lose their flexibility and take on a rigid structure (Yin et al., 2016; Zhang et al., 2017). At the same time, the surface areas of the fibers become narrower and they lose their fiber-fiber bonding potential (Biermann, 1993; Clark, 1978; Mckee, 1971; Minor, 1994).

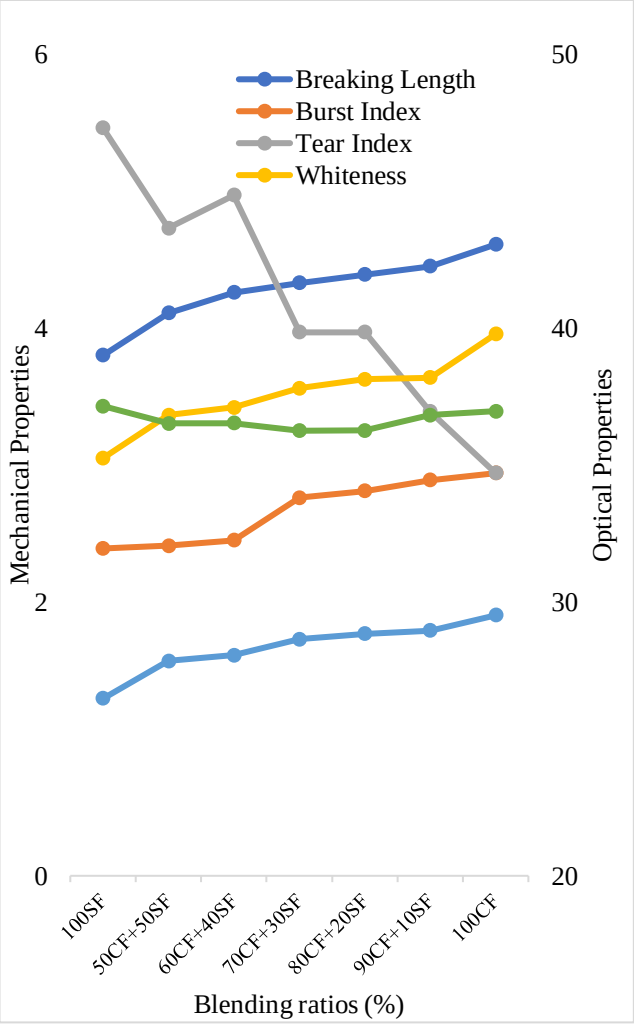

Figure 6. The mechanical and optical properties of the papers produced with CF and SF blends

The mechanical properties of old corrugated cardboard (OCC) pulps consisting of secondary fibers were lower than that of CF (Fig. 6). The fiber lengths and surface areas of SF pulps are lower than virgin pulps as mentioned above. Since the optical properties of CF are higher than that of SF, there has been an increase in optical properties in parallel with the CF addition ratio. This variation is caused mostly by differences in the fibers themselves, as well as the existence of different pollutants that have been properly eliminated from the pulp during processing (Obradovic & Mishra, 2020). The presence of pollution in paper made from secondary fibers can influence the optical properties as well as mechanical properties, though the effect of the changing characteristics of the fibers themselves is considerably more substantial in this case (Yin et al., 2016).

4. CONCLUSION

Optimum results in pulp production from chickpea stalks with a modified kraft method were obtained by adding 0.7% AQ to the cooking liquor. The breaking length and burst index of the pulp obtained by AQ added to the cooking liquor increased from 4.10 km to 4.62 km, and 1.84 $kPa.m^2.g^{-1}$ to 1.94 $kPa.m^2.g^{-1}$, respectively. The total pulp yield was enhanced by 15.8% with the addition of 0.7% AQ. The utilization of chickpea stalks in pulp production would increase fiber availability in countries with limited wood resources. The mechanical and optical properties of the papers obtained by mixing the chickpea stalk with primary and secondary fibers in certain proportions were not significantly affected. The strength properties of the papers produced with the mixture of the fibers recovered from the old corrugated cardboards and the chickpea stalk fibers increased in parallel with the chickpea stalk fiber content. It was observed that the optical properties of the produced papers increased when the primary and chickpea stalk fibers were blended. Short fiber chickpea stalk pulps can be used in the production of many kinds of paper by blending it with long fibers in certain proportions. Besides, the losses in the strength properties of the paper produced in waste paper recycling can be eliminated by adding chickpea stalk fibers. Correspondingly, the possibility of using chickpea stalk fibers in the production of different papers is quite high.

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Sustainable Production of New-Graded Bitumen with Waste Styrofoam Modification

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Abstract: Styrofoam is a recyclable petroleum origin product. However, releasing it and/or its waste into nature causes permanent damage to environment and human health owing to toxic materials that it contains. This study was set out on sustainable recycling of waste styrofoam (WS). To recycle the waste, it is used as a modifier in bitumen. In this respect, three type of bitumen in different penetration grade, which are one used for modification the other two used for reference in optimization analysis and WS in five different rate ranging from 1% to 5% by weight of bitumen were utilized. Different conventional test methods were applied on each samples to identify the effect of WS rate on bitumen basic characteristics. Optimum rate of WS required to produce new grade bitumen was evaluated based on modification index found for all test results. The test results showed that WS modification changes the bitumen properties, significantly. It can be possible to produce new graded bitumen using certain rate of WS. Test method was found a critique factor, since optimum rate of WS considered based on modification index changes due to the test method. Overall, the recycling WS using as a modifier in bitumen can an alternative, energy efficient, economic, and eco-friendly method.

Keywords: Sustainability, recycling, waste, Styrofoam, bitumen, modification

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1. INTRODUCTION

Sustainability is a kind of institutional operating practices and physical development that meet the needs of users without compromising the ability of future generations to provide their own requirements with the waste of natural resources (Anonymous, 2019). Therefore, sustainable practices must enhance a health environment for all livings and support the economic activities (Hart, 1997; Norton, 1992; Sikdar, 2003). Economic activities that vital to meet the human being needs extensive amount of energy to be used, and therefore significant amount of greenhouse gas is emerged beside of waste generation (Tonelli et al., 2013). Greenhouse gases released into the air and the energy used by human and industrial facilities may be possible to be reduced using advance technology. But wastes arising from vital activities and increasing population remains constant (Singh et al., 2012; Tseng et al., 2018). Fortunately, the wastes can be recycled instead of natural resource and can be used for different purposes (LaGrega et al., 2010; Reed et al., 1995).

Wastes are valuable materials in today world and significant economic gains can be provided with their recovery. Moreover, certain benefits can be obtained by means of environmental, health and aesthetic aspects. Waste resources might be residential, industrial, commercial, institutional, constructional, clinical, agricultural etc. based. They might in the form of solid, liquid and gas (Awoyera and Adesina, 2020; Chung and Lo, 2003; Oliveira et al., 2008; Reed et al., 1995; Thomas and Rahman, 2006). In the scope of the study, one of waste generally in occurs in solid form is evaluated to its feasibility to be recycled as a bitumen modifier. The waste utilized in this paper is of styrofoam, which is also known as expanded polystyrene (EPS). It is oil-originated synthetic polymer such as plastic consisting of styrene monomers (Ramadan et al., 2020).

Pandemic of coronavirus disease 2019 (COVID-19) increases dramatically usage of styrofoam based materials. Not only being widely used in packing jobs but also used in building and construction works, marketing, and some domestic applications, significant amount of post-use waste is generated during the period (Dev and Sengupta, 2020). Styrofoam can be destroyed if it is exposed to extremely high temperatures. It is known that heating it with fire releases toxins such as carbon black and carbon monoxide, which are hazardous product and effect the human and the other livings

health (LaGrega et al., 2010; Ramadan et al., 2020). The degradation of styrofoam material after usage is possible even if it is thrown into nature without any precautions. However, the time for degradation may be at least 500 years up to 4500 years (Awoyera and Adesina, 2020). Rapid rate in urbanization and technological development has increased the generation of waste material. It is possible to recycle such kind of wastes and make them valuable and versatile raw source for different engineering applications (Kaya and Kar, 2016; Grinnell, 1996; Nciri et al., 2020; Ngugi et al., 2017; Thakur et al., 2018).

Using waste styrofoam (WS) as a modifier in bitumen to improve their certain engineering properties has been a subject being attractive since the beginning of the 21st century with development polymer technology. However, the studies on this subject has been mostly restricted with comparisons of the effect of WS on bitumen characteristics including physical, and rheological ones beside of performance of hot mix asphalt produced bitumen with WS modification. Numerous examples from earlier studies are presented at the following to highlight in those manners.

Nassar et al. (2012) reported the effect of WS contribution on bitumen is significant in mechanical properties, particularly on viscosity. Baker et al. (2016) did an experimental study on physical properties of bitumen modified with WS three different rate from 5% to 15% with 5% increments by weight of bitumen. Researcher pointed out: decrease in ductility and penetration and increase flashing and fire points are significant. Al-Haydari and Masued (2017) investigated the potential benefits of WS usage in HMA properties. One of the results indicated by the authors is that adding WS into bitumen did not produce a modified bitumen. The other is introducing WS to bitumen and making a homogeneous mixture enhancing the mechanical performance of HMA. Nciri et al. (2020) investigated the potential usage of WS as modifier for hot mix asphalt (HMA) to produce flexible road pavements. The scope of the study is limited with the chemical and microscopic analysis. The authors found that WS addition increases the resin concentration in asphalt matrix, but does not affect the crystalline phase structure of asphalt. Microscopy base analysis shows asphalt and WS are compatible. Ramadan et al. (2020) investigate the mechanical properties of bitumen modified with different rate of WS. They reported that addition of WS into bitumen for modification purpose improve the stiffness of the bitumen. Moreover, there is significant improvement in high-temperature performance of bitumen modified with WS. This result associated with rutting resistance of the pavement produced using WS modified bitumen. Yıldız et al. (2021) investigated the usability of waste EPS foam in asphalt modification at the rate of 2%, 4%, 6%, and 8% by weight. The bitumen used in the study has 50/70 penetration grade and the conventional test methods were implemented for evaluation of the changes with modification, which were found significantly in certain contribution rate. They showed the feasibility of using EPS foam additive to be used in bitumen modification for hot regions, where low penetration is required.

1.1. Motivation and Objective

Together these studies provide important insights into the effect of WS addition on bitumen properties, but do not give extensive scientific understanding the effective or optimum rate of WS. According to the presented study, different contribution of WS rate change the grade of bitumen by means of either penetration or viscosity, in other words, new-graded bitumen was produced with WS modification. Although the changes in bitumen properties were determined with the available studies, there was not any study evaluating the optimum contribution rate of WS used for producing new bitumen, especially, according to test methods, bitumen type, modification processes (Vinodhkumar and Vinodhkumar, 2022, Porot, et al., 2021, Pérez-Lepe, et al. 2003). Therefore, it can be said that it is a little-known subject focused on the feasibility of usage for modification purpose and effect of it on bitumen properties. These cases indicate that there is a need to investigate the usage of WS in bitumen various perceptions.

In this respect, this study was established to investigate engineering properties WS modified bitumen based on penetration, softening point, flashing point, and viscosity test results and to make a comparison between the base and the WS modified ones. Determination the changes in temperature susceptibility compression and mixing temperature of bitumen modified with WS being used in hot mix asphalt, and the optimum rate of WS included in 100-150 penetration grade bitumen to reflect the properties of 70-100 and 50/70 on the basis of test methods in order for producing new graded bitumen are the other objectives.

2. MATERIAL AND METHOD

Three type of bitumen were used. These bitumen binders are in grade of 100/150, 70/100 and 50/70 penetration rate. All bitumen samples were supplied from Kırıkkale Refinery with deputy of Adana Bitumen Chief of General Directorate of Highways located in Republic of Turkey. The basic properties of bitumen are given in Table 1. The WS samples are supplied from waste collection and recycling containers located on the university campus. Some physical and chemical properties of WS are presented in the light of the study done by Nciri et al., 2020 (Table 2). It is worth to describe an acronym or abbreviation for each sample type used throughout the study for each bitumen form to distinguish the sample results from each other in figure or table bases. In this respect, Table 3 is prepared to notify the sample ID in which bitumen in 100/150 penetration grade is used for modification purpose, 70/100 and 50/70 penetrate grade are the reference ones used for optimization to determine the rate of WS that reflects the same characteristics for the modified bitumen for each case.

Table 1. Fundamental properties of bitumen samples

Tests	Unit	Standard	Test result of bitumen		
			A	B	C
Penetration	dmm	EN 1426	56	74	129
Softening Point	°C	EN 1427	47	44	39
Flashing Point	°C	ISO EN 2592	315	305	285
Ductility (25 °C)	cm	EN 12589	>108	102	58
Viscosity (135 °C)	cP	ASTM D4402	698.3	563.3	307.5
Viscosity (165 °C)			180.0	160.0	95.8

Where, A: PG:50/70, B: PG:70/100, C: PG:100/150

Table 2. Fundamental properties of WS

Chemical Parameter	Unit (%)	Result
Carbon	wt.	92.34
Hydrogen	wt.	6.93
Nitrogen	wt.	0.51
Sulfur	wt.	0.00
Oxygen	Wt.	0.22
Physical Parameter	**Unit**	**Result**
Molecular Weight	g/mol	~300,000
Density	Kg/m^3	17
Thermal Conductivity	W/ mK)	0.040
Flexural Strength	N/cm^2	24
Compressive Strength	N/cm^2	10

Table 3. Identification form for bitumen samples

Sample ID	Bitumen Composition	Form of Bitumen
BB	Base Bitumen	PG:100/150
CB-1	Base Bitumen	PG:50/70
CB-2	Base Bitumen	PG:70/100
WS-1	Base Bitumen + 1% WS	Waste Styrofoam Modified Bitumen
WS-2	Base Bitumen + 2% WS	
WS-3	Base Bitumen + 3% WS	
WS-4	Base Bitumen + 4% WS	
WS-5	Base Bitumen + 5% WS	

Where, PG is penetration grade

2.1. Production of WS Modified Bitumen

In order to prepare the WS modified bitumen, the recent literature linked with the polymer modification processes were analyzed (Gökalp, et al., 2019, Lin t. al. 2019, Anwar et al., 2020, Yıldız et al. 2021). In the light of the cited studies, production processes of WS modified bitumen were determined. The followed processes is presented step by step at bellow.

- Initially, WS samples are collected from waste storage area. Because WS samples are in different and big size, it is needed to break them in small sizes, approximately, 2-3 cm particles. The broken WS samples are stored in a bag and weighted for the studied rates ranging from 1% to 5% with 1% increment by weight of bitumen.
- During size reduction of WS, the bitumen is heated at 140 °C for one and half hour. 500 gram of heated bitumen transferred to a metal box. The box with bitumen is located on a heater works at 170 °C for the next half hour.
- After the heat of the bitumen samples reaches to 170 °C, the prepared WS samples was placed one by one in a container filled with hot bitumen.
- Subsequently, mixing phase is started for WS and bitumen with a propeller mixer that works at 200 revolutions per minute (rpm). The weighted for the specified contribution rate WS samples are introduces in bitumen while the mixer works.
- After all WS samples are introduced and coated with bitumen and the mixer is worked at 1000 rpm for 30 minutes for soften and penetration of the WS throughout the bitumen sample.
- The last step for the mixing is that arrangement mixing the mixture at 100 rpm for 15 minutes to provide homogeneity of WS modified bitumen. The other reference base bitumen samples are also subjected to the same mixing processes to provide the same aging level for the modified ones.

2.2. Test and Analyzing Methods

Some basic properties of bitumen were planned to determine, and the test methods categorized as conventional were performed. These are penetration, softening point, flashing point, viscosity at 135 °C and 165 °C. To observe the effect of WS contribution rate on thermal susceptibility, penetration indexes for each bitumen sample are calculated with internationally accepted formulation. The test methods are briefly summarized at the following.

Penetration test: The test is applied on the bitumen samples to determine their consistency or hardness under a certain load, temperature by utilizing a specific needle for certain time. In this study, EN 1426 standard was followed to conduct the test on samples. The three test results are considered acceptable if the range of the results does not exceed the relevant value given in the standard. Finally, the arithmetic mean of the acceptable results is reported (Gökalp et al., 2019).

Softening point test: Similar to penetration test, this test method is also one of conventional test used mostly to determine the softening point of the bitumen. EN 1427 was followed to apply the test. If the difference between the two temperatures, that the bitumen samples fall down does not exceed 1 °C for softening points, the test results is taken as acceptable. Finally, the mean of the temperatures is expressed to the nearest 0.2 °C (Gökalp et al., 2019).

Viscosity: Bitumen is a visco-elastic material and its viscosity changes with heating. To make the bitumen workable in HMA production and application it on the field, bitumen should be heated, sufficiently. Viscosity test is used to determine the sufficient heat. Brookfield rotational viscometer test is implemented on the samples according to ASTM 4402 standard under two specific temperatures: 135 °C and 165 °C (Mirsepahi et. al, 2020) within the scope of the study.

Flashing Point: Bitumen has certain volatile component and this component evaporates as exposed a fire source.

Following to heating, flashing can occur, temporarily. The temperature at this point is called flashing point. The flashing point for each bitumen samples may change due to their origin, chemical composition and the additive used to modify them. In this regard, flashing point test is implemented on each samples according to EN 22592 standard (Yaşar, 2015)

Penetration index: Bitumen is a thermal susceptible construction material. Thermal activities effect bitumen state and therefore its physical and mechanical properties. To identify the level of sensitiveness of bitumen, penetration index is developed on basis of penetration and softening point test results. To determine the index value, the following equation is used.

$$PI = \frac{1952 - 500\log Pen - 20SP}{50\log Pen - SP - 120} \quad (1)$$

where,
PI refers to penetration index,
Pen refers to penetration value obtained at 25 °C, and
SP refers to softening point value.

As the bitumen PI index increases the thermal susceptibility is decreases. The range of PI value for the bitumen to be used in HMA production is (+) 2 and (-) 2. However, evaluation of the thermal susceptibility of bitumen with PI is made with (-) 2. PI values below the (-) 2 indicates that the bitumen more resistant to thermal activity than those above (-) 2 (Firoozifar, et al. 2011, Sengoz and Işıkyakar, 2008).

Modification index: Bitumen modification change the bitumen characteristics. To determine the change in properties and make a comparison between the modified and base bitumen, an index called modification index value is established in the light of the earlier studies (Gökalp and Uz, 2019, Gökalp, et al., 2019). The index value can be calculated with the following equation.

$$MI = \left(\frac{VR - VMi}{VR}\right) x100 \quad (2)$$

Where,
MI refers to modification index,
VR refers to test results for reference bitumen, and
VM refers to test results for modified bitumen,
i WS rate indicator.

Optimization rate analysis: Modification index results are used to predict the optimum rate of WS for the new bitumen that stimulate the properties of reference bitumen used throughout the study. This analysis is done for all test methods studied in this paper, individually. As the modification index reaches to zero, the rate remarks it is considered as the optimum rate for the reference bitumen (Gökalp and Uz, 2019, Gökalp, et al., 2019). To do the analysis, best fit curve method is used. There are three main objectives to do this analysis. These are describing the trend of change in bitumen properties, predicting the rate of WS that provide studied reference bitumen, which are in 70/100 and 50/70 grade, and highlighting the effect of test methods on optimum rate.

3. RESULTS AND DISCUSSION

This section is presented in two parts. The first is test and analysis results and the second one is on the optimization analyses of WS contribution rates that reflect the 70/100 and 50/70 bitumen properties studied within this study.

3.1. Test and Analysis Results

The current study was established on evaluation of the effect of different rate of WS on the bitumen properties as being used modifier. To examine the change in bitumen properties, conventional test methods and thermal susceptibility analysis were performed. Table 4 indicates the results of test and analysis for each type of sample.

Table 4. Test and analysis results

Test Methods	Samples and Test Results							
	CB-1	**CB-2**	**BB**	**WS-1**	**WS-2**	**WS-3**	**WS-4**	**WS-5**
Penetration (dmm)	56.0	74.0	129.0	105.6	85.7	69.4	54.0	48.2
Softening Point (°C)	47.0	44.0	39.0	43.0	46.9	52.5	57.6	59.4
Flashing Point (°C)	315	305	285	290	310.0	320.0	329.0	335.0
Penetration Index	-1,72	-1,94	-2.17	-1.29	-0.67	0.24	0.73	0.82
Viscosity at 135 °C (cP)	698.3	563.3	307.5	337.5	424.1	703.3	791.1	1417.0
Viscosity at 165 °C (cP)	180.0	160.0	95.8	141.8	152.7	171.8	224.9	263.3

The data presented in Table 4 shows that the significant effect of addition of WS to the bitumen. Similar conclusions were highlighted in earlier studies done by Yıldız et al., 2021, Ramadan et al., 2020, Baker et al., 2016, Nassar et al., 2012. But, the level of changes are different due to material origin and/or test methods. Therefore, the general result in the current study and the earlier studies complied with each other. To indicate the results presented in Table 4, briefly, it can be indicated that bitumen penetration rate decreases, the softening point as the WS contribution rate increases. Moreover, flashing point of the bitumen also increases with increasing rate of WS and this case provides that the bitumen may be safer as heating against explosion caused by a source of flame. However, the change is lower than the other test results. On the other hand, the findings for PI results show that the WS modified bitumen exhibits better thermal susceptibility, because PI decreases while WS rate increases. Moreover, looking for the viscosity, as can be easily expected from penetration and softening point results, viscosity of the bitumen samples increases with WS modification.

It is possible to determine the temperature of the bitumen being used in HMA production in mixing and compaction

phases with viscosity test. To evaluate it, the related standard, which is ASTM D4402 recommends mixing and compaction as the viscosity of bitumen matches 170±20 cP and 280±30 cP, respectively (Öner, 2018). This criterion make the bitumen and the HMA workable both in plant and in site. The compression and mixing temperature results gathered from viscosity test is presented in Table 5.

It can be seen from the data presented in Table 5 that the mixing and compression temperatures of bitumen increases with addition of the WS modified bitumen. Comparing with the base bitumen, BB, the increase in temperature is approximately 15 °C in mixing and 25 °C in compression as the WS rate is 5%.

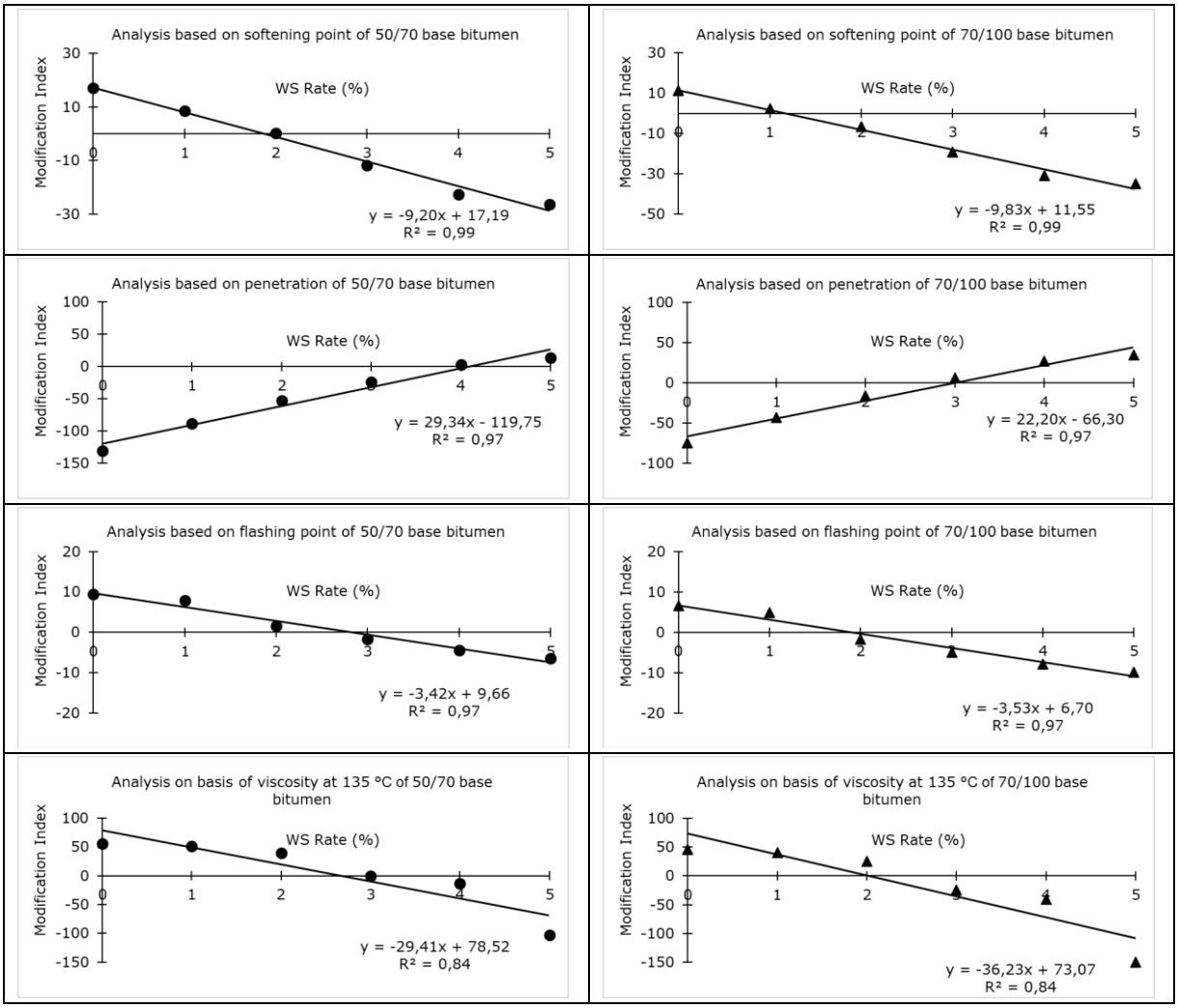
Table 5. Compression and mixing temperature results

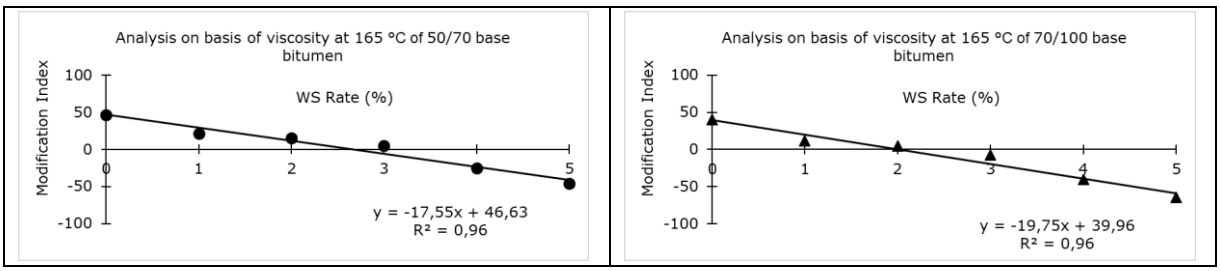
Temperature (°C)	Limits (cP)	Samples and Test Results							
		CB-1	CB-2	BB	WS-1	WS-2	WS-3	WS-4	WS-5
Mixing	170±20	167-164	166-163	157-152	164-158	165-161	166-164	169-167	168-167
Compression	280±30	161-157	158-153	143-135	148-139	154-148	161-157	164-161	165-164

Overall, all the results presented in Table 4 and Table 5 showed that modifying the base bitumen in 100/150 penetration grade with different rate changes significantly the studied properties of bitumen. The properties of modified bitumen approach to the reference bitumen in 70/100 and 50/70 penetration grades. This finding as mentioned before that is the motivation of the current research, which was rarely studied before for WS modified bitumen.

3.2. Optimization Analysis Results

The optimization analyses are established on best line analysis corresponding to the highest accuracy coefficients This case is done for the two, 70/100 and 50/70, grade bitumen. To start the analyses, best-fit line graphs in linear basement are given in Figure 1.





Where, x is WS rate and y is the referenced test or analysis results

Figure 1. Optimization analysis for 50/70 and 70/100 reference bitumen

It is obvious from the optimization analysis presented in Fig. 1 that there are high root square values that more than 0.95 except of the ones occurred for the viscosity at 135 °C found for the drawn best-fit line. As zero value of modification index gives optimum rate of WS that different WS rates are required for providing different bitumen in grade. As expected, optimization analysis corresponding to different test results gives different optimum WS rate. Similar results were found in recent studies used similar optimization approach (Gökalp and Uz, 2019, Uz and Gökalp, 2020, Gökalp and Uz, 2021) to determine optimum rate of additive to be used in modification of bitumen. To summary the optimum WS based on the test method for the two reference bitumen cases, Table 6 is presented.

Table 6. Optimum WS rate based on the test method for the reference bitumen samples

Test Methods	Optimum WS rate (%)		Difference in rate between CB-1 and CB-2 (%)
	CB-1	CB-2	
Penetration	4.08	2.99	26.7
Softening Point	1.87	1.17	37.4
Flashing Point	2.82	1.90	32.6
Viscosity at 135 °C	2.67	2.01	24.7
Viscosity at 165 °C	2.66	2.02	24.1

The results of optimum rate analysis presented in Table 6 indicate that higher rate of WS requires to make the studied bitumen in 100/150 penetration grade properties similar to CB-1 due to lower penetration grade and vice-versa. Moreover, it can be highlighted that the optimum rate of WS changes depend on the result of test method, which are different in numerous points. Looking for the differences between the optimum rate required to enhance properties of CB-1 and CB-2, the highest one is considered for softening point results as 37.4%, while the lowest one for viscosity at 165 °C as 24.1%. Similar conclusions were highlighted in numerous studies done by Gökalp and Uz, (2019), Uz and Gökalp,(2020), and Gökalp and Uz, (2021), which all investigate the subject on basis of both rheological and conventional test methods.

4. CONCLUSIONS

The studied waste material in the current study is that of styrofoam emerged mainly from packing and construction demolition activities. Although, great number of studies carried out on different waste to improve the bitumen characteristics, investigation done on the feasibility WS for use modifier in bitumen take limited place in the literature. Therefore, some uncertainties existed about the relation between the waste and bitumen. To make an original contribution to the current literature, an investigation on optimum contribution rate being used for producing new-grade bitumen in addition to highlight the effect of WS contribution on studied bitumen properties. Therefore, the current study is thought to be one of preliminary study, and the research methodology was stand on the conventional test to show main characteristics of bitumen. To achieve the aim, bitumen in 100/150 penetration graded bitumen was used to modify the bitumen with WS with 1% to 5% with 1% increment by weight of bitumen in rate. 70/100 and 50/70 penetration graded bitumen were used as reference that constructing the optimum rate analyses of WS as used in the studied bitumen. Best-fit line analysis is done for each test results to make optimization analysis. Consequently, it can highlighted that sustainable recovering of WS is reasonable with bitumen modification, WS modification improves the bitumen engineering characteristics, using certain rate of WS can change the grade of bitumen, different WS rate is required while optimization analysis done based on different test methods, higher WS rate is necessary for producing lower penetration graded bitumen.

Since it is a kind of preliminary study, some additional analyses are reqired to make a clear sense about the usage of WS in bitumen as modifier. Some are may be exemplified as following. Different bitumen types, either in origin or in grade should be studied since only one type of bitumen performance was evaluated in the current study. The effect of environment can be studied in detail due to the temperature-sensitivity characteristics of bitumen. Investigation should be expanded with the analysis based on rheological test methods to bring deep scientific understanding of the subject. Finally, the effect of WS modified bitumen on asphalt mixture performance, which is not included in this study should be investigated.

Ethics Committee Approval
N/A

Peer-review
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Conflict of Interest
The authors have no conflicts of interest to declare.

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Kinetic Modeling of Heat and Mass Transfer During Deep Fat Frying of Churro

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Abstract: The effect of deep fat frying temperatures, ranging from 160 to 190 ºC, on frying parameters including the heat transfer coefficient (h_e), mass transfer coefficient (k_e) and effective moisture diffusivity (D_e) were investigated during deep fat frying of churro that was fried as dough pastry. Therefore experimental studies were conducted for both heat and mass transfer phenomena and mathematical model was developed for simultaneous transfer by using Newman technique for churro actual geometry (3-D cylindrical shape). Fourier's and Fick's laws were applied for the computation of coefficients of heat and mass transfer. The h_e coefficients were 437.360-93.535 W/m^{2}K in the temperatures range of 160 to 190 ºC. However, the value of k_e and D_e increased by an increase in oil temperature during frying. The maximum values were determined as 17.36×10^{-5} m/s and 2.48×10^{-5} m^2/s at 190 ºC for k_e and D_e, respectively. Model and experimental data had good agreement and the transfer coefficients followed the first order kinetic model with high R^2 and low root mean square error (RMSE) values. The Arrhenius equation was applied to describe the relationship between the effective moisture diffusivity and deep fat frying temperature, so the value of activation energy was calculated as 63.546 kj/mol.

Keywords: Biot number, first-order kinetic, frying process, heat transfer coefficient, moisture diffusitivity, newman technique.

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1. INTRODUCTION

Churro, the traditional Spanish dessert, is a kind of sweet snack and it is very popular in Spain and Mexico. It is prepared from soft dough which was formed strips by a simple extruder and generally fried between 185-200 ˚C at 3-4 minutes until golden color and the crispy product is obtained (Morales et al., 2008; de Oliveira Silva et al., 2022). After the frying process is completed, Spanish churros are generally coated in sugar or cinnamon powder and served with thick dipping chocolate (Moolwong, 2020). Deep-fat frying or immersion frying is an important process for the preparation of churro in which heat and mass transfer occur simultaneously (Safari et al., 2018). Heat is transferred from the oil to the food surface by convection and then to the core by conduction, and at the same time, water loss by evaporation and absorption of oil by the churro is a complex process (Oke et al., 2018). This complex operation brings about desirable physical, chemical, structural and nutritional changes such as gelatinization of starch, the denaturalization of proteins, inactivation of enzymes, destruction of microorganisms, Maillard reaction, aromatization, shrinkage, etc. (Dourado et al., 2019; Manjunatha et al., 2019). Thanks to the beneficial effect of these physicochemical changes, the most acceptable properties of churro such as golden color, crispy and crunchy crust, soft and moist interior, cylindrical shape, and fine texture are obtained. Moreover, suitable design, control, and optimization of the frying operation are essential to obtain a high-quality product (Dehghannya and Ngadi, 2021). For this reason, knowledge of the frying parameters including h_e, k_e, and D_e are necessary to predict the quality of product and standardize and optimize processing conditions. Several researches of determining heat and mass transfer parameters by different techniques for model foods are presented in the literature. For example, Neethu et al. (2016), developed a simple model to calculation of heat and mass transfer parameters for fried pantoa (Indian dairy dessert), at 125–145 °C. When the frying temperature increased from 125 to 145 °C, the h_e parameter increased from 101.77 to 237.10 W/m^{2}K, while the k_e parameters increased from 7.79×10^{-6} to 9.05×10^{-6} m/s. Sandhu et al., (2016), determined the h_e coefficient for a fried-potato disc with one-dimensional methodology. The h_e parameters were 3617, 4517, and 7307 W/m^2°C at frying temperatures of 150, 170, and 190

°C, respectively. Another investigation was carried out to examine mass transfer parameters for fried green peas (Manjunatha et al., 2019). The kinetic rate constant for moisture loss and as the oil uptake was increasing with increasing the frying temperature.

The reported results showed that there is a lack of knowledge on the calculation of coefficients of heat and mass transfer, accurately. Because heat transfer was evaluated without mass transfer and also the actual dimensions of the food product (3-D) were not taken into account in the modeling studies. Since heat and mass transfer during frying are interrelated, they both need to be considered when investigating the frying process. In addition, using simple geometry rather than the real food geometry may confuse the calculation of kinetic boundary conditions.

In this research, therefore, the kinetics of heat and mass transfer coefficients of the churro with actual geometry during the frying process at four different temperatures (160, 170, 180, and 190 °C) were investigated. There is no scientific literature about the frying kinetic parameters of the churro. For this reason, the aim of this research was to determine the kinetics of convective heat transfer, mass transfer, and effective moisture diffusivity parameters during the frying process. To reach this aim, the plots of dimensionless temperature and concentration ratios against frying time were used to define the parameters with churro geometry (3-D cylindrical shape). Another objective of the present investigation is modeling the frying kinetic parameters. Thus, the kinetic model helps to establish the connections between phases and processing time.

2. MATERIAL AND METHOD

2.1. Preparation of Churro Dough

The main ingredients of churro dough were water 250 g, butter 47 g (Torku), salt 0.5 g (Billur) and wheat flour 300 g (Type 850, Yüksel) (de Oliveira Silva et al., 2022).
When all the ingredients were ready, water, butter, and salt were mixed and boiled and wheat flour was added to the water. After cooling, the dough was filled into the piping bag and squeezed into the hot oil using a star-shaped piping nozzle which is a symbol of churro. Acylindrical shape was given to dough (10 cm in length, 1 cm in diameter) and then fried (Moolwong, 2020).

2.2. Deep Fat Frying of Churro

Deep fat frying of churro was carried out using a deep fryer (Angelo Po, Italy). The frying temperatures and times were selected as 160, 170, 180 and, 190 °C at 420, 360, 300 and 180 s, respectively. Fried samples were filtered for 1 minute and placed on an adsorbent paper to remove surface oil.

2.3. Proximate Composition for Thermophysical Properties

Thermophysical properties of churro such as thermal conductivity (k), density (p) and specific heat (c_p) are necessary to determine the h_e parameter. For this, there are certain improved equations (Eq.1-3) (Cemeroğlu, 2017) and it is necessary to know the proximate composition of churro in using these equations. The moisture, protein, crude fat and ash of churro dough were determined by AACC methods (AACC, 1999). The total carbohydrate content was calculated via subtraction of the sum of moisture, protein, crude fat and ash from 100. These thermophysical parameters assumed constant during the frying.

$$k = \sum k_i \, x \, X_{Vi} \tag{1}$$

$$\rho = \frac{X_i}{\rho_i} \frac{1}{\frac{X_C}{1600} + \frac{X_P}{1320} + \frac{X_F}{920} + \frac{X_A}{240} \frac{X_W}{1000}} \tag{2}$$

$$Cp = 1.6X_C + 2.0X_P + 2.0X_F + 1.1X_A + 4.2X_W \tag{3}$$

2.4. Experimental Study and Kinetic Modeling for Heat Transfer

The inner temperatures of the churro doughs during frying at different temperature-time combinations and also the temperature of the frying oil were measured by a K type thermocouple, for 1 s time interval. These thermocouple sensors were connected to a data logger (Sper Scientific, Scottsdale) and obtain temperature-time graphs with using software program of data logger.

The Fourier's equation for an infinite plane wall (Eq. 4) and the Fourier's equation for an infinite cylinder shape (Eq.5) provides a simplified description of the heat transfer during frying. According to Newman technique, the Eq. (4) and Eq. (5) can be used together to obtain the solution of experimental heat transfer data for the churro geometry (finite cylindirical shape) by making use of the superimposition technique (Eq. 6).

$$Y_z = \frac{T_{(z,t)} - T_\infty}{T_i - T_\infty} = \sum_{i=1}^{\infty} \frac{2 \sin \mu_i}{\mu_i + \sin \mu_i \, cos \mu_i} \exp(-\mu_i^2 \frac{\alpha t}{L^2}) \cos\left(\mu_i \frac{z}{L}\right) \tag{4}$$

$$Y_r = \frac{T_{(r,t)} - T_\infty}{T_i - T_\infty} = \sum_{i=1}^{\infty} \frac{2\beta_i}{(Bi_r{}^2 + \beta_i{}^2)} \frac{1}{(J_0(\beta_i))^2} J_1(\beta_i) \, J_0(\beta_{in_r}) \exp\left(-\beta_i{}^2 \frac{\alpha t}{R^2}\right) \tag{5}$$

$$Yrz = Yz \times Yr \tag{6}$$

$$Y_{0zr} = \frac{T_{0zr} - T_\infty}{T_i - T_\infty} = \frac{2Bi_r}{(Bi_r{}^2 + \beta_i{}^2) J_0(\beta_i)} \, exp\left(-\beta_i{}^2 \frac{\alpha t}{R^2}\right) \frac{2 \sin \mu_1}{(\mu_{1+} \sin \mu_1 \cos \mu_1)} \, exp\left(-\mu_1{}^2 \frac{\alpha t}{L^2}\right)$$

The first order exponential kinetic model was used to describe the h_e parameters for different frying temperatures.

$$h_t = h_e + (h_0 - h_e) e^{-k_h t} \tag{7}$$

where h_0, h_t, h_e are the heat transfer parameters of initial, at time t and at equilibrium respectively and k is kinetic coefficient and t is frying time.

2.5. Experimental Study and Kinetic Modeling for Mass Transfer

Experimental study was conducted of mass transfer with the calculation of moisture contents for fried churro samples by dry matter analysis at 135 °C (AACC, 1999). Fick's second law equation for an infinite plane wall (Eq. 8) and an infinite cylinder shape (Eq. 9) provide a description of the moisture loss during frying. Eq. (8) and Eq. (9) can be used together to obtain the solution of the experimental moisture content data for the churro geometry with the superimposition technique (Eq. 10).

$$C_z = \frac{C_{(z,t)}-C_\infty}{C_{i-}C_\infty} = \sum_{i=1}^{\infty}\left(\frac{2sin\mu_i}{\mu_i+sin\mu_i\,cos\mu_i}\right)\exp\left(-\mu_i^2\,.\frac{D_e\,.t}{L^2}\right)\cos\left(\mu_i\frac{z}{L}\right) \quad (8)$$

$$C_r = \frac{C_{(r,t)}-C_\infty}{C_i-C_\infty} = \sum_{i=1}^{\infty}\frac{2\beta_1}{(Bi_{r^2}+\beta_{1^2})}\,\frac{1}{((J_0(\beta_1))^2}J_1(\beta_1)J_0(\beta_1\frac{r}{R})\exp\left(-\beta_{1^2}\frac{D_e t}{R^2}\right) \quad (9)$$

$$Crz=Cz\times Cr \quad (10)$$

$$C_{0zr} = \frac{C_{0zr}-C_\infty}{C_i-C_\infty} = \sum_{i=1}^{\infty}\left(\frac{2sin\mu_i}{\mu_i+sin\mu_i cos\mu_i}\right)\left(\frac{2Bi_r}{(Bi_{r^2}+\beta_{i^2}).J_0(\beta_i)}\right)exp - \left(\frac{\beta_{i^2}}{R^2}+\frac{{\mu_i}^2}{L^2}\right)D_e\,.t$$

The first-order reaction kinetic model describe the k_e and D_e values for different frying temperatures (Eq. 11 and Eq.12).

$$X_t = X_e + (X_0 - X_e)e^{-k_x t} \quad (11)$$

$$Y_t = Y_e + (hDe_0 - De_e)e^{k_h t} \quad (12)$$

3. RESULTS AND DISCUSSION

3.1. Kinetic Model of Heat Transfer Phenomena

The chemical composition and thermophysical properties of the churro dough are given in Table 1. In order to determine the convective heat transfer coefficients for all frying temperatures, firstly, the time-temperature profiles of the churros were obtained experimentally (Fig. 1). In Figure 1, the experimental study resulted in a slight increase in temperatures towards the boiling point of the water in the center of the churros during approximately the first 100 s of the frying process and then remained constant. The h_e parameters were 437.36, 301.679, 123.256, and 93.535 W/m^2 K at 160, 170, 180 and 190 °C, respectively. It is evident that the heat transfer coefficient and Biot numbers decreased with an increase in frying temperature. This result indicates that a higher temperature frying environment results in higher water loss from the product.

Table 1. Chemical composition and thermophysical properties of churro

Nutrient	Amount(%)	Property	Value
Moisture	60.425	Thermal conductivity, *k*	0.497 W/m °C
Protein	5.252	Specific heat, c_p	3.038 kj/kg °C
Fat	0.101	Density, *p*	1173.014 kg/m^3
Ash	0.351	Thermal diffusivity ,α	1.394×10^{-7} m^2/s
Carbohydrate	33.871		

The greater the rate of water loss, the greater the amount extracted from the incoming energy. This will reduce the amount of energy available for the internal energy increase and consequently the effective heat transfer coefficient will decrease (Yıldız et al., 2007; Erim Köse and Dogan, 2018). These results were in agreement with the findings of Yıldız et al. (2007) for french fries, Erim Kose and Dogan (2018) for tulumba dough, and Franklin et al. (2014) for gulab jamun. On the other hand, the data obtained in the study contradict most of the other studies in the literature, which found that the convection heat transfer coefficient increased with the increase in frying oil temperature (Seruga and Budžaki, 2004; Neethu et al., 2016; Sandhu et al., 2016; Koerten et al., 2017;, Asefi and Roufegarinejad, 2021). This difference could be attributed to the heat transfer was not evaluated with mass transfer simultaneously, product shape and porosity were not actual dimensions of the food product (3-D), and different frying conditions used in these studies.
In addition, the changes in the h_e parameters were best represented by the first-order kinetic model with a good fit obtained by nonlinear regression (R^2=0.9576 and RMSE=0.082). The kinetic rate constant (k) was also calculated for all the frying parameters (Table 3). The negative k value (-0.0552) showed no strong frying temperature dependence, which greater values measured at increased with decreasing the frying temperature.

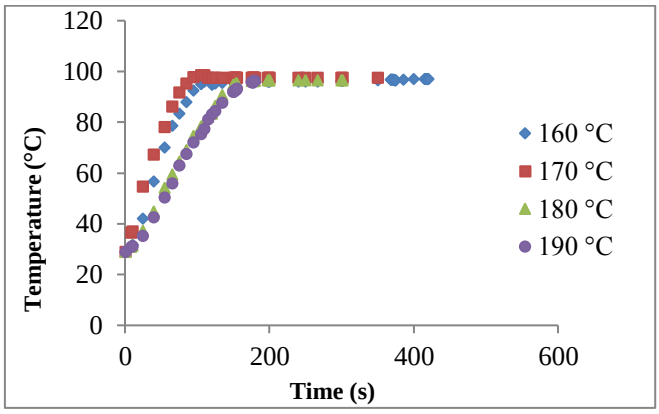

Figure 1. Experimental values for the temperature profiles of churro

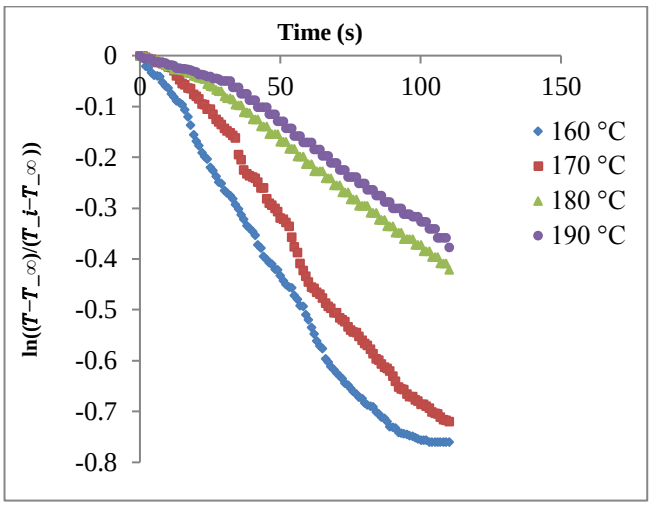

Figure 2. Dimensionless temperature ratio versus time

3.2. Kinetic Model of Mass Transfer Phenomena

Moisture loss is the major mass transfer phenomena during frying operation. The speed of frying operation is closely interested in the combination of frying time and temperature (Ngadi et al., 2006; Nasiri et al., 2011). The data of moisture contents were fitted to Fick's second law of diffusion and the first-order kinetic model, respectively, and the obtained model parameters are shown in Table 2.

Table 2. Biot numbers and frying parameters of churro

T (°C)	Biot number heat transfer	h_e W/ $m^{2o}C$	Biot number mass transfer	$k_e \times 10^{-5}$ m/s	$D_e \times 10^{-5}$ m^2/s
160	Bi_z=44.00 Bi_r=8.80	437.36	Bi_z=0.09 Bi_r=0.018	0.75	1.350
170	Bi_z=30.35 Bi_r=6.07	301.679	Bi_z=0.245 Bi_r=0.049	1.11	5.439
180	Bi_z=12.40 Bi_r=2.48	123.256	Bi_z=0.275 Bi_r=0.055	1.40	7.700
190	Bi_z=9.41 Bi_r=1.88	93.535	Bi_z=9.41 Bi_r=1.88	2.48	17.360

In order to obtain the model parameters, first, experimental studies were carried out. The experimental results of moisture contents at different frying temperatures have been reported in Fig 3. According to Figure 3, there was a fast decrease in all frying temperatures even in the first minutes due to the loss of water in surfaces of churros. The initial moisture content of churros was about 60.425 % (on wet basis, wb), and it was reduced to 35.43, 30.43, 28.73, and 27 % wb at temperatures 160, 170, 180, and 190 °C respectively. Similar results for different fried foods were reported by many researchers in the literature (Mariscal and Bouchon, 2008, Manjunatha et al., 2019, Adedeji et al., 2009, Zhang et al., 2020).

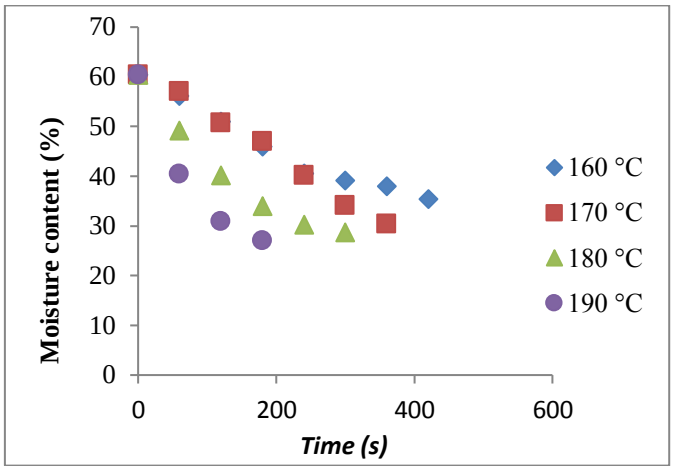

Figure 3. Moisture contents of churro for different frying temperatures

The kinetic data were obtained with the plots of dimensionless concentration ratio against frying times for different frying temperatures. D_e , k_e, and Biot numbers for mass transfer were determined from Figure 4.

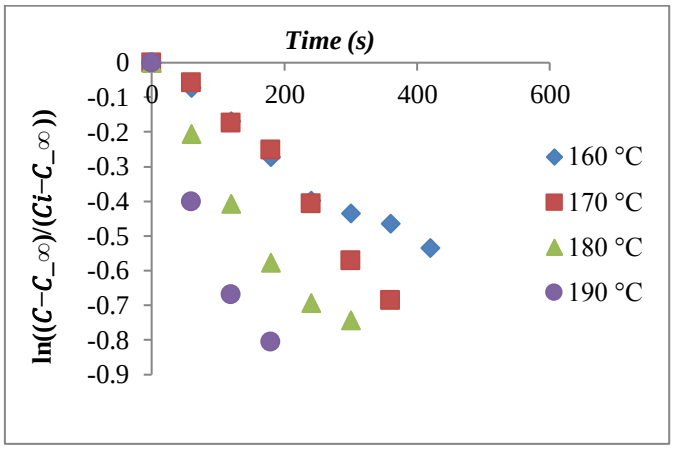

Figure 4. Dimensionless concentration ratio versus time

The D_e values of the frying processes at 160, 170, 180 and 190 °C were 0.75×10^{-5}, 1.11×10^{-5}, 1.40×10^{-5} ,2.48×10^{-5} m^2/s, respectively. This result revealed that the frying temperature had a favorable effect on the moisture diffusivities due to accelerating the moisture loss of the product. As deep-fat frying temperature increased, the moisture transfer coefficients also increased linearly from 1.35×10^{-5} to 17.36×10^{-5} m/s, indicating that maximum diffusion of moisture occurred at 190 °C. Similar results were reported on frying of the breaded fish nuggets (Zhang et al., 2020), krostula dough (Budzaki and Seruga, 2005), tulumba dough (Erim Köse and Dogan, 2008), green peas (Manjunatha et al., 2019), chicken nuggets (Adedeji et al., 2009). Torres-Gonzalez et al., (2018), reported that difusivity and mass transfer coefficients of Arepa Con Huevo increased with the increasing temperatures and its activation energy was calculated at 63.96 kJ/mol. Kinetic modeling for mass transfer of breaded and battered of fish

nuggets during deep-fat frying was performed by Fick's second law and first order kinetic model (Yuan et al.,2018). Catillo et al. (2021), determined the kinetics of moisture loss for chorizo during atmospheric and vacuum frying with Fick's second law for cylinder geometry. The diffusion coefficient increased from 3.50×10^{-8} to 4.28×10^{-8} with increasing temperature for vacuum frying.

The linear relationship between ln (C/C_0) and frying time showed that the changes of effective mass transfer coefficient and moisture diffusivity followed first-order kinetics with high R^2 (0.9417-0.9726) and low RMSE (0.048-0.059) values (Table 3). An Arrhenius equation was used for described of the relationship between the frying temperature and moisture diffusivity (Fig 5). As shown in Fig.5, the slope was equal to the ratio of the activation energy and the universal gas constant and so activation energy was calculated as 63.546 kJ/mol. This result is in agreement with those reported by Mondal and Dash (2017), who found similar activation energy was 67.163 kJ/mol of fried Chhena Jhili, which is a popular deep fat frying dessert in India.

Table 3. Kinetic parameters of frying coefficients

Parameter	Model	k (s^{-1})	R^2	RMSE
h_e	First-order kinetic	-0.0552±0.026	0.9576	0.0821
k_e	First-order kinetic	0.0801±0.104	0.9417	0.0480
D_e	First-order kinetic	0.0382±0.051	0.9726	0.0590

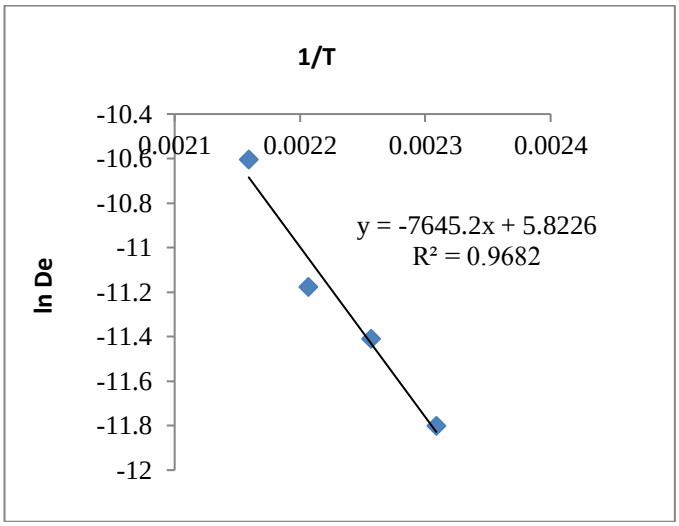

Figure 5. An Arrhenius relationship between temperature and moisture diffusivity

4. CONCLUSIONS

The frying process of churro was investigated for different time-temperature combinations on heat and mass transfer parameters in this study. An experimental study was conducted in the laboratory and the obtained data were analyzed kinetically. For this reason, analysis of the experimental data using the Fourier's and Fick's second law equations for churro geometry shape revealed that the heat transfer coefficient decreased, while the moisture diffusivity and moisture transfer coefficient increased linearly with frying temperature. The first-order kinetic models gave a good fit for all off frying parameters and the Arrhenius plot showed the temperature dependency of mass diffusion in the product. Thanks to this study, knowledge of heat and mass transfer parameters during the frying process accurately will assist in the equipment and process design of churros and similar fried foods. At the same time, thanks to the data obtained as a result of the study, the connection between the phases and the processing time can be established.

Ethics Committee Approval
N/A

Peer-review
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Author Contributions
All authors have read and agreed to the published version of manuscript.

Conflict of Interest
The authors have no conflicts of interest to declare.

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Mineral Exploration and Lithological Mapping Using Remote Sensing Approaches In Between Yazıhan-Hekimhan (Malatya) Turkey

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Abstract: The Remote Sensing processing analysis has become a directing and hopeful instrument for mineral Exploration and lithological mapping. Mineral exploration in general and bearing chromites associated with ultrabasic and basic rocks of the ophiolite complex in particular has been successfully carried out in recent years using Remote Sensing techniques. Yazıhan-Hekimhan (Malatya) region of the East Taurus mountain belt, ranks second in terms of iron mineralization in Turkey are accepted. The area is characterized by high grade iron ore deposits in use, development and exploration. Lithological mapping and chromite ore exploration of this area is challenging owing to difficult access (High Mountain 2243 m) using the traditional method of exploration. The main objective of this research is to evaluate the capacity of Landsat-8 OLI and Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER) satellite imagery to discriminate and detect the potential zone of chromites bearing mineralized in Malatya (Yazıhan). Several images processing techniques, Vegetation Mask, Band Ratio (BR), Band Ratio Color Composite (BRCC), Principal Component Analysis (PCA), Decorrelation Stretch, Minimum Noise Fraction and Supervised classification using Spectral Angle Mapper (SAM) exist in previous studies have been performed for lithological mapping. The obtained results show that BR, PCA and Decorrelation Stretch methods applied on NVIR-SWIR bands of Landsat-8 and ASTER were clearly discriminate the ophiolite rocks at a regional scale. In Addition, SAM classification was applied on a spectral signature of different ultrabasic and basic rocks extracted from ASTER data. The results are promising in identifying the potential zones of chromite ore mineralization zones within the ophiolite region. Thus, the techniques used in this research are suitable to detect or identify the high-potential chromite bearing areas in the ophiolite complex rocks using Landsat-8 OLI and ASTER data.

Keywords: Remote Sensing, Yazıhan - Hekimhan (Malatya) region, iron ore deposits, Vegetation Mask, Band Ratio Color Composite, Principal Component Analysis.

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1. INTRODUCTION

Recently, remote sensing is among the most powerful tools in geological research and mineral mapping (Rowan et al., 2006; Rajendran 2011; Pour et al. 2014 Zoheir et al., 2018; Noori et al., 2019; Pour et al., 2019a; Sekandari et al., 2020; Cardoso-Fernandes et al., 2020). However, it is possible to distinguish many alteration minerals and map them with more accuracy to understand the difference Furthermore, the development of new remote sensing methods, a few image processing methods have been discovered to map geological formations and they are also used in minerals research (Zhang et al., 2007; Hassan and Ramadan, 2015). Mineral exploration and geological mapping using remote sensing have been made effectively in arid, semi-arid and in other regions with little or high vegetation cover (Hellman and

Ramsey 2004; Pour et al., 2018a,b; Sheikhrahimi et al., 2019). several researchers have used spectral signatures (Rowan et al., 2006; Pour and Hashim, 2012; Pour et al., 2018b) for lithological mapping and mineral exploration based on remote sensing techniques. Minerals such as iron, hydroxyl, sulphate, dolomite, clay and carbonate have spectral characteristics in the Visible and Near-Infrared (VNIR) and Shortwave-Infrared (SWIR) domains, for the electromagnetic spectrum, whereas silicate minerals exhibit a spectral characteristic in the Thermal Infrared (TIR) region (Nimoyima 2005; Rowan et al., 2006).

Hydrothermal mineral mapping; which are most common indicators of mineralization is basic to reconnaissance and play an essential role in mineral exploration (Abdelsalam et al., 2000; Crósta et al. 2003; Loughlin 1991; Gabr et al. 2010; Kusky and Ramadan, 2002; Zhang et al., 2007; Madani, 2009; Gabr et al., 2015; Pour et al. 2018c, 2019a, b; Bolouki et al., 2020). The mineral assemblage found in the host rocks by Abrams et al. (1983) contained at least one mineral with diagnostic spectral absorption characteristics according to their study. Lithology describes the physical characteristics of rock units visible in the field, such as color, texture, grain size or composition. Lithological maps include the spatial distribution of rock units on the surface. Digital database produced from surface lithology provides support for the determination of land features such as geological mapping, mineral exploration and environmental characterization (Laake and Insley 2007; Laake, 2011, Rajendran et al., 2017, Özkan et al., 2017; Traore et al., 2020 a, b). Satellite images provide advantages in interpreting lithology the surface and near-surface structures (Laake and Insley 2007; Laake, 2011).

This study uses Aster and Landsat images of 174-033 rows and columns . The most important advantages of these images are that the images of the satellites are provided free of charge, they offer multi-band images to the users, they are suitable for medium-scale geological map production in spatial resolution, and they allow studies that cover large areas. This study aimed to investigate the use of geometric and radiometric corrected images in defining rock types and making maps for different lithological units within the scope of this study. In this context; Vegetation Mask, Band Ratio (BR), Band Ratio Color Composite (BRCC), Principal Component Analysis (PCA) analysis has been made in an area of approximately 1415 km^2 between Yazıhan and Hekimhan districts of Malatya province (Fig. 1).

Figure 1. Map of Malatya located in the Turkey.

2.GEOLOGICAL SETTING

According to the study and examined in the chapter, two different second rows of transgressive stages can be mentioned. First state; In the late Maastrichtian, with the emplacement of the Hocalıkova ophiolite in the region, the encrustation increased, the advancing region increased and some parts of the marine basin turned into land. In this phase, a basin was opened behind the Yuksekova- Baskil arc (ensialic) in the Late Campanian due to the effect of the stress forces; this basin matured in the Late Maastrichtian period and the basin closed in the Middle Eocene, and became terrestrial in the Upper Eocene period.) the first transgressive phase has been the most beautiful. Towards the end of the closure phase of the basin, during the Oligocene period, the region has completely transformed into a terrestrial environment and includes pebbles of Stork volcanics at its base. At the end of the Oligocene, the second period transgressive phase started and the region became the place where lake environments are located in the region. The impact of the Malatya Fault has been observed on young sediments. Soft-deformation structures were developed in the Late Miocene and Pliocene units. Depressions and uplift areas of the Malatya Fault have developed due to transtensional and transpressional movements. Volcanic rocks outcropping around Yazihan (Malatya) are represented by basalt and basalts with olivine. Volcanics show microlithic, flow, hypocrystalline porphyritic and texture, and geochemically alkaline and subalkali. In the rare earth element (REE) diagram of volcanic rocks normalized to the primary mantle, it is seen that they present a significant enrichment of light rare earth elements (LREE). In the spider diagram normalized to the primary mantle, they present enrichment with lithophile elements (LILE) with coarse cations such as Rb, Ba and K and consumption in terms of high valence cations (HFS). In the tectonomagmatic environment separation diagrams based on trace elements stable against alteration, it is seen that volcanic rocks fall in the direction of intracontinental basalt (Fig. 2).

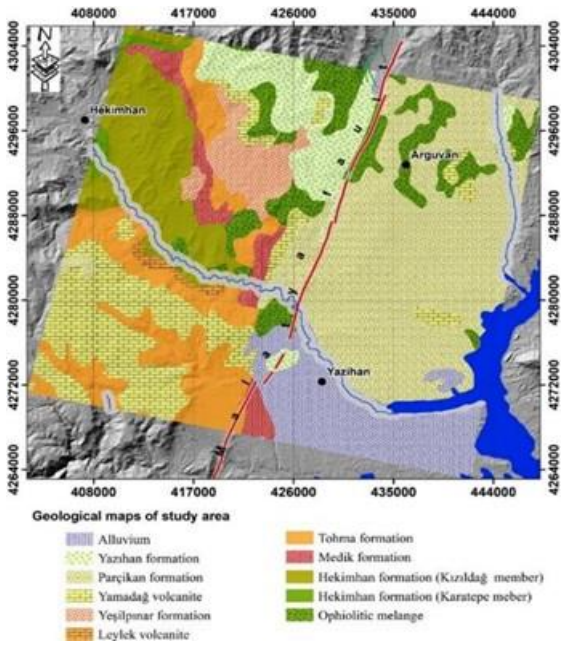

Figure 2. lithology map of the study area (Akbaş et al., 2002; Sevimli, 2009)

3.MATERIAL AND METHOD

3.1. Material and Preprocessing

Used in this study Visible near infrared and short-INFRAROUGE (VNR-SWIR) bands of Landsat-8 OLI and ASTER (Fig.3) the satellite satellite image of the US Geological Service (USGS) it is available as made of geometric and radiometric correction from the website. This image is in the Universal Transverse Mercator (UTM) coordinate system zone 37 and WGS84 datum. Satellite images processing, interpretation and data visualization processes, while field studies include controlling lithological units and contact boundaries detected by satellite images in the field. The contact relations, structural and textural properties of rocks belonging to different lithologies includes the comparison of the results obtained with the geological map and satellite images. The Aster image plays an important role in lithological discrimination. Table 4 gives a clear idea of the use of the Aster bands to explore mineral substances. The ASTER image was acquired in August 2001 during the dry period, with minimal green vegetation coverage These images correspond to images corrected radiometrically and geometrically. ASTER is composed of 14 strategically distributed spectral bands. The spatial resolution varies according to the wavelength, 15 m in the VNIR region (3 bands), 30 m in the SWIR region (6 bands), and 90 m for the three bands in the TIR region (Abrams and Hook, 2002) (Fig. 4).

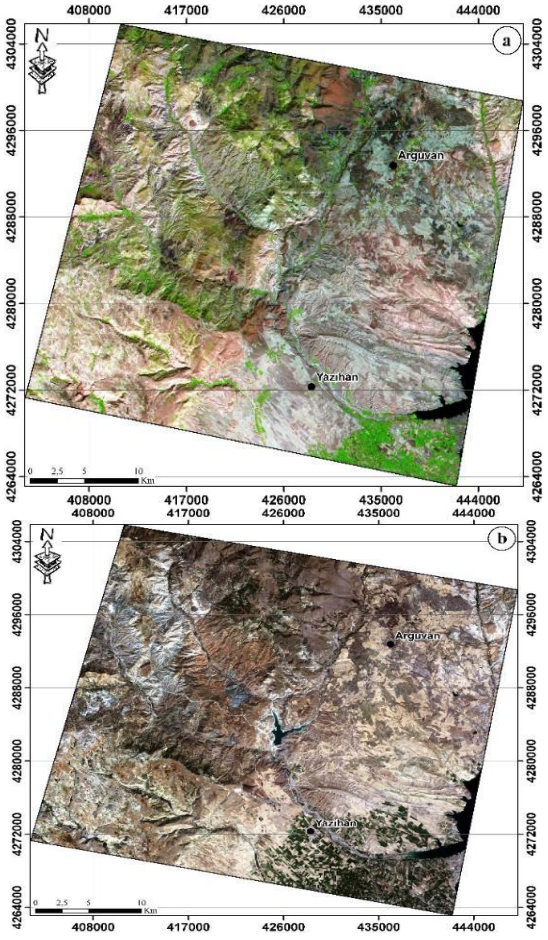

Figure 3. Aster (a) and Landsat-8 OLI (b) data of the study area.

Cross-talk correction does not apply in this research because the ASTER L1T SWIR bands had already been processed this correction. The atmospheric correction using Fast Line-of-sight Atmospheric Analysis of Spectral Hypercubes (FLAASH) algorithm was performed Aster image's VNIR and SWIR bands.

The variance of water vapor associated with different climate models is an important problem for the same bands of satellite data such as band 8 and band 9 of SWIR-ASTER image (Hewson, et al., 2005). Atmospheric correction reduces the influence of these factors (Moore et al., 2008, Pour et al., 2014).

This correction process requires the luminance image and therefore generates a reflectance corrected image. These effects were compensated for using the FLAASH tool of the ENVI software which uses the radiative transfer model M0DTRAN4.

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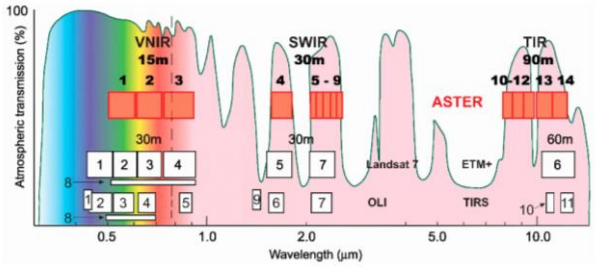


Figure 4. The spectral range and spatial resolution comparison for Landsat-8 (OLI), Landsat 7 (ETM +) and ASTER from USGS documents.

3.2. Methods

3.2.1. Band Raion Color Composite (BRCC)

Band rationing is an image enhancement analysis method in which new pixel values are obtained by dividing the gray color value of a pixel in one band by the value of the same pixel in another band or by applying other mathematical operations (Khan et al., 2007). In addition, by using this image enhancement method, the spectral differences between the bands are enriched and the effect of the terrain roughness on the images is reduced (Gupta et al.2005). The enriched images obtained from band proportions may not give the same results and interpretability in different geological and mineralogical environments and different topographies (Van der Wielen et al., 2004). Therefore, for this study, RGB (Red-Green-Blue) color composites of enriched bands obtained as a result of band proportioning applied to Landsat-8 OLI and ASTER bands were used to separate rock groups from each other and provide areal data. The steps of the working method are presented in Fig. 5.

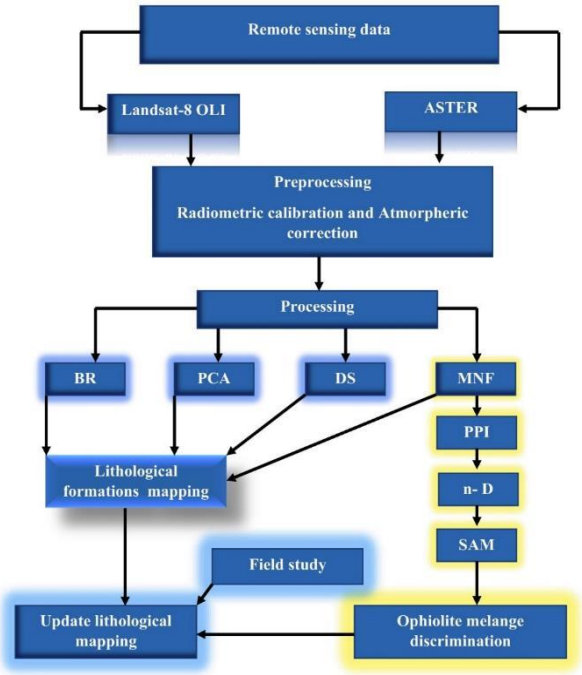


Figure 5. flowchart in this study.

3.2.2. Principal component analysis (PCA)

The principal component analysis is an effective technique for emphasizing a multispectral image for geological interpretation. Principal Component Analysis (PCA) is a mathematical processing method that minimizes information redundancy on different bands by translations and rotations. New axes are thus obtained and the different bands are "decorrelated" in the new reference marks established along these axes. The spatial resolutions of the different bands having been previously homogenized. The PCA was applied on nine bands of ASTER and Sven bands of Landsat-8 OLI in order to discriminate the lithological formations in this research.

3.2.3. Decorrelated stretching

Decorrelated stretching is a method used to interpret thermal infrared spectral data and enhances emissivity values between bands (Hook, et al., 2005). In this same procedure the effects of fringes between images are also eliminated (Guillespi, et al, 1987). Decorrelated stretching was used to determine the distribution of lithologies based on the increase in variations between spectral signature of bands related to differents lithogical formation and particullary in ophiolite melange rocks of the study area.

3.2.4. Supervised Classification

The following procedure replaces the methodology for using pure spectral signatures from fieldwork. This procedure includes extracting pure spectral signatures from ASTER bands in order to obtain a reference for ophiolite rocks of the study area. The first step was to calculate an Minimum Noise Fraction (MNF) on the emissivity bands to reduce noise. MNF is a transformation used to determine the inherent dimensionality of spectral data and to isolate noise (Boardman and Kruse, 1994). The second step was to establish the PPI purity index from the results of the MNF in order to isolate the pure members, and their respective spectral signatures were performed using Spectral Angle Mapper (SVM).

The SAM supervised classification method was applied in this study to map the lithology formations. SAM determines the similarity between a reference (r) and the unknown spectrum of the pixel (t) by calculating the vector angle (a) between the two in n-dimensions (Kruse, et al, 1993). SAM made it possible to determine the similarity between the spectral signature of emissivity of the lithologies extracted by the PPI purity index (reference spectrum) and the pixels of the rest of the image (unknown spectra) from the calculation of the angles between the 2 vectors.

4. RESULTS AND DISCUSSION

4.1. Vegetation Mask

The presence of vegetation and water masks a significant percentage of our study area although we have an acquisition date where water and vegetation are at their weakest cover. To eliminate the vegetation, we produced a mask from using Normalized Difference Vegetation Index (NDVI) and using the ratio of High reflectance (NIR) and high absorption (Red) spectrum carhacterized by the bands 4 and 5 for Landsat-8 OLI, and bands 2 and 3 for ASTER image equation 1. However, To mask the water body, the Normalized Difference Water Index (NDWI). This index uses the near infrared (NIR) and the Short-Wave infrared (SWIR) bands correspond to the bands 5 and 6 for Landsat-8 OLI , and bands 3 and 4 for ASTER image. NDWI can be calculated by following equation 2. Figure 5 shows the water and vegetation masks obtained.

NDVI = (NIR – Red) / (NIR + Red)
NDWI = (NIR – SWIR) / (NIR + SWIR)

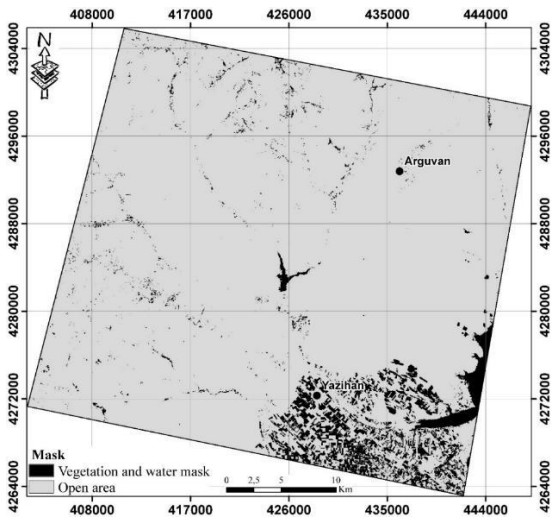
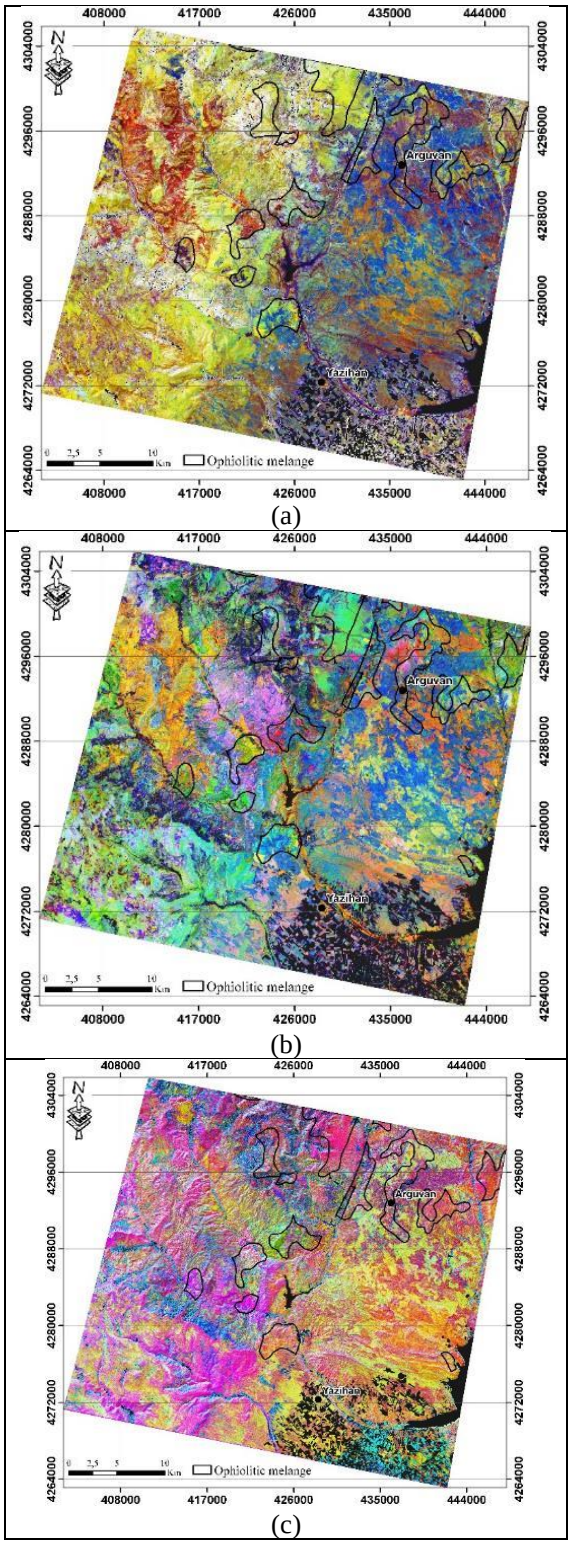


Figure 6. Vegetation and water mask in the study area.

4.2. BRCC

Previous studies have shown the ability of Landsat and ASTER VNIR-SWIR bands to map lithology formations and ophiolitic complexes (Amer et al., 2010; Rajendran et al., 2011; Ramadan 2013; Özkan et al., 2017; Traore et al.,

2020a). Based on the characteristics of the spectral bands of Landsat-8 OLI and ASTER image data, specialized band ratios of (7/4, 6/3, 5/7) and (7/6, 6/5 and 4/2) in RGB (Red, Green and blue) of the Landsat-8 OLI satellite image, and ((2 + 4) / 3, (5 + 7) / 6, (7 + 9) / 8), and a new band ratio (3/5, 4/6, 7/6) in RGB from ASTER image acquired in the dry season, and after different corrections applied in these data, allowed the map the geological formation and distinguished especially the ophiolite rocks of the study area. Its notes that the boundaries of the ophiolitic complexes have been clearly distinguished using these colored BRCC (Fig. 7).



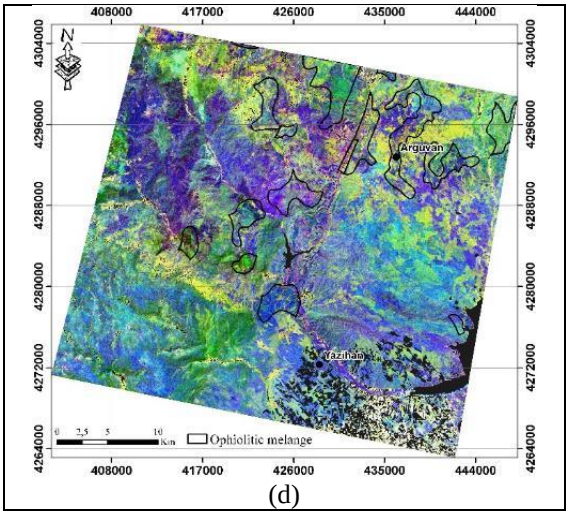


Figure 7. Band ratio (a) (7/4, 6/3, 5/7) and (b) (7/6, 6/5 and 4/2) in RGB from The Landsat-8 OLI, and (c) ((2 + 4) / 3, (5 + 7) / 6, (7 + 9) / 8) and (d) new 3/5 4/ 6 7/6 in RGB from ASTER image.

4.3.PCA

In order to differentiate the ophiolitic complex from other lithological formations in our study area, we used the PCA analysis technique. This technique has been used successfully in recent years by several researchers for the exploration of weathering minerals but especially for the mapping of geological formations (Crosta, et al., 2003, Pour et al., 2014, Rajendran et al., 2017, Özkan et al., 2017, Traore et al., 2020 a, b). The visible and near infrared bands of Landsat-8 OlI and ASTER data were selected and a PCA analysis was applied. The statistics related to the loading of each of the bands for each of the components (eigenvectors) were analyzed. The results allowed us to make a good lithological discrimination between the formations in our study region. Consequently, the band (PC1, PC2, PC3) and (PC1, PC3, PC5) in RGB for Landsat-8 OLI, and the band (PC1, PC5, PC6) and (PC4, PC2, PC1) in RGB for ASTER data were selected for good discrimination of ophiolites rocks among the other lithological formation in the study area (Figure 8). The results of visual image interpretation show that, the high ophiolites rocks are discriminated by yellow color in (PC1, PC2, PC3) and light pink-yellow color in (PC1, PC3, PC5) from Lansat-8 OLI (Fig. 8 a, b). According to the result from ASTER, the potential ophiolites rocks are distinguished by pink and yellow color in (PC1, PC5, PC6), and orange-yellow and light green-yellow color in (PC4, PC2, PC1) (Fig. 8 c,d).

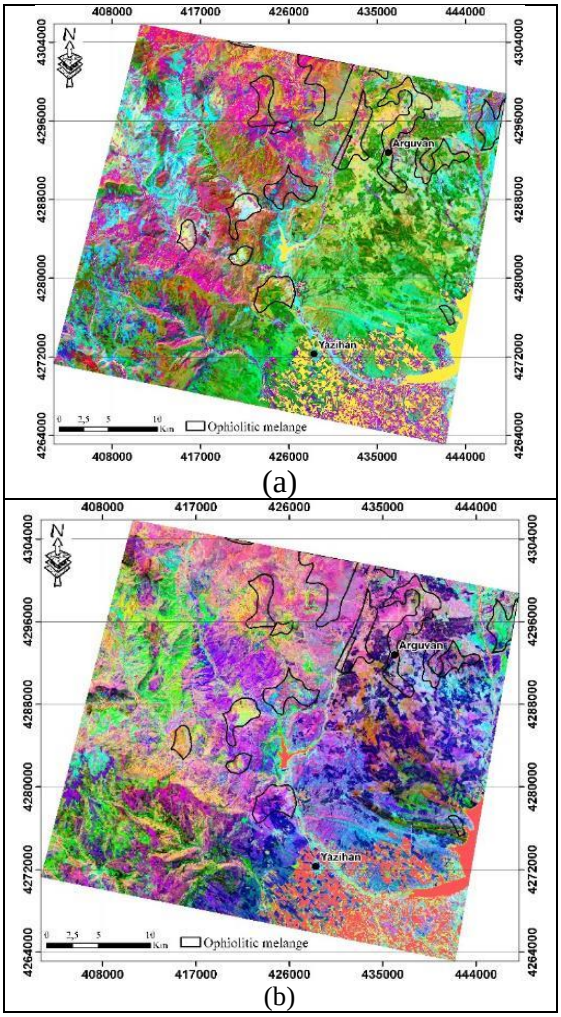


Figure 8. RGB color composites of (a) PCA (1, 2,3), (b) PCA (1, 3, 5) extracted from Landsat-8 OLI image, and RGB color composites of (c) PCA (1, 5,6) and (d) PCA (4, 2, 1) from ASTER data of the study area.

4.4. Decorrelation stretching

Recently, decorrelated stretching is one of the most important methods used in remote sensing research to map the lithology formations. Usually, the data from the spectral VNIR and SWIR bands are used and analyses the effect of enhancing the emissivity values between the bands (Hook, et al., 2005). In this same procedure, the impact of fringes between images are also eliminated (Guillespi et al, 1987). In this study, decorrelated stretching was used only from VNIR-SWIR bands of Landsat-8 OLI to map the distribution of lithological rocks. Based on the spectral signature of rocks in the study area, the (2, 4, 6) and (7, 6, 5) bands were selected (Hubbard et al., 2007; Rajendran et al., 2011). The results of this method are presented in figure 9. to better appreciate the results and distinguish the ophiolite rocks from other lithology formations, the spatial observation of the results obtained was superimposed on the contour of the ophiolitic complexes of the reference geological mapping of our area. The dark blue and red colored areas correspond to ophiolitic rocks when using bands (2, 4 and 6) in RGB. On the other hand, these same formations appear in light green and yellow color in the bands (7, 6, 5) in RGB (Fig. 9).

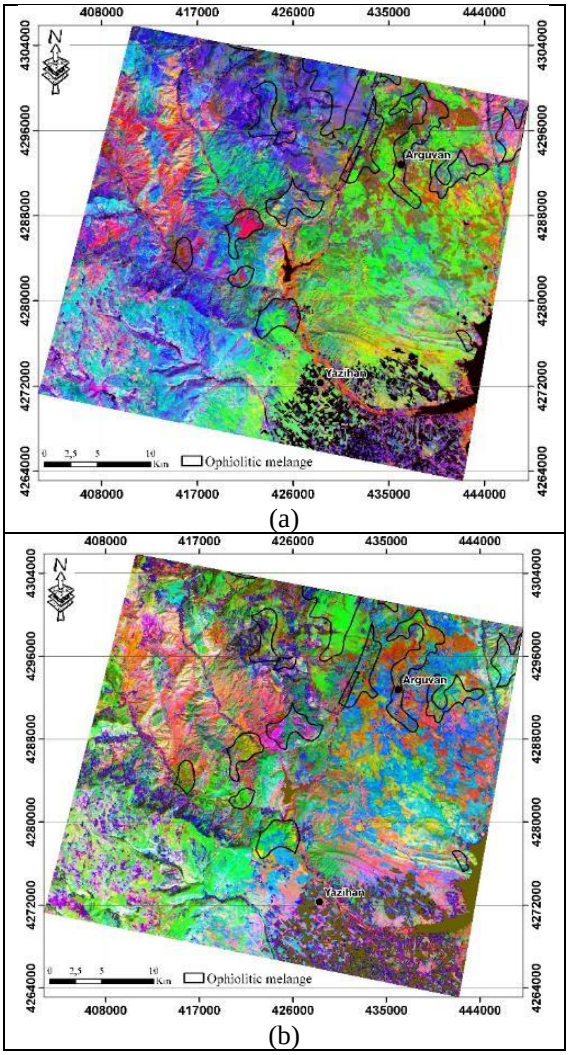


Figure 9. Landsat-8 OLI RGB (a) (2, 4, 6) and (b) (7, 6, 5) bands decorrelated image of study area.

4.5. Minimum Noise Fraction (MNF)

The ability to map rocks using multispectral satellite data is enhanced by the different bands, which are sensitive to differences in rock mineralogy. The calculation of MNF is likely to provide the maximum lithological information. The importance to use the MNF technique is to distinguish all possible color combinations of Landsat-8 OLI and ASTER data bands for lithological mapping. The MNF technique was applied to VNIR-SWIR Landsat-8 OLI and ASTER bands. Table 1 shows the statistical results for MNF components of the seven bands of Landsat-8 OLI and nine band of ASTER SWIR bands. The MNF shown in RGB allowed us to distinguish the colors that correspond to the different rocks in our study area (figure 10). The result of band 1,2 and 3, and 4,3 and 2 in RGB of Landsat-8 OLI showed that ophiolite melange rocks appear as a melange of color characterized by green to yellow and dark blue color in North of the study area (Fig. 10 a,b). However, the result of band 1, 2 and 3, and 4,2 and 3 in RGB of ASTER data showed that ophiolite complexes rock manifest as dark pink and light-yellow color in this research (Fig. 10 c,d).

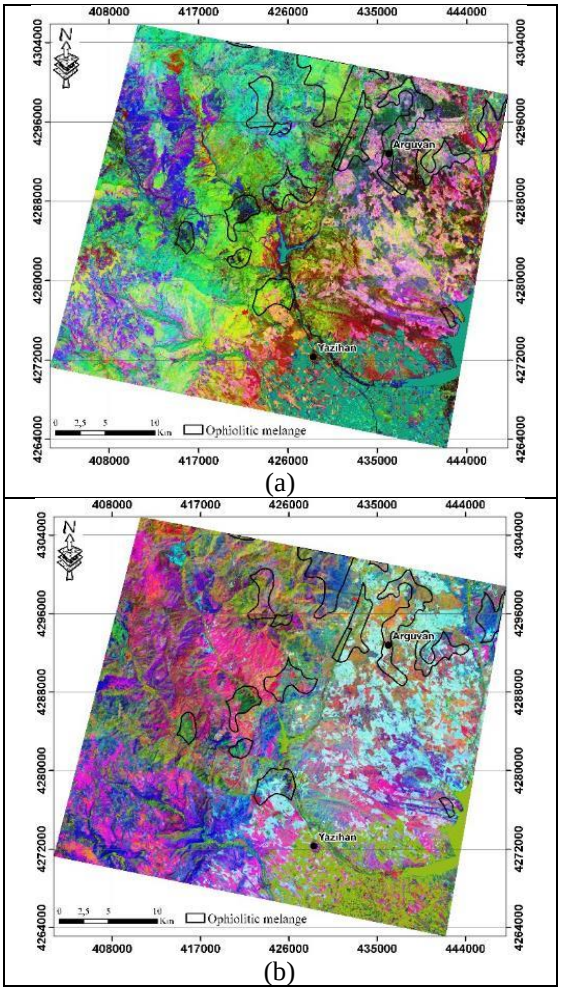


Figure 10. RGB color composites of (a) MNF (1, 2,3), (b) MNF (4, 2, 1) extracted from Landsat-8 OLI image, and RGB color composites of (c) MNF (1, 2,3) and (d) MNF (4, 3, 2) from ASTER data.

4.6.Supervised classification

MNF is a transformation used to determine spectral data's inherent dimensionality and isolate noise (Boardman and Kruse, 1994). The second step was to establish the PPI purity index from the results of the MNF to isolate the pure members, the Identification of Endmembers was applied, their respective spectral signatures of ophilites complex rocks (Fig.11b) were selected and finally, themap distribution and abundance were performed using Spectral Angle Mapper (SVM) (Fig.11 a).

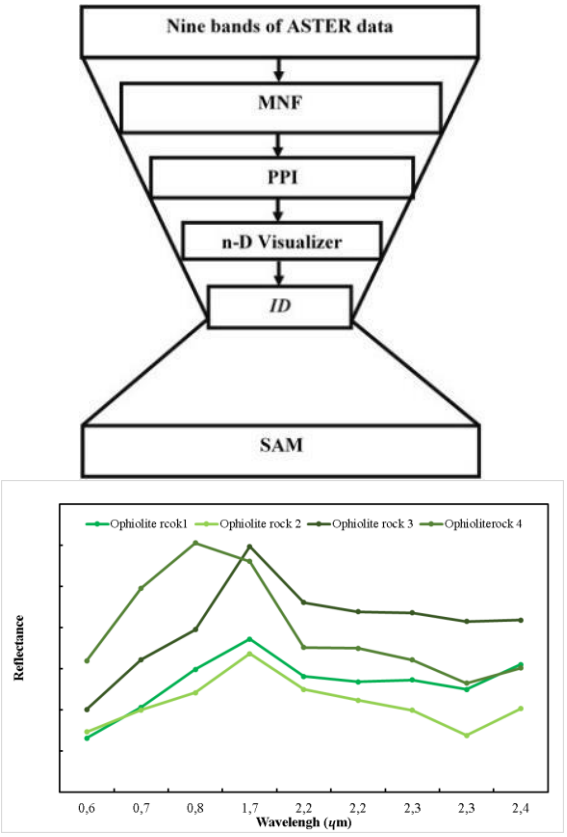


Figure 11. (a) Standardized ASTER analysis scheme for ophiolite mapping using the spectral hourglass approach and (b) the selected n-D classes (end-member spectra) extracted from ASTER data of ophiolite melange rock.

The analysis of the results of Decorrelation stretching (DS) and PCA allows us to makes the interpretations and comparation of map ophiolite melange rock with the available geological of study area. The result of DS such as (7, 6, 5) (Fig. 12a) display to the RGB color showed Ophiolite melange rocks were clearly in light to maroon color. However, the ophiolites melange rocks were also differentiatedin light green-yellow color using PCA (4, 2, 1) in RGB (Fig. 12 b). Based on the result of DS and PCA bands, It is important to note.

The result of this classification is illustrated in figure 12 c. The reference vector is constructed to perform the SAM classification.The angle between the reference vector and the pixel vector is calculated to compare with the determined threshold angle value. In total, the pure spectral signatures were determined to correspond to the lithologhy of the ophiolitic complex rocks. These signatures were compared to the USGS spectral library to associate a lithology name with the calculated signature. The ophiolitic rocks were mapped with the SAM method using the references obtained from the previous step. A visual representation of the SAM results is shown in Figure 12c where these rocks have been identified in green color. The comparison of the result of this classification with an old delineation of the ophiolitic rocks of our study area, showed an excellent correspondence.

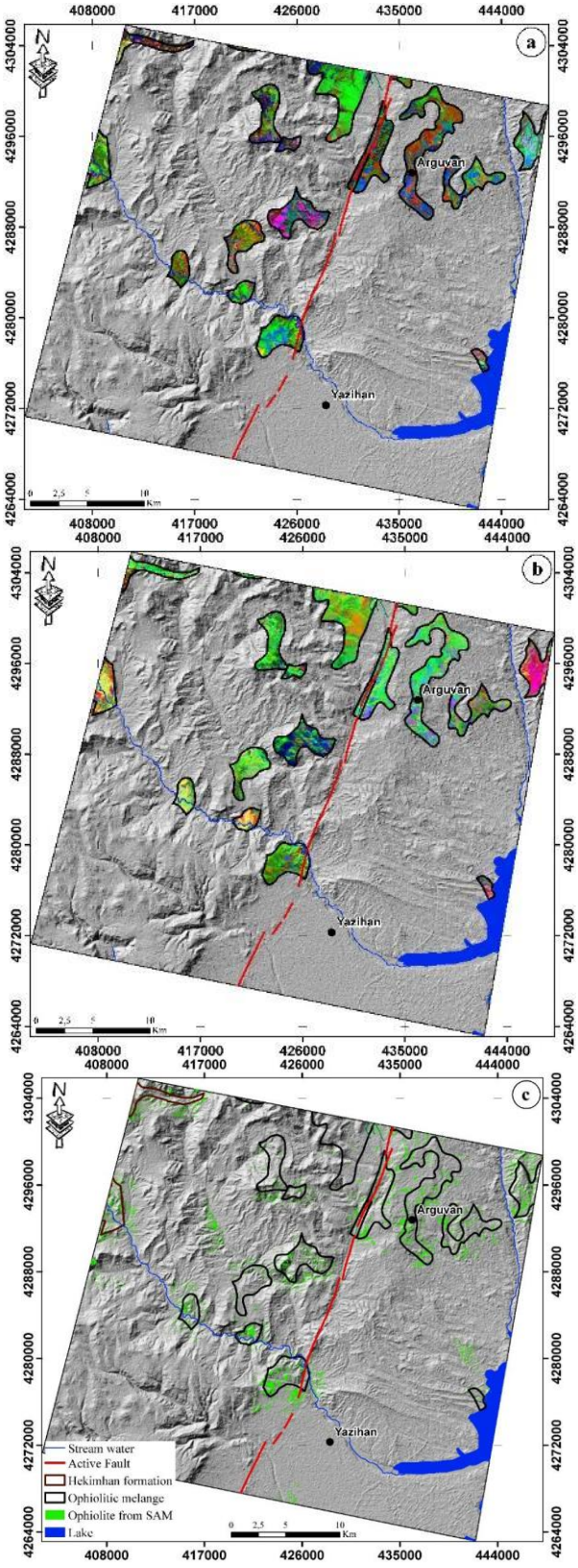


Figure 12. Ophiolite melange zone extracted from (a) Decorrrelated stretch (7, 6, 5), (b) PCA (4, 2, 1) in RGB and Ophiolite mapping using SAM in the study area.

5. FIELD INVESTIGATION

The ophiolite unit, which crops out in large areas in the study area, consists of dunite, harzburgite, pyroxenite, gabbro and spilite. The images of the formations that are common in the region in the field studies carried out in the study area are shown in Figure 13. Most of the ultramaphic and mafic rocks are serpentinized in the ophiolite regions. Volcanites are generally of Spilitic type and red pelagic deposits are located in the uppermost levels of the ophiolite. Sediments; consists mainly of calcitic dolostone, radiolarite and mudstone. It is easily distinguished from other rocks of ophiolite with its red, brown and pink colors. (Fig. 13 a, b and c).

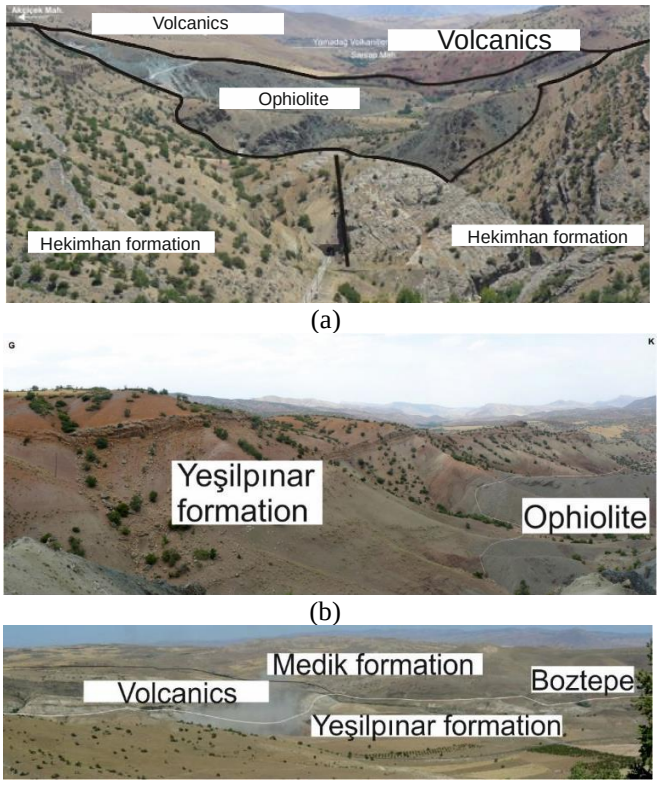


Figure 13. Field photographs of the study area.

6. CONCLUSION

The study illustrates the capability and how RS and GIS techniques can be used to correlate the previous lithological and mineral, between the integration multispectral data (ASTER, Landsat-8 OLI and ASTER, fieldwork, Geochemistry and Petrographic analysis. In this study, ASTER and Landsat-8 OLI satellite image data for Malatya were processed and tested for detected the potential zones for chromite bearing mineral and updating lithological map of the study area. The special distribution of ophiolite melange rocks were mapped using band combinations, BR, PCA, DS, MNF and SAM image processing techniques. The result indicates that, the different methods performed on spectral bands (VNIR-SWIR) of Landsat-8 OLI and ASTER data demonstrates successful in discrimination of ophiolite melange rocks and depiction of the zone of chromite ore bearing zone within ophiolites in this region. The results obtained from SAM showed high ability of this techniques for in ophiolite differentiations. It shows also a good virtual correlation with the previous geological map of the study area. The statistical results derived from virtual verification, the previous geological map and geological formations in the field shows that the overall accuracy and Kappa Coefficient 82.51% and 0.86 for SAM respectively. This research suggests that, the discrimination of ophiolitic complexes and exploration of potential of chromite ore deposits zone can be

performed using the processing techniques applied in this study.

Ethics Committee Approval
N/A

Peer-review
Externally peer-reviewed.

Author Contributions
Conceptualization, Investigation, Material and Methodology, Supervision, Visualization, Writing-Original Draft, Writing-review & Editing: İ.G. Other: The author has read and agreed to the published version of manuscript.

Conflict of Interest
The authors have no conflicts of interest to declare.

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Identification and Validation of Reference Genes for RT-qPCR Normalization in *Nauphoeta cinerea* (Olivier, 1789) (Blattodea, *Blaberidae*)

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Abstract: Quantitative RT-PCR (q-RT-PCR) is a powerful tool that allows large-scale analysis of very small changes in gene expression. For the calculation of gene expression, such as the delta-delta Ct method, different PCR primer efficiencies (E) may affect the result, as PCR primer yields are assumed to be comparable for the gene of interest and housekeeping gene. Therefore, identification of a suitable reference gene for data normalization is an important step in the development of qPCR assays. Furthermore, accurate and reliable results depend on the use of stable reference genes for normalization. The aim of the current study is the identification and validation of a set of six housekeeping genes (*GADPH, RPS18, α-TUB, EF1α, ArgK,* and *ACTB*) in cockroach species *Nauphoeta cinerea* adults using five different algorithms (ΔCt method, Bestkeeper, geNorm, Normfinder and RefFinder) to evaluate the stability of selected reference genes expression. Our results show that *α-Tub* use provides accurate normalization of gene expression levels in *N. cinerea* adults. In addition, since the *GADPH* is selected as the second most stable reference gene, *GADPH* can be also used for transcript analysis *N. cinerea* adults. Our study also showed that *ACTB (β-actin)* should not be used for normalizing transcript levels when examining *N. cinerea* adults. Additionally, validation studies for reference genes in cockroaches are very few (only one) in the literature. Therefore, the results highlight the need for validation of reference genes under biotic and abiotic conditions in q-RT-PCR studies in cockroaches.

Keywords: RT-qPCR, reference genes, validation, normalization, *Nauphoeta cinerea*.

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1. INTRODUCTION

Cockroaches are a highly diverse insect with ~4000 described species (Velez et al., 2006), most of which live in terrestrial habitats and have good adaptations to survive in extreme conditions (Bohn et al., 2010). A Blaberidae, ovoviviparous cockroach, the *Nauphoeta cinerea* (Olivier) is known as the gray cockroach and is the only representative of its genus. In addition, *N. cinerea* is a model species for sexual selection (Bouchebti et al., 2016) and correlations between metabolic rate and fitness studies (Schimpf et al., 2012). Because cockroaches can live in habitats with varying amounts of toxic substances such as insecticides, environmental pollutants, microbial toxins and other xenobiotics, they can be a good experimental model for studying the stress response and detoxification abilities (Bell et al., 2007; Zhang et al., 2016). Among cockroaches, the lobster cockroach *N. cinerea* has been used as an experimental model for toxicology (Adedara et al., 2016; Adedara et al., 2015; Rodrigues et al., 2013) and has been proposed to be a valid alternative model for basic toxicological studies. *N. cinerea* is more advantageous to other cockroach species in scientific studies because it is easy to care for and does not fly.

Gene expression studies are essential for molecular biology research. Knowledge about gene expression helps us better understand its regulation and functions. Massive sequence data in the form of transcriptomes and genomes of several organisms can be used to understand the transcription of genes (Heid et al., 1996). However, these analyses became more accurate and robust after the development of reverse transcriptase quantitative PCR (RT-qPCR), a gene expression quantification method. This method is highly sensitive, reproducible, and accurate enough to detect even minute changes that are not often detected. RT-qPCR data is influenced by many factors, including the quality and quantity of starting material, RNA extraction, cDNA synthesis, and other laboratory procedures. Even pipetting errors and reverse transcription efficiency can significantly affect Ct values (Bustin et al., 2005; Yeung et al., 2004).

The use of reference genes with stable expression as internal control has become one of the most common methods of data normalization (Feuer et al., 2015; Huggett et al., 2005; Pabinger et al., 2014). Normalization becomes necessary when one wants to express results as relative quantities by application of the well-known $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) or modified versions this method (Hellemans et al., 2007; Pfaffl, 2001; Schmittgen and Livak, 2008; Vandesompele et al., 2002). In order to analyze the results accurately, it is necessary to calculate the PCR primer yields. For the calculation of gene expression, such as the delta-delta Ct method, different PCR primer efficiencies (E) may affect the result, as PCR primer yields are assumed to be comparable for the gene of interest and housekeeping gene. Therefore, identification of a suitable reference gene for data normalization is an important step in the development of qPCR assays. It is very important that the reference gene is not affected by experimental conditions like changing in developmental stages, tissues. Since correct normalization is considered an important part of gene expression analysis, it has been recommended to verify the expression stability of the reference gene prior to each RT-qPCR experiment. (Huggett et al., 2005; Vandesompele et al., 2002). Therefore, normalization has been a precondition in gene expression studies as it limits the variability by comparing target gene expression with housekeeping genes (HKGs). Normalization recognizes that expression of HKGs is stable across various biotic and abiotic stresses and treatments. Recent studies highlight the need to identify a condition-specific reference gene for accurate measurements of gene expression (Ponton et al., 2011b). Furthermore, it is also not recommended to use a single reference gene in gene expression studies (Chandna et al., 2012; Koramutla et al., 2016), as the use of a single reference gene may produce up to 20-fold errors in expression data (Vandesompele et al., 2002). In most expression studies, actin is chosen as a universal HKG (Li et al., 2010), or HKGs approved for particular systems are administered directly without proper verification of their stability in that particular system. Stability of gene expression is defined as the smallest variation in successive levels of stability of the samples analyzed. Therefore, different software programs have been developed to identify many of these stable genes (Galiveti et al., 2010). These programs include GeNorm (Vandesompele et al., 2002), BestKeeper (Pfaffl et al., 2004), NormFinder (Andersen et al., 2004), ΔCt Method (Silver et al., 2006), and RefFinder (http://www.leonxie.com/referencegene.php).

Molecular tools, particularly RT-qPCR, have been frequently used in insect systems to quantify differences in gene expression. RT-qPCR has speed up research progress in biomedicine and has gained equal importance in entomology Gene expression analysis is increasingly important in the field of insect molecular biology as it can be used to examine changes in gene expression between insect developmental stages, tissues, and other samples from various assays. Studies to evaluate the selection and validity of reference genes in various biotic and abiotic conditions in insects are summarized in reviews by Lü et al. and Shakkel et al. (Lü et al., 2018; Shakeel et al., 2018). In studies in the literature, different types of housekeeping genes, including traditional and novel genes, were selected for gene expression stability analysis in different insect species. Although RT-qPCR is widely used for the detection of gene expression in insects, there is not yet a suitable HKG and stable gene quantification system for *N. cinerea*. Our current study aims to identify suitable reference genes and evaluate their expression stability in *N. cinerea* before they are used as internal controls in functional genomic studies of six reference genes commonly used to normalize qRT-PCR data in *N. cinerea*.

2. MATERIAL AND METHODS

2.1. Sample and ethics statement

The *Nauphoeta cinerea* (Blattodea, Blaberidae) species included in the study were purchased commercially in Antalya/Turkey (Antalya Çekirge, https://www.antalyacekirge.net/). Adults of *N. cinerea* (n=10) were transported to the laboratory, preserved in the RNA stabilization reagent RNAlater® (Qiagen, Cat. No: 76106) and stored at -20°C for use in further studies.

2.2. Total RNA extraction and cDNA synthesis

Total RNA was isolated using the commercially purchased GeneAll® Hybrid-R™ kit (GeneAll Biotechnology, Seoul, Korea) according to the manufacturer's recommendations. cDNA synthesis was performed using The GeneAll® HyperScript™ first chain synthesis kit (GeneAll Biotechnology, Seoul, Korea) to obtain the cDNA product containing 1500 ng/µL of RNA in accordance with the manufacturer's protocol.

2.3. Candidate reference genes selection

In the current study, six candidate housekeeping genes (*GADPH, RPS18, α-TUB, EF1α, ArgK* and *ACTB*) were selected among the most preferred reference genes in insects by taking the studies in the literature as reference. The specification information of each primer included in the study is given in Table 1. PCR amplification efficiencies (E) and correlation coefficients (R^2) were determined to validate the primers. Standard curves were generated using serial dilutions of cDNA (1, 1/5, 1/25, 1/125 and 1/625) for each primer pair.

2.4. Quantitative real-time PCR (RT-qPCR)

qPCR experiments were performed according to the previously reported protocol (Berk and Pektas, 2020). Briefly, a 5-fold dilution series of 1:625 from an undiluted cDNA of *N. cinerea* adult cDNA samples was analysed to determine the amplification specificity and efficiencies of each primer pair used in the qPCR analysis. For expression analysis of six candidate reference genes, all experimental samples for *N. cinerea* were analysed simultaneously in the same reverse transcription process.

RT-qPCR analyses in a 96-well plate (ABI-Type) using commercial qPCR Master Mix (iGreen, 2X, Biomatik, Canada) were performed using the StepOnePlusTM Real-Time PCR system (Applied Biosystems, USA). With a final volume of 20 µL, the reaction conditions are as follows: [10 µL Master Mix (2X), 0.7 µL forward and reverse primers (10 µM), 2 µL cDNA, and 6.6 µL nuclease-free water].

The RT-qPCR schedule is as follows: initial denaturation at 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C for 10 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 15 seconds. For melting curve analysis, it is as follows: a cycle of decomposition steps (58°C -1 minute- followed by 0.5°C up to 95°C for 10 seconds). A melting curve was composed at per PCR reaction final to confirm a single peak and remove the primer-dimer possibility and formation of non-specific product. Efficiency of PCR amplification (E) was determined in terms of the equalization: $E = (10^{[-1/slope]}-1) \times 100$.

2.5. Data analysis

The Cq values of six housekeeping genes were exanimated using the statistical analysis statistical software GraphPad Prisim 6.0 (GraphPad software, San Diego, C), and a boxplot of these Cq values was generated. The stability of these six housekeeping genes was determined by GeNorm (Vandesompele et al., 2002), BestKeeper (Pfaffl et al., 2004), NormFinder (Andersen et al., 2004), ΔCt (Silver et al., 2006) and the comprehensive web-based analysis tool integrating all four software algorithms (https://www.heartcure.com.au/reffinder/?type=reference). BestKeeper evaluates the coefficient of variance (CV) and standard deviation (SD) of the Cq values of each housekeeping gene, and the gene with the lowest CV and SD is defined as the most constant gene (Pfaffl et al., 2004). GeNorm reveals the mean expression stability value (M2) of each candidate (M value) to demonstrate expression stability. The gene with the lowest M2 value is determined as the most stable gene (Vandesompele et al., 2002). In NormFinder, expression stability (M1) is revealed by the Cq values obtained by RT-qPCR analysis of candidate genes and are ranked. The gene with the lowest M1 value is the most stable (Andersen et al., 2004). RefFinder appoints a relevant weight to each gene and evaluates the geometric mean of these weights to make an overall final ranking (https://www.heartcure.com.au/reffinder/?type=reference).

Table 1. Overview of the six candidate housekeeping genes evaluated in RT-qPCR analysis.

Symbol	Gene name	Description	Primer sequence (5'→3')	GenBank accession number	Length (bp)	R^2	Reference
ACTB	β-actin	Cytoskeleton	F: TCCATCATGAAGTGCGATGT R: CCACATCTGTTGGAATGTCG	NM_001172372	228	0.872	(Sang et al., 2015)
RPS18	Ribosomal protein S18	Ribonucleoprotein; Ribosomal protein; RNA-binding; rRNA-binding	F: TACACCTTTGATCGCTGTGAG R: GGCTCTGGTCATTCCAGATAAG	XM_045615265	108	0,914	(Yang et al., 2015c)
EF1α	Elongation factor 1α	Translation elongation factor activity; GTPase	F: ACCAGATTTGATGGCTTTGG R: CACCCAGAGGAGCTTCAGAC	XM_003705302	194	0,957	(Rodrigues et al., 2014)
ArgK	Arginine Kinase	Phosphotransferase activity	F: CTCGTGTGGTGCAACGAAGA R: GGTGGCTGAACGGGACTCT	NT_037436	130	0,972	(García-Reina et al., 2018)
GADPH	Glyceraldehyde-3-phosphate	Oxidoreductase in glycolysis & Gluconeogenesis	F: GCCAAGGTGATCCATGACAA R: GTCTTCTGAGTGGCAGTTGTAG	NC_007420	80	0,965	(Pan et al., 2015)
α- TUB	Alpha-tubulin	Microtubule	F: TCAAATGCGACCCACGTCAT R: GGCAATAGCCGCGTTGACAT	XM_970811	191	0,978	(Altincicek et al., 2008)

3. RESULTS

3.1. The quality of total RNA

In the current study, the A260/A280 ratio of total RNA from *N. cinerea* adults ranged from 1.90 to 2.12, while the A260/A230 ratio was found to be over 1.90. It shows that the product does not contain organic salts and protein contamination. The total RNA concentration was between 1218ng/uL and 2000ng/uL, and this value range was considered suitable for expression of the cDNA template.

3.2. Primer specificity and efficiency

All PCR amplification of each primer used in the present study was confirmed by the presence of a single peak in melting curve analyses and specific band of expected size based on 1.5% agarose gel electrophoresis (data not shown). Initial screening of six potential reference genes showed that all genes were expressed in *N. cinerea* adults. The primer efficiency (E) for each of the six candidate genes was calculated using the slope obtained when measuring fluorescence at 5-fold cDNA dilutions of a calibrator cDNA sample using the formula: $E=10^{1/-slope-1}$. All primers in the study were found to have a correlation coefficient (R^2) varying between 0.87 and 0.98. In addition, a primer efficiency value of between 98.6% and 110.05% was obtained, and the specificity of each primer was verified by the BLAST program (Table 1). No fluorescent signal amplification was detected in the negative control samples (−RT); this demonstrated that RNA extraction methods and DNase treatment procedures effectively removed genomic DNA from RNA samples.

3.3. Expression profiling of candidate reference genes

The expression profiles of all RT-qPCR products for all primers and both experiments are shown in Figure 1. Cycle threshold (Cq) values obtained amplifying the six putative housekeeping genes from *N cinerea* adult were plotted (Figure 1). Cq values for the six genes ranged from 21.29 to 36,67 in *N. cinerea*. *GADPH* showed the lowest Cq values in *N. cinerea* (24,72 ± 2,238, mean Cq ± std. dev.). Amplification of *α-TUB, RPS18, ArgK, EF1α* and *ACTB* showed mean Cqs of 28,96 ± 2,056, 30,36 ± 1,418, 31,78 ± 3,633, 31,96 ± 1,418 and 33,36 ± 4,081, respectively.

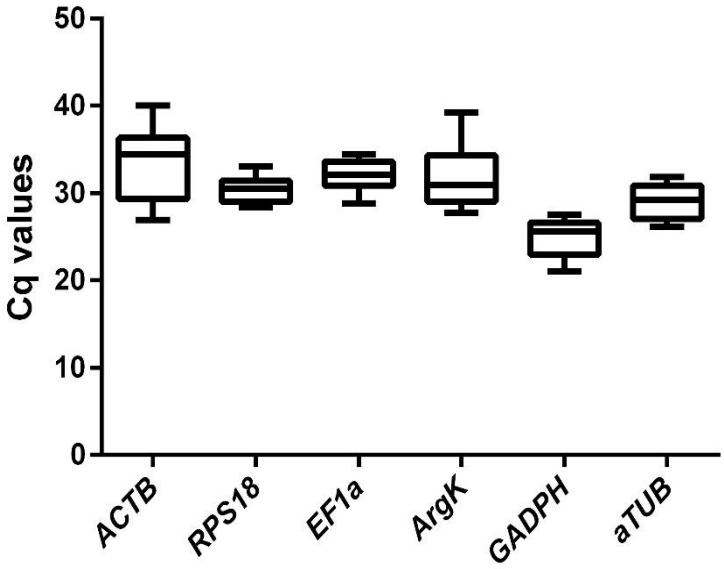

Figure 1. Amplification profiles of candidate housekeeping genes. Box plot of qPCR cycle threshold values (Cq) for housekeeping genes in *Nauphoeta cinerea*.

3.4. Expression stability of selected reference genes

Four statistical Excel macro programs (geNorm, NormFinder, BestKeeper, and Δ-Ct method) were used to evaluate the stability of six candidate genes, in order to find optimal reference genes in *N. cinerea* for RT-qPCR normalization. Additionally, six reference genes were compared and ranked by web-based analysis tool RefFinder (Table 2 and Figure 2).

Table 2. Expression stability of the putative housekeeping genes in *Nauphoeta cinerea* adults.

Reference gene	Genorm		Bestkeeper			Delta Ct		Normfinder		RefFinder	
	M	**Rank**	**SD**	**R**	**Rank**	**SD**	**Rank**	**SV**	**Rank**	**Stability**	**Rank**
ACTB	1.957	6	3.40	0.973*	6	2.59	6	2.262	6	6.00	6
RPS18	0.992	4	1.12	0.957*	1	1.73	4	1.046	2	2.38	2
EF1α	0.841	3	1.50	0.902*	2	1.72	3	1.123	3	2.71	4
ArgK	1.639	5	2.92	0.903*	5	2.52	5	2.146	5	5.00	5
GADPH	0.699	1	1.76	0.902*	4	1.70	2	1.133	4	2.38	1
α-TUB	0.699	1	1.68	0.977*	3	1.48	1	0.401	1	1.32	3

SD, standard deviation; **SV**, stability value; **r**, Pearson correlation coefficient; *****p≤0.001.

The geNorm algorithm assumes that the candidate genes are not co-regulated. For each reference gene tested, the gene expression stability value M is normally calculated as the mean pairwise variation for that gene with all other reference genes. Genes characterized by low M have stable expression, while the gene with the highest M value is eliminated, and this process continues until the two most stable genes are identified. This last gene pair is recommended as the optimal reference gene pair. It is recommended to use at least two reference genes to ensure correct normalization in geNorm. In *N. cinerea* adults, geNorm ranked the set of candidate reference genes: *α-TUB, GADPH >EF1a >RPS18 >ArgK > ACTB*. *α-TUB* and *GADPH* were found to be the most stable genes with an expression stability value M of 0.699. The gene with the least stable expression was *ACTB* with an M-value of 1.957 (Table 2 and Figure 2A).

Normfinder analysis is based on an ANOVA (analysis of variance) model that identifies genes with the least variation in expression over the entire sample set. Analyzes taking into account systematic differences between sample subgroups. In *N. cinerea* adults,

Normfinder ranked the six reference genes as follows: *α-TUB* >*RPS18*> *EF1α*> *GADPH*> *ACTB*> *ArgK* > *ACTB* with a stability value SV of 0.401, 1.046, 1.123, 1.133, 2.146, 2.262, respectively. *α-TUB* was found to be the most stable with a stability value of 0.401 and *ACTB* the least stable with a stability value of 2.262 (Table 2 and Figure 2B).

The stability of the candidate genes was analyzed with BestKeeper, which can calculate the standard deviation (SD) based on the Cq values of all candidate reference genes. Reference genes exhibiting the lowest standard deviation (SD) were taken as the most stable genes. Also, values exceeding the cut-off value (SD < 1) are considered unstable across all samples. According to this analysis, all housekeeping genes [*RPS18* (SD: 1.12), *EF1α* (SD:1.50), *α-TUB* (SD: 1.68), *GADPH*, (SD: 1.76), *ArgK* (SD: 2.92), *ACTB* (SD: 3.40)] exceeded the threshold in *N. cinerea* adults (Table 2 and Figure 2C).

Based on relative pairwise comparisons, the ΔCt method calculates the mean SD of each gene set using raw Ct values, and this SD value is inversely proportional to the expression stability of the gene. Standard deviation below 1 indicates appropriate stability. As the mean SD of each gene set was over 1, none of the available reference genes were found to be stable enough for *N. cinerea* adults according to the ΔCT method. The overall ranking based on the ΔC t method of reference genes was: *α-TUB, GADPH, EF1a, RPS18, ArgK, ACTB* (Table 2 and Figure 2D).

RefFinder is a comprehensive algorithm that combines all the above-mentioned software tools (geNorm, NormFinder, BestKeeper and Δ-Ct method) to rank candidate reference genes according to their stability. The overall ranking based on RefFinder of reference genes in *N. cinerea* was: *GADPH, RPS18, α-TUB, EF1α, ArgK*, and *ACTB* (Table 2 and Figure 2E).

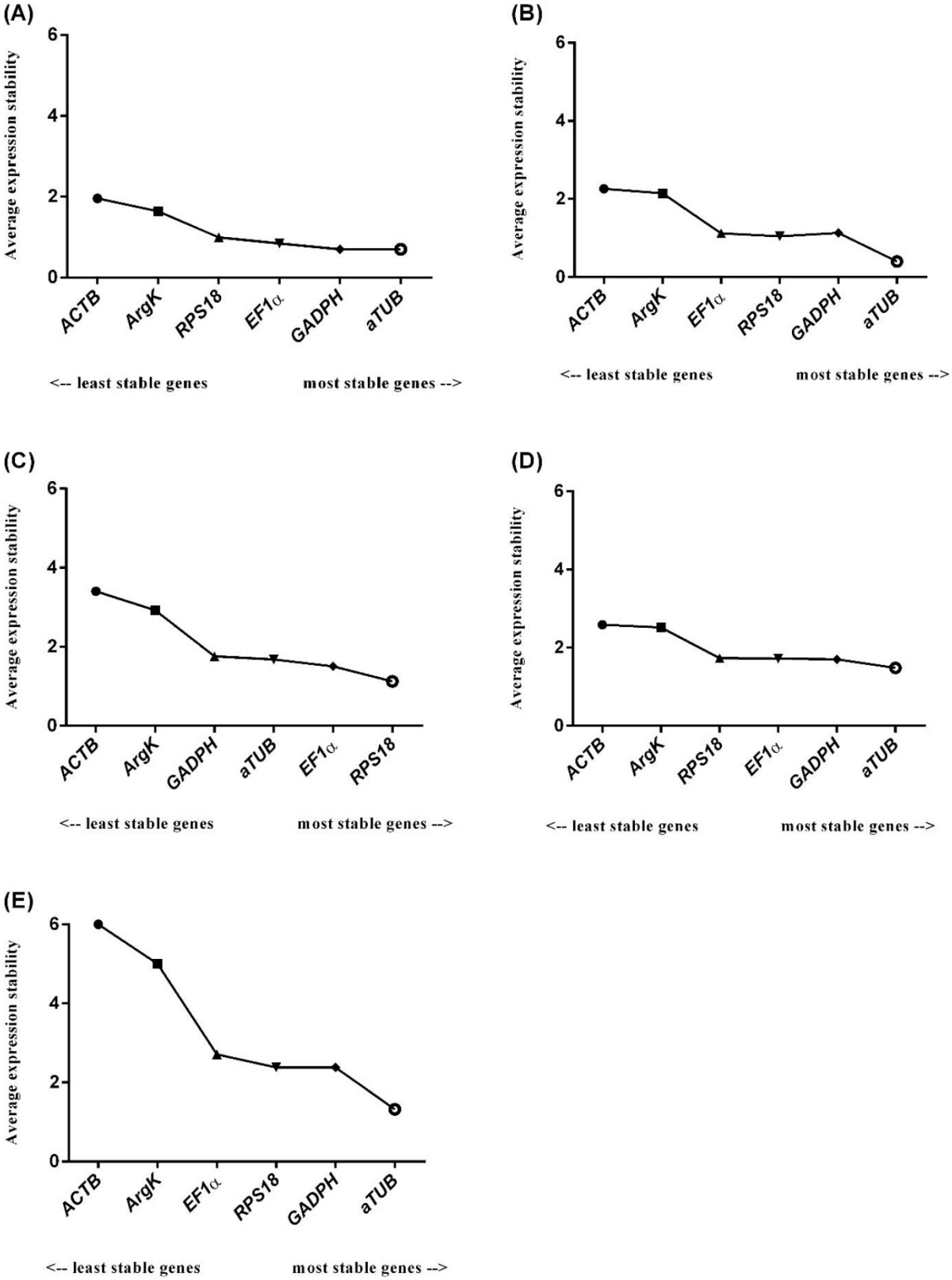

Figure 2. Expression stability and ranking of the six housekeeping genes evaluated by Genorm (A), Normfinder (B), Bestkeeper (C), ΔCt metod (D), RefFinder (E).

4. DISCUSSION AND CONCLUSIONS

Housekeeping genes structurally expressed to maintain essential cellular functions are the traditional choice for a standard reference (Vandesompele et al., 2002). However, there are no "universal" reference genes that are stably expressed and applicable for all cell and tissue types under various experimental conditions (Fu et al., 2013b; Li et al., 2013; Liang et al., 2014; Pan et al., 2015; Yang et al., 2014; Yang et al., 2015a; Zhu et al., 2014). Especially in studies on

the selection of reference genes in various biotic and abiotic conditions in insects, different stability of different reference genes may vary under each condition. In insect studies, actin has been identified as the most frequently used reference genes, including *ACTB, RPL, Tubulin, GAPDH, RPS, 18S, EF1A, TATA, HSP*, and *SDHA* (Lü et al., 2018). *ACTB*, which encodes a large structural protein, is expressed at various levels in many cell types and is considered the ideal reference gene for RT-qPCR analysis and is the most frequently investigated reference gene. Many studies evaluating the stability of reference genes in insects have shown that *ACTB* expression is the most stable among other reference genes at different developmental stages of many insects, including *Apis mellifera, Chortoicetes terminifera, Schistocerca gregaria, Plutella xylostella, Drosophila melanogaster, Liriomyza trifolii, Chilo suppressalis,* and *Diuraphis noxia* (Chang et al., 2017; Chapuis et al., 2011; Ponton et al., 2011a; Scharlaken et al., 2008; Sinha and Smith, 2014; Teng et al., 2012; Van Hiel et al., 2009). On the other hand, *ACTB* expression in some insect species was reported to be less stable (Pan et al., 2015; Yang et al., 2015d; Yang et al., 2016). Ribosomal protein (RP), an essential component of ribosomes, is among the highest conserved proteins in all life forms, and RPL and RPS family genes have been the most preferred reference genes for expression studies, especially in insects (Lü et al., 2018). *RPS24* and *RPS18* were identified as the two most stable reference genes across different developmental stages and sex treatments of *C. maculate* (Yang et al., 2016); *RPS13* and *RPS23* have been reported as stable reference genes at different developmental stages of *P. xylostella* (Fu et al., 2013a); the three most stable reference genes *RPL11, RPS8* and *RPL14* at different developmental stages and different temperature conditions of *Aphis craccivora* were identified (Yang et al., 2015b). Tubulin (α-tubulin, β-tubulin and γ-tubulin), which encodes cytoskeletal structure proteins, another most studied reference gene, has been found to have variable stability under different treatments for the same species in many studies. For example, α-tubulin exhibited stable expression in different tissues and sexes of *C. maculata*, while its expression was found to be unstable throughout different developmental stages and following dsRNA treatments (Yang et al., 2015d). Similarly, the stability of *GAPDH* expression has also been found to be variable under different treatments within the same species. For example, *GAPDH* expression was not affected by tissue type, sex, photoperiod, or dsRNA treatment in *H. convergens*, but was found to vary at different developmental stages and at different temperatures (Pan et al., 2015). Furthermore, the *EF1A* gene was selected as the least stable reference gene in *A. craccivora* during different developmental stages and at different temperatures (Yang et al., 2015b). On the other hand, EF1A expression level in *H. convergens* was not affected by three biological factors (developmental stage, tissue type, and sex) and three abiotic conditions (temperature, photoperiod, and dietary RNAi), and EF1A was chosen as the most stable gene *H. convergens* (Pan et al., 2015). In summary, the expression of some commonly used housekeeping genes may vary under different experimental setups. Genes that play important roles during insect metamorphosis and affect different tissues serve as target genes for manipulations that kill the insect or retard its growth. Therefore, gene expression profiles should be widely evaluated in different developmental stages and in different tissues, and gene stability should be determined (An et al., 2016; Chang et al., 2017; García-Reina et al., 2018; Pan et al., 2015; Ponton et al., 2011a; Rodrigues et al., 2014; Sang et al., 2015; Yang et al., 2015c).

To our knowledge, only one validation study has been reported for reference genes in cockroaches (Marchal et al., 2013). They aimed to identify and validate a set of eight housekeeping genes (*β-actin, EF1a, GAPDH, Arm, RpL32, SDHa, AnnIX* and *α-Tub*) during the first gonadotropic cycle of the cockroach *Diploptera punctata*. They used two different algorithms (geNorm and Normfinder) to evaluate the stability of its expression. *α-Tub* concluded that the combined use of *EF1a* and *RpL32* resulted in an accurate normalization of gene expression levels in the *D. punctata* ovary. They found that *Actin* and *AnnIX* should not be used to normalize transcript levels when examining the first gonadotropic cycle in the corpora allata or the ovary of *D. punctata.*

In the current study, a validation study was performed for reference genes (*GADPH, RPS18, α-TUB, EF1α, ArgK*, and *ACTB)* in cockroach species *N. cinerea* adults using five different algorithms (ΔCt method, Bestkeeper, geNorm, Normfinder and RefFinder) to evaluate the stability of selected reference genes expression. The six possible reference genes included in our current study were selected based on their traditional use and stability as described in validation studies reported in other insects. Our results show that *α-Tub* use provides accurate normalization of gene expression levels in *N. cinerea* adults. In addition, since the *GADPH* gene is selected as the second most stable reference gene, we can suggest that it can be used for transcript analysis. As Marchal et al. (2013) suggested, our study also showed that actin should not be used for normalizing transcript levels when examining *N. cinerea* adults. Additionally, validation studies for reference genes in cockroaches are very few (only one) in the literature. In the continuation of this study, as in other insect species, it is planned to investigate especially cockroaches under the conditions of many experimental factors such as the stability of reference genes, developmental stage, tissue, temperature, pesticide, diet, population, virus, sex, photoperiod and starvation. The result of the present study and existing studies in the literature highlights the need for validation of reference genes under biotic and abiotic conditions in q-RT-PCR studies in cockroaches.

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N/A

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Author Contributions
Conceptualization: Ş.B, A.N.P, K.Ö Investigation: Ş.B, A.N.P, K.Ö.; Material and Methodology: Ş.B, A.N.P, K.Ö,

Supervision: Ş.B; Visualization: Ş.B, A.N.P, K.Ö; Writing-Original Draft: Ş.B; Writing-review & Editing: Ş.B; A.N.P; K.Ö.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest
The authors have no conflicts of interest to declare.

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Başlık ve özet (İngilizce): Özet 500 kelimeyi geçmeyecek şekilde yazılmalıdır. Araştırmanın gerekçesini, amaçlarını, uygulanan yöntemi, sonuç ve önerileri içermelidir. Özet sonuna 3-6 kelimeden oluşan anahtar kelimeler eklenmelidir.

Ana metin: Makale ana metni tek satır aralıklı olarak yazılmalı, çizelge ve şekillerle birlikte toplam 15 sayfayı geçmemelidir. Konu başlıkları 1., 1.1., 1.1.1., şeklinde numaralandırılmalıdır.

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