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Review

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A new approach to the horse nutrition: Nanoparticles

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A B S T R A C T

There has been a gradual increase in research on alternative feed materials and feed additives in animal nutrition. Since the purpose of animal nutrition is to ensure healthy and sustainable animal production, the primary objective is to ensure that the alternative substances are not only beneficial to disposal of waste, but also to the health and development of the animals. Particularly in horse farming, feeding is based on commercial diets supplemented with some vitamin additives. However, the specific digestive anatomy and physiology of horses create obstacles in the methods, which used to compensate for deficient feedstuffs and nutrients. Nanoparticles, which are widely used especially in human nutrition and discovered in search of alternative sources after various legal regulations in animal nutrition, have not yet opened a field for itself in equine nutrition. In this study, the aspects and possibilities of using nanoparticles, which are frequently used in ruminant and poultry nutrition, in equine nutrition were discussed and the pros and cons of nanoparticles were criticized.

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1. Introduction

Horses, which have been in people's lives since ancient times, started to be raised for sportive purposes in developed countries following the Industrial Revolution. Feeding in horses raised for sportive and hobby purposes has been shaped depending on parameters such as the physiological state, age, gender and performance level of the horse and has become almost traditional. Misformulated horse diets lead to serious issues by causing some metabolic problems and this situation is negative for both horse health and the benefits of the producer.

Nanoparticles are the substances that contain at least 50 % of natural substances and vary in size between 1 to 100 nanometers (Garcia-Barrasa et al., 2011). The gradual development of nanoparticles has led to the expansion of their use in animal nutrition. Nanoparticles can be used as feed additives instead of antibiotics which are banned due to residue risk and microbial resistance (Reddy et al., 2020). Some nanoparticles (e.g. nanozinc and nanosilver) are being considered potential alternatives to antibiotic feed additives because of their bactericidal capabilities. Moreover, unlike their macro counterparts, the fact that nanoparticles reduce mineral excretion and environmental pollution has played a role in the evaluation of their usability as feed additives (Gopi et al., 2017). The total dose required to achieve competent serum concentrations is lower for nanoparticles than for non-nanoparticles. This allows the desired level of nutrition to be achieved by using fewer additives (Wang et al., 2018). The application of nanotechnology in animal nutrition, specifically through the use of nanoencapsulation and nanoparticles, allows for precise and minimal addition of materials while still achieving the same function as their bulk-sized counterparts. This is commonly referred to as 'nano nutrition' or 'nano additives'. Research in animal nutrition has utilized metal and phyto-based synthesized nanoparticles, including silver, gold, calcium, iron, selenium, silicon, titanium, and zinc, in various fields of nanotechnology (Adegbeye et al., 2019).

Although there are various studies on the use of nanoparticles in livestock species, they are generally limited to ruminants and laboratory animals. The use of nanomaterials in equine nutrition has been mostly related to preventive or therapeutic medicine (Gopi et al., 2017; Elghandour et al., 2018; Xie et al., 2018; Adegbeye et al., 2019). The use of nanoparticles as feed additives, particularly in immunotherapy, is supported by their easy digestibility through the intestinal lumen (Hameed, 2021; Hill and Li, 2021). Polymeric, liposome, dendrimer, micellar, and ceramic nanoparticles are commonly used in animal production and feed additives. Carbon-based nanoparticles, such as fullerene and carbon nanoparticles, as well as metallic inorganic nanoparticles, have also been used. Among the metallic nanoparticles, gold, silver, cobalt, copper, chromium, and magnesium are frequently employed in livestock production. Metallic nanoparticles, including gold, silver, cobalt, copper, chromium, magnesium oxide, ferrous oxide, zinc oxide, titanium oxide, and selenium, are frequently utilized in livestock production. It is important to note that this statement is objective and does not contain any subjective evaluations (Adegbaye et al., 2019;

Nanoparticles commonly induce oxidative damage by generating reactive oxygen species, disrupting cell membranes, and inhibiting cell division and death (Zhu et al., 2007; Rudramurthy et al., 2016). The formation of excessive reactive oxygen species, including hydrogen peroxide, leads to oxidative stress and subsequent cell damage (Allen et al., 2009). Other effects include depletion of intracellular ATP production and disruption of DNA replication (Donato et al., 2010).

Nanomolecular additives such as gold nanoparticles, iron-oxide nanoparticles, gelatin nanoparticles are frequently used to improve performance in horses (Reddy et al., 2020). This group includes gut-floral stabilizers, digestibility enhancers and environmental modifiers and other additives that improve the nutritional status of horses. The beneficial effects of antibiotics used as feed additives in various livestock species have not been achieved in horses due to differing dosing requirements (Elghandour et al., 2018). Because of the capability of metal nanoparticles to disrupt gram-positive and gram-negative bacterial cell walls, nanotechnology is considered as an alternative to several antibiotic feed additives (Xie et al., 2018). Bacterial (e.g. Clostridium perfringens, Clostridium difficile, Escherichia coli, Bacteroides fragilis, Enterococcus, and Aeromonas spp.) foal diarrheas are highly contagious infections and negatively affect growth performance (Mallicote et al., 2012).

Mallicote et al. (2012) also reported that 800 mg kg^{-1} nano-ZnO supplementation to the diet decreased diarrhea rate and positively affected daily body weight gain in foal diarrhea. Spherical silver nanoparticles are also known to decrease diarrhea incidence through bacteriostatic and bactericidal effects on Salmonella and Shigellas (Tiwari et al., 2018). Although, according to the literature review, there is no similar study conducted in adult horses, the reported results for foals suggest that similar effects can be achieved in adults by adapting these substances. In this study, various empirical researches and comprehensive literature reviews on the use of nanoparticles in nutrition of horses were reviewed.

2. Production of nanoparticles for animal feed additivities

Nanoparticles can be synthesized in the laboratory or purchased commercially. Nanoparticles can be produced using traditional chemical and physical methods or using green and sustainable biosynthetic methods that use plant extracts or microorganisms (Gopi et al., 2017; Yusof et al., 2019; Abdelnour et al., 2021). The methods used to obtain nanoparticles and their classification are given in Table 1.

Table 1. Production methods of nanoparticles (Michalak et al., 2022)

Physicial Methods	Evaporation and condensation, Physical Evaporation, Chemical Deposition, Electric Arc Discharge (EAD), Ballmilling-Annealing, Gas Phase SynthesizingLaser ablation
Chemical Methods	Reduction methods Chemical Indirect
	Sedimentation methods Sol gel, alkaline, and co-precipitation hydrothermal
	Inert gas condensation
Biological Methods	Using plants, bacteria, virus, fung, etc.

Microencapsulation techniques involve physically entrapping sensitive bioactive compounds in a matrix of macromolecules, allowing for safe delivery through deleterious environments until assimilated in the proper organs (Galland, 2013). However, this method's large particle size does not solve the problem of low bio-accessibility of the material after digestion and release processes. In this context, nano-encapsulation could be an effective approach to increase the bioactivity and bio-accessibility of the compounds, as well as to enhance their stability under digestive conditions and protect them from interacting with other components of digestion and premature degradation before reaching the target site (Neilson et al., 2007). Nano-encapsulation enhances cell permeability and solubility during the digestion process, resulting in proper absorption and bioavailability of nano-encapsulated compounds (Hu et al., 2002). Polysaccharides, including starch and its derivatives, pectin, glucans, cellulose, and protein-based materials such as polypeptone, soy protein, milk proteins, and gelatin derivatives, are the most widely used materials for encapsulation in food or pharmaceutical applications (Ahmad et al., 2017; Ahmad et al., 2018).

3. Using nanoparticles as feed additives

Feed additives can be defined as the substances that improve the digestibility, utilization or storage of basic diet components (Elghandour et al., 2018). Primary components of the diets (i.e. amino acids, vitamins, minerals, and poly-unsatureted fatty acids (PUFA)) are used for direct consumption in small-scale quantities. Nanoparticles are also under investigation in the food industry as a delivery system for nutraceuticals. Because of the different gastrointestinal tract of horses compared to other livestock species and the different pH levels between parts of the digestive tract, feed additive or nutraceutical evaluation is mandatory. Nanocarriers have shown good results in delivering nutrients for efficient absorption at the small intestine (Singh et al., 2017). Cause of their ability to emulsify and their well gelling properties, proteins are considered to be safe nanocarriers. An in vitro release study simulating the gastrointestinal tract displayed that the cruciferin (i.e. canola protein) is a prospering carrier for beta-carotene. It is released at intestinal pH (Akbari and Wu, 2016).

It is known that processed cereals used in horse feeding are subjected to processing-related loss of many vitamins and minerals besides causing some metabolic disorders (Reddy et al., 2020). Bioavailability is directly related to bioaccessibility, absorption and molecular transformation (Salvia-Trujillo et al., 2016). Therefore, delivery systems on the basis of nanomaterials increase nutrient bioavailability. Draught and racehorses have a higher physiological need to nutritional supplements. However, because of the other elements that interfere with absorption, inorganic mineral salts have poor bioavailability. The addition of these inorganic minerals with low utilization levels to horse feeds leads to increased excretion rates and environmental pollution. The use of nano-sized minerals can increase bioavailability by reducing the amount of these waste materials.

The expectation of improved performance as a result of increased absorption rates of nutrients with the use of mineral nanoparticles is related to this physiological transformation. The increased surface area and the reduction in size of the mineral nanoparticles lead to improvements in certain physico-chemical characteristics (Boyles et al., 2016). The nanoencapsulation technique used in the delivery of vitamins A, C and E to body tissues has been developed by utilizing this capability. In addition to the increase in absorption and diffusion efficiency, the nano-encapsulation technique also contributes to the reduction of inflammation and acceleration of the recovery process (Bunglavan et al., 2014). Although all these physiological and biochemical processes have been tested in vivo and in vitro in different livestock species, the literature on their use in horses is quite limited.

Although there are very few nano-elements that have ever been studied in equine nutrition, the most widely studied is the nanoform of vitamin E (Ezhilarasi et al., 2013; Bunglavan et al., 2014; Sinatra et al., 2014). To support optimal neurological and muscular activity in performance horses, an adequate intake of vitamin E is necessary. Readily available commercial forms of vitamin E are commonly recommended for expectant or nursing mares, stallions, racehorses and convalescent grown horses. Coenzyme Q10 (CoQ10), being an indispensable nutrient for mitochondrial energy generation, is also a nanosuspension besides vitamin E. Nonetheless, CoQ10 is barely bioavailable because of its low water solubility. The tenside nanosuspensions cause CoQ10 enzyme constituents to be more soluble and bioavailable (Sinatra et al., 2014). Therefore, excepting in case of ubiquinone deficiency, CoQ10 suspensions, which can collaboratively function with vitamin C and vitamin E, are often used to combat oxidative stress in racehorses. Casein micelles are widely regarded to be natural nanoparticles. They bind to proteins, calcium and other nutrients to enable them to be transferred from expectant mother to her foal. Casein nanoparticles are described to be effectual in delivering oleophilic nutrients and they can increase growth rates by facilitating nutrient delivery in weaned foals (Hill et al., 2017).

Nanostructured MgO is a low-cost and easily manipulable nanoparticle that exhibits intrinsic biocompatibility (Auger et al., 2018). It has been shown to possess antimicrobial activity against Escherichia coli and Pseudomonas aeruginosa at concentrations of 0.7, 1.0, and 1.4 mg/mL by damaging the cell wall, cell membrane, and destroying formed biofilms (Nyuguen et al., 2018). Additionally, these nanoparticles may be used as prebiotics in animal feeding (Fondevila et al., 2009). Similarly, the use of copper-loaded chitosan nanoparticles results in an increase in the population of Lactobacillus and Bifidobacterium in cecal digesta, as well as a decrease in coliforms population (Wang et al., 2011). Additionally, supplementation with graded levels of 0, 25, 50, and 75 mg/kg copper nanoparticles leads to an increase in the growth of total bacteria, ranging from 45.97% to 105% compared to the control (Refaiye et al., 2015). These findings suggest the potential probiotic benefits of nanoparticles. Furthermore, the levels of pathogenic *E. coli* and *Clostridium* spp. were reduced by approximately 1.6- to 2.7-fold and 1.37- to 2.86-fold, respectively, compared to the control group when supplemented with nanoparticles (Adegebeye et al., 2019).

Ruminant feeds containing urea may be tolerated to a limited extent. The NPN compounds can be supplemented into horse diets at specific amounts, as urease activity in the equine caecum is approximately 25% of that in the rumen for ruminants (Martin et al., 1996). Nevertheless, horses are known to have a narrow range of tolerance to urea, around 15% of plasma urea levels, so excessive use may lead to undesirable results. Although urea feeding to mature horses with marginal protein intake is not widely recommended because it may have beneficial effects in terms of feed cost, it is often used in practice (Martin-Rosset and Tisserand, 2004).

However, excretion of unused nitrogen results in a significant environmental nitrogen load (Reddy et al., 2019a; Reddy et al., 2019b). Kottegoda et al. (2017) proved that urea release was reduced by including it in hydroxyapatite nanoparticles. It can be concluded that by delivering urea with the mentioned nanoparticles, the protein deficit in the basal diet can be completed and a safer and healthier feeding can be achieved in horses.

Equine stomach cannot fully digest starch. Therefore, when the incompletely digested starch enters the caecum-colon chamber (wherein anaerobic fermentation region), as a result of hindgut environmental pH depression, usual microbial balance and/or microbial activity is disrupted. Consequently, digestive disorders in horses are associated with diets containing high starch grains (Cipriano-Salazar et al., 2019). However, increased stomachal carbohydrate digestion results in a reduced starch flow into the hindgut. In this case, yeasts are often used to help, but nanoparticles are known to function in a similar way (Adegbeye et al., 2019). Nanoparticles can also be safely added into equine diets for a better starch digestibility. The activities of enzymes involved in digestion (e.g. amylases, lipase, protease) can be regarded as hallmarks of nutrient utilization capability, and to some degree, digestibility based on the diet provided (Gomez-Requeni et al., 2013).

It was revealed that alpha-amylase activity increased in coexistence with citrate-reduced gold and biosynthesized silver nanoparticles (Saware et al., 2015). While the coexistence of gold and silver nanoparticles resulted in a 1.5-fold increase in α-amylase activity, gold nanoparticles alone brought about higher activity. Subsequent researches indicated that a higher starch solution resulted in a higher enzymatic activity. In the presence of nanoparticles, there appears to be an increased breakdown of starch into sugars, resulting in a catalyzing effect (i.e. nanocatalyzing effect) on starch uptake, more likely. This nanocatalytic activity may reduce starch granules flowing into the hindgut of horses, and therefore microbial dysbiosis. The immobilized enzyme overrides the common collision frequency between free enzymes and substrate molecules, and then the enzyme binds to the nanoparticles instead of the reducing sugars, albeit the enzymatic activity still sustains (Jiang et al., 2005). This may also be administered to fibrolytic enzymes in equine feeds to increase fiber digestibility. On the other hand, depending on the enzyme activity, it may be necessary to use lower levels of fibrolytic enzyme in the horse feeds. Likewise, it was observed that averagely 11 nm-sized gold nanoparticles (stabilized with citrate) resulted in higher alpha-amylase activity with incrementing starch concentration (Deka et al., 2012). In the presence of gold nanoparticles in the medium and when probed with starch, the concentration of α-amylase is found to be 5.5 to 9.6 times higher, approximately. The unexpected lower enzymatic activity accompanied with the higher alpha-amylase is a consequence of binding of the enzyme to the nanoparticles and following agglomeration (Gosh et al., 2013).

One of the feed additive groups is sensory additives. Sensory additives enhance animal palatability and appetite by providing sensory attitude of aroma, palate and odor. Food flavorings are often used in commercial horse diets to combat with hesitancies against any nutritional novelty (neophobia) and encourage the consumption of tasteless additives, water and anthelmintics.

It was shown that, tasteless pellets, when they were aromatized with fenugreek or banana, the feed intake is promoted (Goodwin et al., 2005). Although never tested previously in horses, it is well documented that, some common nanoencapsulation methods result in increased aroma release in foods. The capability of silicon dioxide nanomaterials to carry odors has been demonstrated empirically for food applications (Malheiros et al., 2010; Bokkers et al., 2011). Malheiros et al. (2010) revealed that nano-based liposome entrapment method provides more effective delivery of flavorings rather than other encapsulation methods. This is a method that contributes to the provision of basic flavors in horse feed.

It is well documented that feed additives often play a role in the improvement of animal health. Therefore, nanoparticles are also used as feed additives in the prevention of protozoal diseases observed in horses (Raguvaran et al., 2015; Dubey and Bauer, 2018).

An in vitro study showed that silver nanoparticles (4.6 nm in size) produced by *T. harzianum* reduced Fasciola hatchability by 28.71% compared to conventional triclabendazole use (Gherbawy et al., 2013; Wu et al., 2016).

4. Using nanoparticles in feed hygiene

Technological methods are constantly used to ensure the hygiene of feeds or feed additives. Substances used for feed hygiene may include antioxidants, preserving agents, emulsifying agents, stabilizing agents, silage additives and acidity regulator. Developments in the food science and technology have paved the way for the study of nanoparticles being biocides to preserve feeds (Hill and Li, 2017). Contaminations that may occur in feeds and especially mycotoxins can have serious negative impacts on animal health (Kottegoda et al., 2017). The use of plastic containers implanted with silver nanoparticles can minimize the risk of contamination with their antimicrobial properties (Bunglavan et al., 2014). Although it has been suggested that metal-based nanoparticles are transferred from feed containers, on the other hand recent studies have shown that silver nanoparticles in metal-impregnated feed boxes are untraceable. Therefore, the use of multiple silver-based zeolite products for disinfection purposes has been certified by the US Food and Drug Administration bureau (Reddy et al., 2020). Another study revealed that mycotoxin contamination is very common in equine feed mixtures sold in the market. Although determined mycotoxin concentrations were below the toxicity threshold level, it was found that they caused skin allergies and various inflammations; as the accumulation level increased, they caused serious health problems (Horky et al., 2018). The use of nanoparticles that can bind to toxins in reducing or completely preventing toxicity can be considered as a critical progress in terms of animal health (Ajdary et al., 2018).

Propionic acid is a common supplement to reduce dust and mold, and to extend expiry date in equine feeds. The conservatory nanotechnology products (e.g. chitosan films containing silicon dioxide nanocrystals, alginate nanolaminate coatings, gelatin-based cellulose nanocrystals, and nano-silica coatings) seem to be more hopeful method rather than the rest of the conventional methods to sustain feed quality even along an extended shelf-life (He and Hwang, 2016). Aluminum is preferred to prevent caking of horse feeds during storage, but it is also known to have a negative effect on phosphorus absorption. Although there is no alternative product or substance yet, the European Union has registered nano-sized silicon dioxide being an anti-caking agent; on the other hand, it has not yet been validated in horse feeds (Ezhilarasi et al., 2013). It is well documented that the vast majority of the nanometallic particles generate oxidative stress through the release of ROS (reactive oxygen species), and this is one of the negative characteristics of nanoparticles. However, a small number of reactive nanomaterials (e.g. polymer coatings and silicon dioxide gallic acid) are used to deliver antioxidants in the food industry (Horky et al., 2018). Lipid-based nanoencapsulation systems, which contribute to increased particle solubility and bioavailability, may result in higher antioxidant performance (Wolny-Koładka and Malina, 2017).

5. Pros and cons of nanoparticles

The narrow safe and toxic dose range of nanoparticles may cause some concerns in the use of nanoparticles. There is a need for safe studies on the use of nanoparticles in equine nutrition, as this is an area that has not yet been adequately studied. When used as feed additives, the interaction between nanoparticles and diverse biological compounds should be well understood. Within this framework, the evaluation of the haemolytic capacity of zinc oxide nanoparticles showed that clustering depends on the concentration in horse erythrocytes (Raguvaran et al., 2015). Empirical studies are still a long way from determining exactly the appropriate dose. Since the digestive system in horses is very sensitive, and therefore reacts quickly to any change in the favorable flora (particularly *Lactobacillus* equi species flora). These bacteria additionally counteract specific enzymatic functions of the microbial flora in the equine large bowel and pro-carcinogenic bodies produced during digestive processes (Tiptiri-Kourpeti et al., 2016). It is not yet fully understood whether antimicrobial nanoparticles act on these favorable bacteria. Therefore, they need to be properly investigated before they can be used as a supplement in horse feeds. Discrepancies in disclosed results are another issue to be dissected. In an in vitro study, silver nanoparticles were found to be efficacious against coliforms without any adverse effect on Lactobacilli species (Fondevila et al., 2009). Contrarily, in another study it was revealed that the same compounds had a negative effect on Lactobacilli species but not on the pathogens (e.g. *E. coli*, and *S. aureus*) (Tian et al. 2018).

6. Conclusion

Nowadays, nanoparticles are increasingly used in ruminant and poultry nutrition, and ideas for their use in horses not only in health applications but also as feed additives are promising. Results supported by in vitro and in vivo applications show that the use of nanoparticles in horses can contribute positively to both digestive system health and physiological satiety. Considering the importance of waste management in sustainable livestock production, the fact that nanoparticles scarcely contribute to environmental pollution, and also costs and alternative protective aspects, the use of nanoparticles in horse nutrition can be considered as a new era. It is believed that with the studies on the use of nanoparticles in animal nutrition, the cost analyses will reach realistic dimensions. Although it is considered that these materials, which can be used in small quantities and can contribute positively to health, do not have an economic production considering the economic value of the horse today, the price analyses will be updated depending on the development of materials and methods to be used in the synthesis of nanoparticles with further studies. However, the physicochemical properties and biochemical effects of nanoparticles need to be further investigated, considering the scarce number of studies on this topic, and the inconsistency of the previous reports.

Compliance with Ethical Standards

Conflict of Interest

As the author of article declare that there are no conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Şevket EVCİ: Conceptualization, methodology, investigation, data curation, writing – review & editing.

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Comparative seasonal analysis of IC50, total antioxidant capacity, phenolics, and flavonoids of some vegetable plants from the aquaponics system

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Seasonal factors such as temperature, solar UV-light intensity, and daylight length can induce changes in the water quality properties and, hence, the nutritional compositions of plants. This comparative study was carried out for the consecutive four (4) seasons (winter, spring, summer, and autumn) to determine the influence of seasonal variations on the 50% inhibitory concentration (IC50), total antioxidant capacity (TAC), total phenolics content (TPC), and total flavonoids content (TPC) of the red chili fruit (RCF), red tomato fruit (RTF), green leafy spinach (GLS), and green leafy lettuce (GLL) collected from a coupled commercial aquaponics system. The IC50, TAC, TPC, and TFC concentration levels indicated a significant ($P<0.05$) difference in the summer compared with the winter, spring, and autumn. The RCF extract indicated the lowest IC50, thus greater scavenging power in comparison to RTF, GLS, and GLL extracts. Similarly, the RCF showed the highest TAC and TPC, while the GLL showed the highest TFC. In this study, variations in seasons have induced changes in the IC50, TAC, TPC, and TFC concentration levels of the RCF, RTF, GLS, and GLL extracts.

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1. Introduction

An aquaponics system is a bio-integrated ecosystem. It links recirculating fish aquacultures with hydroponics plants production (Pattillo, 2017; Goddek et al., 2019). Hydroponic plants such as vegetables, flowers, or herbs, and aquatic species such as fish can be grown together in this soilless, water-based system (Rakocy et al., 2006). The fish in the aquaponics technology produces wastes that different bacterial species can convert into nutrients to be used up by plants, which in turn improves the water quality for the fish. This closed-loop system provides a renewable and sustainable method of food production (Medina et al., 2016; Rakocy et al., 2006).

Climatic change is one of the factors that can affect food production and supply at local, regional, and global levels. For instance, abiotic environmental factors such as high temperatures, precipitation pattern changes, and reduced water availability can influence food productivity, quality, accessibility, and availability (US EPA, 2017). Secondary metabolites, bioactive compounds or phytochemicals in plants varies because of changes in the environmental factors (Nchabeleng et al., 2012; Jayanthy et al., 2013; Sampaio et al., 2016). The most successful adaptation of plants is the synthesis of different phytochemicals to withstand or sustain both biotic and abiotic stress (Mohiuddin, 2019; Huang et al., 2020). Thus, secondary metabolites production in plants allows them to survive different seasonal conditions (Yadav and Agarwala, 2011).

Different temperature levels due to seasonal shifts have indicated an effect on the secondary metabolite compositions in plants (Usano-Alemany et al., 2014). Djurdjevic et al. (2012), detected an optimal total phenolics content of Conyza Canadensis L. plants during the flowering and fruiting period (rainy season). In another study, Akiode et al. (2021), reported cool period as the best season for tannins and alkaloids synthesis from *Azadirachta indica* and *Eucalyptus globulus* plants.

Red chilies contain many essential nutrients such as carotenoids (Marisa et al., 2001), tocopherols (Ling and Suhaila, 2001), phenolics and flavonoids (Ananthan et al., 2014). The pungent flavor of red chili is related to the compound capsaicinoid (Garces-Claver et al., 2006). The carotenoids are the pigments synthesized during tomato fruit ripening and responsible for the final red color (Peryeen et al., 2015). A tomato is a vital sources of lycopene (Agarwal and Rao, 2000), tocopherols, and β-carotene bioactive compounds (Burns et al., 2003; Hwang et al., 2012). It is also a good source of phenolics compound (Martinez-Valverde et al., 2002). Thus, tomato plays a critical function against various eyesight disorders, tumors, cancer, and cardiovascular disease (Agarwal and Rao, 2000; Martinez-Valverde et al., 2002; Peryeen et al., 2015). This fruit (tomato) can additionally reduce obesity and hyperglycemia (Cummings and Schwartz, 2003).

The dark green color of the leafy spinach indicates a high concentration of health-promoting carotenoids and chlorophyll (Ramaiyan et al., 2020). Carotenoids are anti-inflammatory, anti-cancerous (Ramaiyan et al., 2020), and prevent macular degeneration and cataracts (Wu et al., 2015). Leafy spinach also contains kaempferol; which reduces the risk of cancers and chronic diseases (Naoki et al., 2009; Ramaiyan et al., 2020), nitrates; which enhance heart health and lower blood pressure (Nathan and John, 2015), flavonoids; which are anti-carcinogenic and promote cardiovascular well-being (Ramaiyan et al., 2020). The antioxidant compounds reported in the leafy lettuce include phenolics (Liu et al., 2007), flavonoids (Llorach et al., 2008), β-carotene, and α-tocopherol (USDA, 2019). Evidence from in vitro, preclinical, and clinical studies suggest that lettuce has potential anti-inflammatory (Pepe et al., 2015), blood pressure-lowering (Lee et al., 2009), anti-diabetic (Cheng et al., 2015), and anti-cancer (Brennan et al., 2000) properties. Hence, consumption of different types of edible vegetable plants can be of crucial advantages, especially in developing countries with high-rate poverty, nutritional marginalization, and ever-increasing human populations (Braglia, 2022; Kumar et al., 2020).

There were no reports in the existing literature on the impacts of seasonal differences on the 50% inhibitory concentration (IC50), total antioxidant capacity (TAC), total phenolics content (TPC), and total flavonoids content (TPC) of vegetable crops from the aquaponics system. Notwithstanding, reports were detected on the influence of cultural shifts on pre-harvest and post-harvest factors on vitamin C content of horticultural crops (Lee and Kader, 2000), effect of plant growth temperature on antioxidant capacity in Strawberry (Wang and Zheng, 2001).

Impacts of cultural cycles and nutrient solutions on plant growth, yield, and fruit quality of alpine strawberry (*Fragaria vesca* L.) grown in hydroponics (Caruso et al., 2011), influence of cultural cycles and nutrient solution electrical conductivity on plant growth, yield, and fruit quality of 'Friariello' pepper grown in hydroponics (Amalfitano et al., 2017). Impact of seasonal and temperature-dependent variation in root defense metabolites on herbivore preference in *Taraxacum officinale* (Huang et al., 2020.), effect of seasonal changes on the quantity of secondary metabolites from neem and eucalyptus plants (Akiode et al., 2021), and influence of different seasons on polyphenol content and antioxidant potential of ethanolic, methanolic, ethyl acetate, and aqueous extracts of leaves, stems, and roots of *Premna integrifolia* (Singh et al., 2022). Hence, the present study intends to reports or identify the impacts of seasonal changes on IC50, TAC, TPC, and TFC contents of some vegetable plant extracts from the aquaponics system.

2. Materials and methods

2.1. Study site

The site of the study aquaponics system was Makhanda town, Eastern Cape of South Africa. The research was conducted for consecutive four (4) seasons (winter, spring, summer, and autumn). The winter study started on the 31st of August 2020. In the spring period, the experiment commenced on the 30th of November 2020. The summer investigation began on the 1st of March 2021. Lastly, the autumn analysis was initiated on the 27th of May 2021.

2.2. Plant materials

The sample plants of this research were Bird's eye red chili (*Capsicum frutescens* L.), red cherry tomato fruit (large) (*Solanum lycopersicum*), green leafy silver-beet spinach (*Spinacia oleracea*), and green leafy Locarno lettuce (*Lactuca sativa* L.) (Figure 1).

Figure 1. Vegetable plants for the comparative seasonal evaluation of 50% inhibitory concentration, total antioxidant capacity, phenolics, and flavonoids, a = Thai or Bird's eye red chili; b = Cherry tomato (large); c = Silver-beet spinach in a wicking bucket to support the growing roots; d = Locarno lettuce.

The Bird's eye red chili was obtained from gravel stone media-bed I denoted as GMB-I. Each collected red cherry tomato fruit and green leafy silver-beet spinach was from gravel stone media-bed II and III, represented as GMB-II and GMB-III, respectively. Besides, the green leafy Locarno lettuce was sourced from a polystyrene sheet on deep-water culture-1 (DWC-1). Each plant sample was then placed in a clean polythene bag, transported to the laboratory, and rinsed separately with Milli-Q water to remove unwanted materials or contaminants. Finally, each plant material was preserved at -20 ^{o}C before analysis.

The study vegetable plants selection is based on their nutritional value composition and or antioxidant properties. Also, these plants are among the most common vegetables regularly consumed and or used for different types of food menus preparation all over the globe.

2.3. Chemical reagents and apparatus

The chemical reagents used for this study include ascorbic acid (>99.5%, Merck, Lot No. 1047302, South Africa), quercetin (Willow Outcrop, South Africa), Gallic acid (CAS-No.149-91-7, Germany), 2,2-diphenyl-1-picrylhydrazyl (Glentham Life Sciences, CAS No. 1889-66-4, England), Folin-Ciocalteu's phenol reagent (Merck, Lot No. HC6043320, Germany), aluminum chloride (Saarchem, Batch No.1021022, South Africa). Other chemical reagents were sodium hydroxide (Merck, Batch No. MB1M610352), ammonium molybdate and sodium molybdate (Saarchem, South Africa), sodium carbonate (Merck, Batch No. QG1Q610988), and HPLC grade methanol (CAS-No. 67-56-1, Germany). The consumables include Milli-Q water (EMD-Millipore, Model 13681), filter paper (Whatman No. 1, Maidstone, England), micropipettes, Eppendorf tubes, and falcon tubes. The equipment consists of an analytical balance (Radwag, 220 g × 0.1 mg, Model, AS/220/C/2, Poland), a 96-well plate reader (Epoch Model, USA), a vortex machine (Model No. S10100A, BioRAD, USA), a BÜchi heating water bath (B-491, Switzerland), and a BÜchi rotavapor (R-210, Switzerland).

2.4. Preparation of standard stock and working solutions

Each standard stock solution for the 50% inhibitory concentration (IC50) and total phenolics content (TPC) was prepared by dissolving 2.0 mg of Gallic acid in 1.0 mL of methanol. However, the stock solution for the total antioxidant capacity (TAC) was generated by dissolving 2.0 mg of ascorbic acid in Milli-Q water (1.0 mL). Nevertheless, the standard stock for total flavonoid content (TFC) was made by dissolving 2.0 mg of quercetin in 1.0 mL of methanol.

The IC50 working solution (500 μg mL^{-1}) was prepared from its standard stock to produce concentrations of 2.5, 10, 50, 100, 150, and 300 μg mL^{-1} by dilution with Milli-Q water. The TAC working solution (1,000 μg mL^{-1}) was obtained from its stock solution, different concentrations of 50, 100, 150, 200, 300, and 600 μg mL^{-1} were made by dilution with Milli-Q water. Lastly, each standard working solution (500 μg mL^{-1}) for TPC and TFC was prepared using each respective standard stock solution to generate concentration levels of 10, 50, 100, 200, 400, and 500 μg mL^{-1} by dilution with Milli-Q water.

2.5. Preparation of samples

Each sample (10.0 g) of multiple fresh-weight red chili fruit (RCF), red tomato fruit (RTF), green leafy spinach (GLS), and green leafy lettuce (GLL) was homogenized using a mortar and pestle. Each homogenate was incubated in 50 mL of the HPLC-grade methanol, allowed to stand for 1 h for complete extraction, and centrifuged at 4oC at 4,000 rpm for 20 min. Each extract was filtered using a Whatman No. 1 filter paper (11 μm pore size). Each filtrate was then evaporated to dryness with rotary evaporator under reduced pressure at 35 ^{o}C. The obtained dried powder of each sample was suspended (1.0 mg mL^{-1}) in HPLC-grade methanol for 50% inhibitory concentration (IC50), total antioxidant capacity (TAC), total phenolics content (TPC), and total flavonoids content (TPC) analysis. Finally, the remaining dried fractions of each sample was stored at -4 ^{o}C. All preparations and reactions were carried out under penumbra of light.

2.5.1. DPPH scavenging activity assay

This assay was performed as reported by Kalita et al. (2014), with modification in reagent stock and working concentrations. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) stock solution was made by dissolving 6.8 mg of the DPPH in 100 mL of HPLC-grade methanol. The absorbance of the DPPH stock reagent solution was obtained as 0.9982 ± 0.05 at 517 nm.

To a 200 µL of each sample extract with different concentrations (2.5, 10, 50, 100, 150, and 300 µg mL^{-1}) in the eppendorf tube, DPPH stock reagent (1.2 mL) was added. Each mixture was vortexed vigorously for 30 s and incubated in the dark at room temperature (25±5 °C) for 30 min. The absorbance of each mixture was read photometrically in triplicate at 517 nm against the reagent blank using a 96-well plate reader.

2.5.2. Phospho-molybdenum assay

The phospho-molybdenum assay procedure for the total antioxidant capacity (TAC) test was performed as described by Umamaheswari and Chatterjee (2008) with modifications in the incubation temperature and time. To 100 µL of each sample extract, 1.0 mL of the reagent solution (0.1 M phosphate buffer, 0.6 M H_2SO_4, 28 mM Na_2MoO_4, and 4.0 mM $Al_2(MoO_4)_3$) were added and mixed. The reaction mixtures were incubated at 95 °C for 120 min in a water bath and cooled to room temperature. Each mixture absorbance was read in triplicate at 765 nm wavelength against the reagent blank, using a 96-well microplate reader

2.5.3. Total phenolics content analysis

The total phenolics content (TPC) of each extracts was assayed using the Folin-Ciocalteu (FC) reagent as reported by Kim et al. (2003) and Blainski et al. (2013) with modification in extracts concentration and incubation time. Each sample extract (200 µL) was mixed with 400 µL Folin-Ciocalteu reagent solution. Each mixtures was kept at 25 °C for 15 min, 0.2 mL of 7% Na_2CO_3 reagent was then added and mixed. Finally, each mixture was diluted with 10 mL of Milli-Q water and re-incubated at 25 °C for 2 h, 15 min. Each mixture absorbance was read in triplicate at 725 nm wavelength against a reagent blank.

2.5.4. Total flavonoids content analysis

The total flavonoid content (TFC) was determined using a procedure described by Chang et al. (2002), with modification in the extract concentration and volume of reagents. To 100 µL of each extract, 400 µL of Milli-Q water, 35 µL of 5% $NaNO_2$, and 35 µL of 10% $AlCl_3.6H_2O$ were added, mixed, and incubated for 6 min. In addition, 215 µL of NaOH (1.0 M) was added to each reaction mixture, diluted with 250 µL of Milli-Q water, and mixed. Finally, the reaction mixtures were allowed to stand for 15 min at room temperature. The absorbance of each sample mixture was read at 510 nm in triplicate against a reagent blank using a 96-well microplate reader.

2.6. Statistical analysis

Data evaluations were conducted using repeated-measures analysis of variance (RM ANOVA). The level of significance used was 5%. When the RM ANOVA indicated a significant difference among the comparative four (4) seasons, a post-hoc test using an unpaired student's t-test was performed to determine the significantly different season(s).

3. Results and discussion

3.1. DPPH scavenging activity

The DPPH scavenging activity of each extract was calculated with the following relation.

$\%I=(AC-AS)/AC\times100$

Where: I = inhibition, AC = absorbance of the control, AS = absorbance of the sample

Finally, the 50% inhibitory concentration (IC50) of each study plant extracts was calculated using GraphPad Prism (Software version 9.2.0332, San Diego, CA 92108, USA). The IC50 concentration level of each sample extract of red chili fruit (RCF), red tomato fruit (RTF), green leafy spinach (GLS), and green leafy lettuce (GLL) was expressed as µg Gallic acid equivalent (GAE) mg^{-1} fw. Charts for the IC50 were depicted in Figures 2 to 5. There was a significant ($P<0.05$) difference in the IC50 level of the RCF, RTF, GLS, and GLL extracts among the comparative four (4) seasons (Table 1). The detected significant ($P<0.05$) difference was between winter and spring (Table 1). Similarly, the winter was significantly ($P<0.05$) different from summer as well as the winter in comparison to autumn (Table 1).

In addition, there was a significant (P<0.05) difference in the spring comparison summer likewise, between the spring and autumn (Table 1). Finally, a significant (P<0.05) difference existed in the summer compared with autumn (Table 1). The lowest IC50 level was revealed in the summer period. The red chili fruit (RCF) extract demonstrated the lowest IC50 value in this period (summer), hence the highest inhibitory action compared with the standard (Gallic acid). However, the highest (lowest inhibitory action) IC50 value was in the winter season, revealed by the green leafy lettuce (GLL). Seasonal variations on the IC50 level of the RCF, RTF, GLS, and GLL extracts from aquaponics system was lacking in the existing literature. Although, Higher or warmer temperature enhances photosynthesis (Jamloki et al., 2021) and reduced water availability (Goddek et al., 2019) in plants. Both cases inevitably encourages the synthesis and increased level of the secondary metabolites in plants (Jamloki et al., 2021).

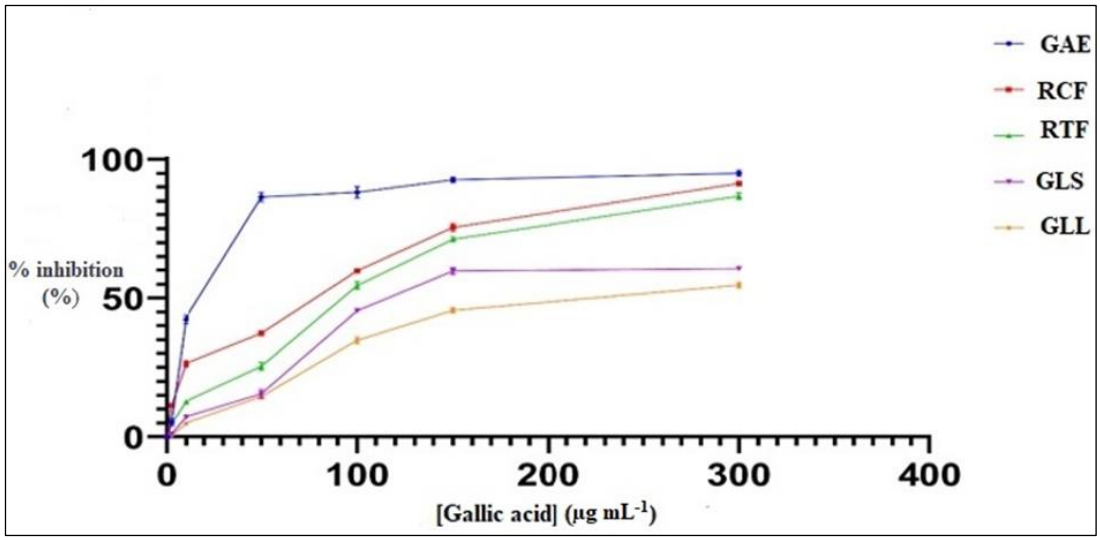

Figure 2. Winter season IC50 charts. The IC50 of each sample extract was determined with a GraphPad Prism. GAE denotes Gallic acid external standard, RCF, RTF, GLS, and GLL are methanolic extracts of red chili fruit, red tomato fruit, green leafy spinach, and green leafy lettuce, respectively.

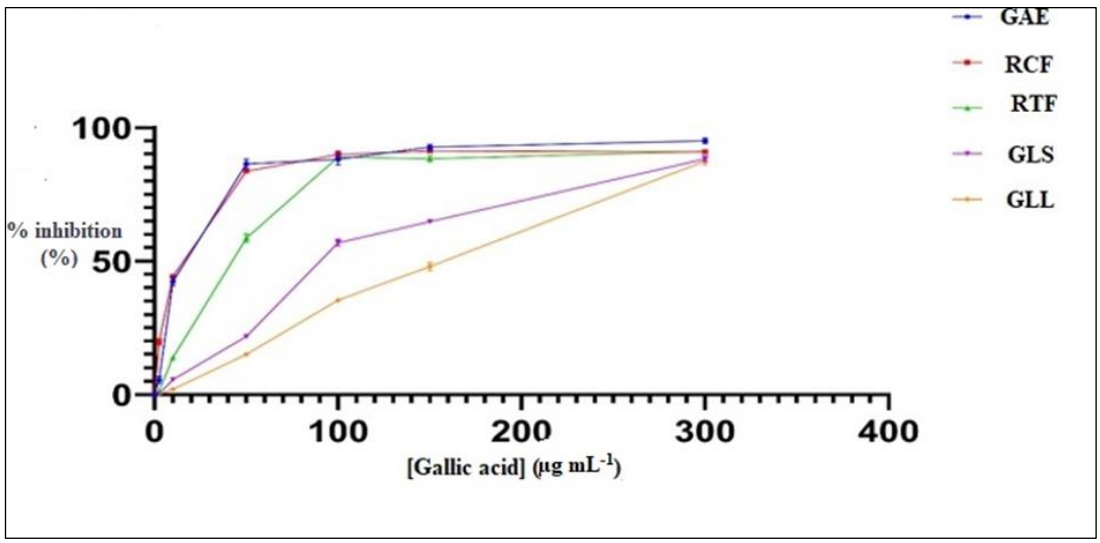

Figure 3. Spring season IC50 charts. The IC50 of each sample extract was determined with a GraphPad Prism. GAE represents Gallic acid external standard, RCF, RTF, GLS, and GLL are methanolic extracts of red chili fruit, red tomato fruit, green leafy spinach, and green leafy lettuce, respectively.

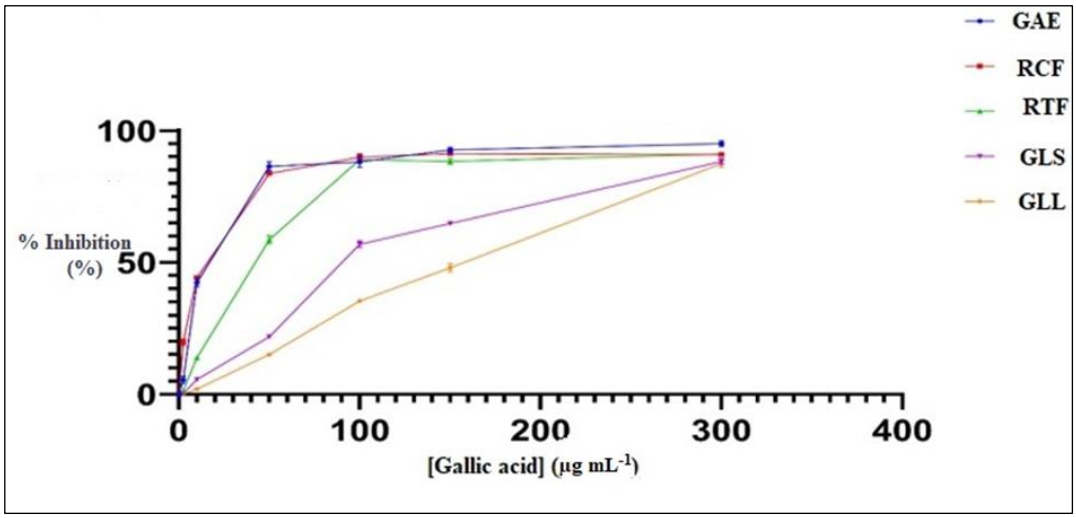

Figure 4. Summer season IC50 charts. The IC50 of each sample extract was determined with a GraphPad Prism. GAE denotes Gallic acid external standard, RCF, RTF, GLS, and GLL are methanolic extracts of red chili fruit, red tomato fruit, green leafy spinach, and green leafy lettuce, respectively.

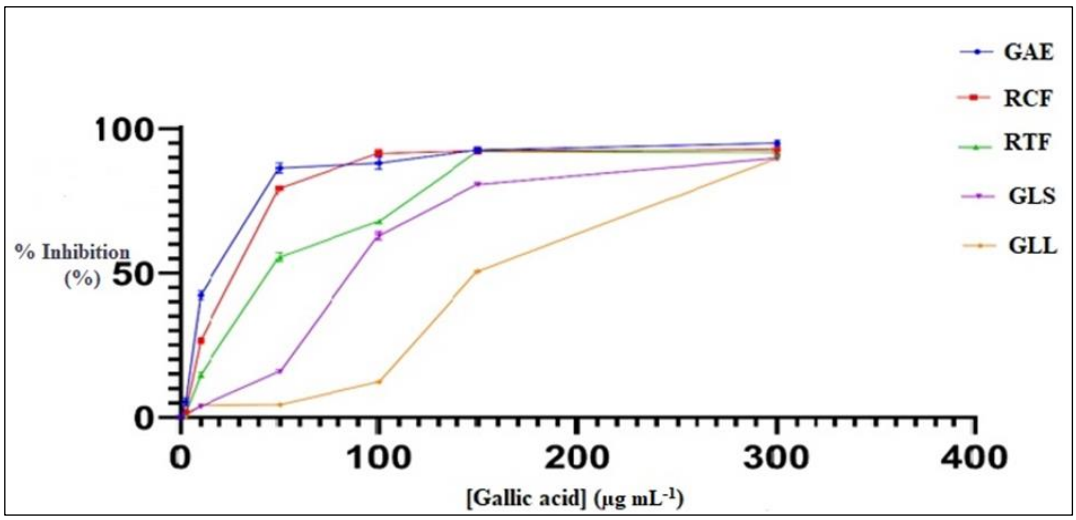

Figure 5. Autumn season IC50 charts. The IC50 of each sample extract was determined with a GraphPad Prism. GAE presents Gallic acid external standard, RCF, RTF, GLS, and GLL are methanolic extracts of red chili fruit, red tomato fruit, green leafy spinach, and green leafy lettuce, respectively.

Table 1. Seasonal variations in the 50% inhibitory concentration of the plant extracts

	IC_{50} (µg mg^{-1} fw)			
	GA = 19.52±9.42			
	Sample extracts (n = 3)			
Seasons	RCF	RTF	GLS	GLL
Winter	98.44±0.42[a]	170.59±4.41[a]	259.29±6.62[a]	286.34±4.44[a]
Spring	69.80±1.20[b]	92.72±2.75[b]	115.00±0.81[b]	175.16±0.52[b]
Summer	18.40±0.05[c]	37.62±0.84[c]	90.74±1.77[c]	152.56±3.75[c]
Autumn	24.19±0.61[d]	47.36±1.29[d]	98.34±1.27[d]	165.28±1.36[d]

IC50 = 50% inhibitory concentration, fw = fresh weight, GA = Gallic acid, RCF = red chili fruit, RTF = red tomato fruit, GLS = green leafy spinach, GLL = green leafy lettuce, and n = number of repeats for each sample extract. Results were presented as a mean±SD. Values with different superscript letters between seasons in a column are significantly ($P<0.05$) different.

The secondary metabolites sometimes called the defensive compounds possessed the capability to defend biotic and abiotic stresses in plants as well as helping plants to survive oxidative stress-mediated damages (Jamloki et al., 2021). Thus, the lower IC50 level detected in the summer could be related to increased synthesis of these defensive compounds. In this study, variations in the IC50 levels of the RCF, RTF, GLS, and GLL extracts can be linked to the influence of seasonal changes.

3.2. Total antioxidant activity

Figure 6 depicts the linear calibration curve for total antioxidant activity. The total antioxidant capacity (TAC) level for each of red chili fruit (RCF), red tomato fruit (RTF), green leafy spinach (GLS), and green leafy lettuce (GLL) extracts was expressed as µg ascorbic acid equivalent (AAE) mg^{-1} fw. A significant ($P<0.05$) difference in the TAC level of the RCF, RTF, GLS, and GLL extracts was detected among the comparative four (4) seasons (Table 2). The TAC level for each RCF, RTF, GLS, and GLL extracts revealed a significant ($P<0.05$) difference in the winter compared with the spring and between the winter and summer (Table 2). Comparably, a significant ($P<0.05$) difference was detected in the winter in comparison to autumn (Table 2). Furthermore, the spring is significantly ($P<0.05$) different from summer (Table 2). Similarly, a significant ($P<0.05$) difference was showed between the spring and autumn (Table 2). Lastly, there was a significant ($P<0.05$) difference in the summer compared with autumn (Table 2).

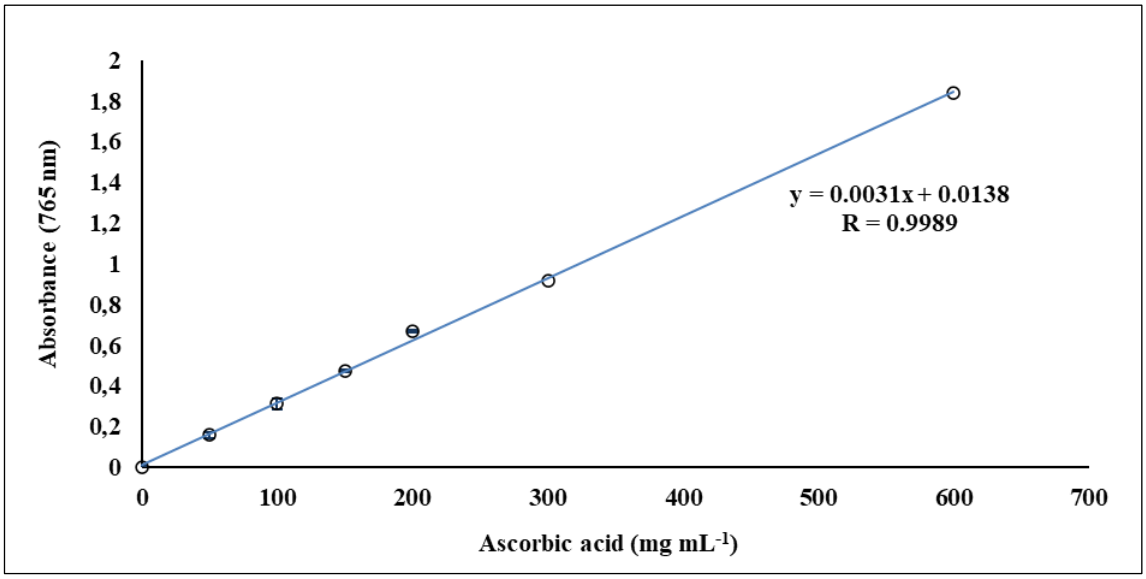

Figure 6. A standard curve for total antioxidant capacity (phospho-molybdenum assay). The standard curve was generated over a concentration range of 50 to 600 mg mL-1 using ascorbic acid as a standard, prepared in the Milli-Q water. Various standard concentrations were evaluated photometrically in triplicate.

Table 2. Seasonal differences in the total antioxidant capacity of the vegetable extracts

	TAC (µg mg^{-1} fw)			
	Sample extracts (n = 3)			
Seasons	RCF	RTF	GLS	GLL
Winter	527.10±0.01^{a}	158.06±0.03^{a}	94.95±0.01^{a}	69.68±0.03^{a}
Spring	566.13±0.03^{b}	179.78±0.01^{b}	122.80±0.03^{b}	107.20±0.01^{b}
Summer	591.18±0.07^{c}	232.37±0.02^{c}	249.57±0.05^{c}	137.42±0.01^{c}
Autumn	503.98±0.10^{d}	225.27±0.03^{d}	180.11±0.05^{d}	98.14±0.01^{d}

TAC = total antioxidants capacity, fw = fresh weight, RCF = red chili fruit, RTF = red tomato fruit, GLS = green leafy spinach, GLL = green leafy lettuce, and n = number of repeats for each sample extract. Results were presented as a mean±SD. Values with different superscript letters between seasons in a column are significantly ($P<0.05$) different.

The summer season revealed the highest TAC level, the RCF extract possessed the highest TAC amount (Table 2). The lowest TAC activity was in the winter period, the GLL extract indicated the lowest activity. There were no reports on the effect of seasonal differences on the TAC level of the RCF, RTF, GLS, and GLL extracts from the aquaponics source. However, Kamath et al. (2015) reported the TAC activity of fresh weight red chilli (40.28±2.18 mg g^{-1}), tomato (8.88±0.73 mg g^{-1}) and spinach (30.49±2.26 mg g^{-1}) extracts sourced from local foodstuff. From the above report, the farming method and cultivar type of the plant material were not indicated. Additionally, Khanam et al. (2012), cited the TAC activity of salad spinach (3.66 µg g^{-1}) and lettuce (11.56 µg g^{-1}) of a dry weight basis from non specified farming system and cultivar, purchased from supermarket. Furthermore, the TAC activity of the fresh tomato fruit from organic (2.59±0.06 mol g^{-1}) and aquaponics (2.87±0.09 mol g^{-1}) farming was detected by Braglia et al. (2022), with unidentified cultivar. Crops culture solution/farming method and cultivar differences influences allelochemicals levels in plants (Nida et al., 1999; Kawaoka and Funabashi, 2020). Variations in TAC levels of the investigated sample extracts are positively associated with seasonal changes.

3.3. Total phenolics content

The total phenolics content standard curve was showed in Figure 7. The total phenolics content (TPC) level of the red chili fruit (RCF), red tomato fruit (RTF), green leafy spinach (GLS), and green leafy lettuce (GLL) extracts was presented as µg Gallic acid equivalent (GAE) mg^{-1} fw. The investigated RCF, RTF, GLS, and GLL extracts indicated a significant ($P<0.05$) difference in the TPC among the comparative four (4) seasons (Table 3).

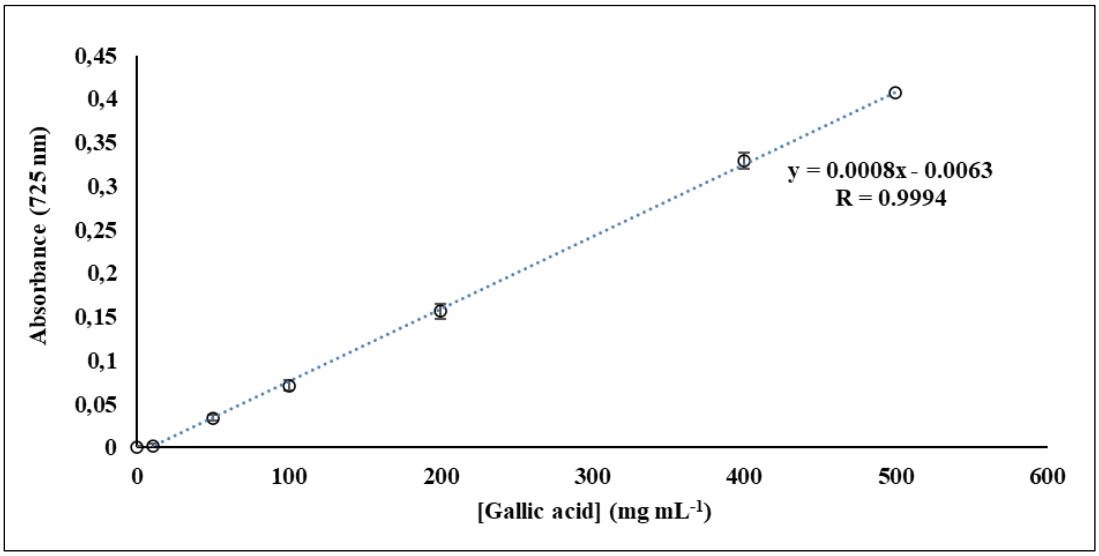

Figure 7. A total phenolics content standard curve. The standard curve was generated over a concentration range of 10 to 500 mg mL^{-1} with gallic acid as an external standard, prepared in the Milli-Q water. Different standard concentrations were determined using Folin-Ciocalteau calorimetric method in triplicate.

There was a significant ($P<0.05$) difference in the RCF, RTF, GLS, and GLL extracts in the winter compared with spring, between the winter and summer, and the winter in comparison to autumn (Table 3). In addition, the spring is significantly ($P<0.05$) different from summer (Table 3). Similarly, a significant ($P<0.05$) difference was detected between spring and autumn (Table 3). The RCF extract had the highest TPC level in the summer season (Table 3). However, the GLL extract indicated the lowest TPC in the winter period. There was no report of the influence of seasonal variations on the TPC of the investigated sample extracts from the aquaponics system. Notwithstanding, Kamath et al. (2015) reported the presence of phenolics in fresh red chili (2.87±0.18 mg g^{-1}), tomato (4.71±0.32 mg g^{-1}), and spinach (5.84±0.42 mg g^{-1}) extracts sourced from the local foodstuff. The cultivar and farming method of samples were not revealed. Moreover, Braglia et al. (2022) detected phenolics from the fresh weight tomato extract sourced from organic (0.61±0.09 mg g^{-1}) and aquaponics (0.41±0.07 mg g^{-1}) farming with different concentration level measurement unit.

Furthermore, Al-Mamary (2002) revealed the presence of phenolics in the fresh weight chili (116.03±2.47 mg 100 g^{-1}), tomato (28.85±0.93 mg 100 g^{-1}), spinach (10.31±1.46 mg 100 g^{-1}), and lettuce (56.45±0.76 mg 100 g^{-1}) extracts sourced from local market. The cultivar name and farming system of the vegetable plants were not provided. Furthermore, Khanam et al. (2012) revealed the presence of phenolics in fresh weight spinach (95.78±2.95 µg g^{-1}) and lettuce (80.84±4.75 µg g^{-1}) plants obtained from supermarket. The farming method and cultivar type were also not revealed. Cultivar type and farming practice determine the secondary metabolite amounts in plants (Nida et al., 1999; Kawaoka and Funabashi, 2020). This study has indicated that seasonal changes have induced differences in the TPC level of the examined sample (RCF, RTF, GLS and GLL) extracts.

Table 3. Seasonal dynamics in the total phenolics content of the plant extracts

	TPC (µg mg^{-1} fw)			
	Sample extracts (n = 3)			
Seasons	RCF	RTF	GLS	GLL
Winter	251.25±0.02^{a}	59.58±0.01^{a}	73.75±0.03^{a}	16.25±0.01^{a}
Spring	404.17±0.01^{b}	100.75±0.04^{b}	137.08±0.01^{b}	65.67±0.03^{b}
Summer	422.50±0.02^{c}	117.17±0.06^{c}	185.42±0.10^{c}	66.00±0.06^{cb}
Autumn	338.33±0.00^{d}	144.00±0.00^{d}	220.17±0.011^{d}	97.08±0.00^{d}

TPC = total phenolic content, fw = fresh weight, RCF = red chili fruit, RTF = red tomato fruit, GLS = green leafy spinach, GLL = green leafy lettuce, and n = number of repeats for each sample extract. Results were presented as a mean±SD. Values with different superscript letters between seasons in a column are significantly ($P<0.05$) different.

3.4. Total flavonoids content

The linear regression curve of the total flavonoid content (TFC) was depicted in Figure 8. Each of red chili fruit (RCF), red tomato fruit (RTF), green leafy spinach (GLS), and green leafy lettuce (GLL) extracts TFC value was calculated as µg quercetin equivalent (QE) mg^{-1} fw. A significant ($P<0.05$) difference was detected in the TFC level of the RCF, RTF, GLS, and GLL extracts among the comparative seasons (Table 4). There was a significant ($P<0.05$) difference in the TFC level of the RCF and RTF between winter and spring, the winter compared with summer, and winter in comparison to autumn (Table 4).

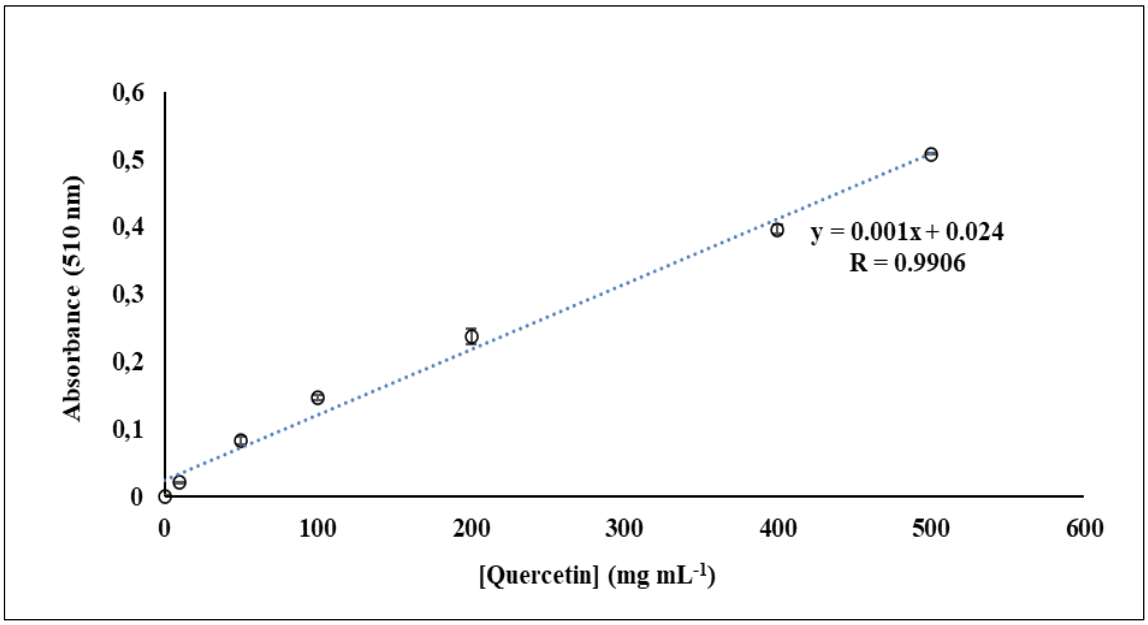

Figure 8. A total flavonoids content standard curve. The standard curve was generated over a concentration range of 10 to 500 mg mL^{-1} with quercetin as a reference standard, prepared in the Milli-Q water. Various standard concentrations were analyzed in triplicate photometrically. A method of $NaNO_2$-$AlCl_3$-NaOH was used.

Table 4. Seasonal variations in the total flavoniods content of the vegetable extracts

	TFC (μg mg^{-1} fw)			
	Sample extract (n = 3)			
Seasons	RCF	RTF	GLS	GLL
Winter	80.33±0.02[a]	11.00±0.01[a]	51.33±0.01[a]	149.67±0.03[a]
Spring	256.33±0.18[b]	18.69-±0.02[b]	134.00±0.02[b]	427.33±0.01[bd]
Summer	272.33±0.01[c]	44.67±0.02[c]	129.00±0.02[cb]	475.33±0.07[c]
Autumn	263.67±0.01[bd]	023.33±0.00[bd]	187.33±0.02[d]	419.00±0.07[d]

TFC = total flavonoids content, fw = fresh weight, RCF = red chili fruit, RTF = red tomato fruit, GLS = green leafy spinach, GLL = green leafy lettuce, and n = number of repeats for each sample extract. Results were presented as a mean±SD. Values with different superscript letters between seasons in a column are significantly (P<0.05) different.

Additionally, a significant (P<0.05) difference was indicated between the spring and summer (Table 4). Furthermore, the summer was significantly (P<0.05) different from the autumn period (Table 4). The GLS extract TFC level indicated a significant (P<0.05) difference in the winter in comparison to spring and winter compared with summer (Table 4). Similarly, the winter was significantly (P<0.05) different from the autumn (Table 4). In addition, a significant (P<0.05) difference was observed in the spring in comparison to autumn and between the summer and autumn (Table 4). The GLL extract TFC amount indicated a significant (P<0.05) difference between the winter and spring, the winter compared with the summer, and the winter in comparison to autumn (Table 4). Lastly, there was a significant (P<0.05) difference in the TFC value of GLL between spring and summer as well as summer compared with autumn (Table 4). The highest TFC amount was detected in the summer (Table 4). The GLL extract revealed the highest TFC level in this period (Table 4). The RTF showed the lowest TFC amount in the winter season. Reports on the effect of seasonal changes on the TFC levels was lacking. However, Khanam et al. (2012), reported the presence of flavonoids in the fresh spinach (102.77±3.95 μg g^{-1}) and lettuce (44.85±1.56 μg g^{-1}) extracts purchased from supermarket. The cultivar name and farming practice of the vegetable plants were not disclosed. Farming system and cultivar variation affects the phytochemical levels in agricultural production (Nida et al., 1999; Kawaoka and Funabashi, 2020). Variation in the TFC concentration level of the researched sample extracts was observed among seasons.

5. Conclusion

This research investigated the effect of seasonal differences on the IC50, total antioxidant capacity (TAC), total phenolics content (TPC), and total flavonoid content (TFC) of the red chili fruit (RCF), red tomato fruit (RTF), green leafy spinach (GLS), and green leafy lettuce (GLL) extracts from the aquaponics system. The summer indicated the highest IC50, TAC, TPC, and TFC amounts compared with winter, spring, and autumn. The RCF extract revealed the highest IC50, TAC, and TPC. While the GLL extract showed the highest TFC value in this season (summer). Thus, seasonal variations have induced changes in the IC50, TAC, TPC, and TFC concentration levels of the RCF, RTF, GLS, and GLL extracts. Therefore, this research findings demonstrated that aquaponics system food production and or harvesting in warmer periods (with increased light intensity and duration) can enhance antioxidants value composition, required for proper growth, development, and protection against diseases when consumed appropriately and sufficiently.

Compliance with Ethical Standards

Conflict of Interest

The author declares no conflict of interest.

Authors' Contributions

The methodology, investigation, conceptualization, data analysis, and data curation were done by the author. In addition, the author write, review, edit, and validate the original draft of this research article.

Ethical approval

Not applicable.

Funding

This research received no financial support.

Data availability

Not applicable.

Consent for publication

Not applicable.

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Modification and performance evaluation of yam peeling machine

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A B S T R A C T

Yam is a versatile crop and plays a vital role in tropical regions, where it can be transformed into various food products. Peeling is an essential step in the processing of yam, as it increases its value. While manual peeling with knives is common in households, larger-scale production requires the use of yam peeling machines. The objective of this study was to analyze and enhance the performance of an existing yam peeling machine. The key components of the machine include the machine frame, a wire mesh-like drum, a speed reduction gear motor, a pumping machine, sprockets, pipes, and a water container. The yam peeler is powered by a three-phase 2 hp electric motor. The machine was tested on three different types of yams with varying moisture contents and at different speeds. Various performance parameters such as peeling efficiency, peeling capacity, flesh loss, and time efficiency were evaluated. Statistical analysis and SPSS models were utilized to examine the relationship between different factors and the performance metrics of the machine. The study revealed that as the peeling speed increased, the efficiency decreased. The peeling efficiency ranged from 63.27% to 92.74%, peeling capacity ranged from 2.28 kg/h to 11.34 kg/h, and flesh loss varied from 7.26% to 36.73%. The moisture content of the yams, peeling speeds, and tuber morphology were found to have significant effects on peeling efficiency, flesh loss, peeling capacity, and time efficiency. The yam peeler production cost is estimated to be $150. The machine demonstrated suitability for small-scale food industries due to its low cost and minimal maintenance requirements.

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1. Introduction

The term "yam" refers to a group of Dioscorea-related tuber crops. Around 72.6 million tons of yams were produced worldwide in 2018 (FAOSTAT, 2018). *Dioscorea rotundata*, *D. alata*, *D. trifida*, *D. polystachya*, and *D. esculenta* are some of the common yam species (Arnau et al., 2010). The most significant yam in the world is the white Guinea yam (*D. rotundata*), which is mostly produced in West and Central Africa, particularly in the "yam belt" countries of Côte d'Ivoire, Ghana, Togo, Benin, Nigeria, and Cameroon (FAOSTAT, 2018). The yam plays significant roles in the lifestyle and culture of the people in the main yam-growing regions and is a staple crop in many tropical nations (Obidiegwu and Akpabio, 2017; Obidiegwu et al., 2020). But because of its limited global distribution, yam has been labeled an "orphan crop" and has gotten far less research funding than other main crop species.

The majority of yam tubers produced have been lost due to manual processing steps caused by a lack of machinery or equipment, which has limited yam processing to a home scale. For most uses, peeling-the process of removing the hard, scaly, brown skin is an essential step in the yam processing process. Because it is challenging to manufacture a peeling machine locally, tuber peeling is best done by hand (IITA, 2008). Most peeling is still done in Nigeria using traditional way. In the traditional method, the yam is peeled using a knife or small cutlass and this led to huge loss of the yam and also be very unhygienic. Due to lack of adequate, reliable and faster means of processing, apart from the manual, most of these yams are wasted. This informs the design of this machine, for effective processing of yam tuber into various food items. The research thus far on the creation of material peeling systems has identified five general peeling techniques: mechanical, human, heat, and abrasive action. Mechanical peeling is the category under which this work fits. Using abrasive techniques, this mechanical peeling may still be divided into manual and automated categories.

Additionally, due to the considerable diversity in root diameters and cortical thickness, existing tuber peeling machines are only moderately efficient and suffer from substantial tuber losses (Egbeocha et al., 2016). As a result, the peel is not correctly or entirely removed. Large tubers have been peeled inefficiently, and in some cases, roots have broken or been crushed (Adetan et al., 2005). Additionally, it had been noted that large-scale tuber peeling operations result in high labor input and substantial processing losses (Ukatu, 2005; Egbeocha et al., 2016).

Some yam peelers have been developed overtime with low efficiency, and appreciably high-speed damage were associated with the operation of the yam peelers and also most of the yam peeler cannot peel all varieties of yam efficiently. Ukatu et al. (2005) developed an industrial yam peeler with three spring-loaded peeler arms and peeler blades that scrape the tuber body to a predetermined depth. The material recovery rate ranged from 82.7 to 88.8%. The efficiency of peeling ranges from 62.7 to 80%. Adetan et al. (2005) designed a spring-loaded cassava peeling machine with five spring-loading points spaced at 140 mm intervals. Onorba (2010) used pressurized steam method to create a home yam peeling machine. restricted to yam tubers with a peeling efficiency of 47.8% that are no longer than 30 cm and have a somewhat curved shape. A yam peeling machine was developed by Adetoro (2012). It has a drum that is eccentrically attached on a shaft and rotates at different speeds, ranging from 20 to 50 rpm. The peeling efficiency, peeling capacity, flesh loss, power rating, and operating speed were 95%, 38.88 kg/ha, 3.90%, 0.973 kW and 20-50 rpm respectively. It cannot be used on yam of less than six tubers of yam. For a yam processing plant, Ayodeji et al. (2014) developed a yam peeling and slicing machine. With an average peeling time of 12.2 seconds, the machine's efficiency was 87-86%. A dual-functioning yam peeling machine was designed by Ojolo et al. (2016). During the peeling process, the machine uses power screw mechanics and spring-loaded peeling knives. The peeling efficiency, peeling capacity, flesh loss, power rating, and operating speed were 71-100%, 218 kg/h, 3.67-14.29%, 1.50 kW and 200 rpm respectively. The yam tuber's diameter has an impact on how well it peels. Bello et al. (2020) developed a yam peeling machine with two functions. It is a revolving drum with wire gauze that is constructed on a frame composed of iron rods and flat bars that are arranged longitudinally for a yam processing plant. In a motorized operation of the yam peeling machine, peeling loss ranged from 3.67-14.29%, while manual operation resulted in peeling loss ranging from 3.91 %-16.96 % respectively.

Ukatu (2005) reported a higher flesh loss of 17.30% while Bello et al. (2020) reported a flesh loss of 20-25%; Fadebiyi and Ajao (2020) achieved a flesh loss of 18% in their multi-tuber peeling machine. Adetoro (2012) achieved a peeling capacity of 38.88 kg/h, while the highest peeling capacity of 920.00 kg/h was achieved by Isa and Olukunle (2021). The peeling efficiency, peeling capacity, flesh loss, power rating, and operating speed were 90%, 700 kg/h, 3.67-14.29 %, 2.50 kW, and 350-750 rpm respectively. The peels or waste are passed through the perforated portion of the drum, while the tubers are fed and discharged through its single opening. Based on the surface scratching principle, Fadebiyi and Ajao (2020) designed and fabricated a batch loading tuber-peeling machine with a capacity of 10 kg/min. The peeling efficiency, peeling capacity, flesh loss, power rating, and operating speed were 40.20-61.10%, 600 kg/h, 18%, 3.0 kW, and 350 - 750 rpm respectively. As the shaft speed increased, so did the amount of flesh loss and the percentage of peel weight (Isa and Olukunle, 2021). The peeling efficiency, peeling capacity, flesh loss, power rating, and operating speed were 62.27-83.16%, 920 kg/h, 14.13%, 5.25 kW, and 1400 rpm respectively. Its performance evaluation revealed that the tuber size and auger-brush speeds had a significant impact on peeling efficiency, material recovery, and tuber loss.

Thus, the development of an efficient mechanical yam peeler with more good output results, cost effectiveness and also a yam peeler that can peel two to three varieties of yam. The development of this yam peeler would reduce the time consumed when peeling manually and also will increase the standard of living of local farmers by increasing the quality of crops produced and also make life easier and more comfortable for them by solving the challenges encountered when peeling the yam manually. For this reason, effective and reasonably priced tuber peeling machines are needed for the critical peeling stage of tuber processing. The purpose of the study is to modify the existing machine and evaluate its performance.

2. Materials and methods

2.1. Description of the existing machine

The yam peeling machine was originally developed in the Agricultural and Environmental Engineering department of the Federal University of Technology, Akure, Ondo State, Nigeria (coordinates: 7.2872° N, 5.1968° E). The machine features a wire mesh-like drum that is 45.3 cm in length and 15.5 cm in diameter and rotates on a shaft that is 92 cm in length and 3 cm in diameter. The distance between the drum-carrying shaft and the gear shaft is 45 cm. Figure 1.1A shows the design of the machine. The exploded view of the machine (1-handle; 2-right drum head; 3-rod; 4-wire gauze; 5-left drum head; 6-v-belt; 7- pulley; 8-motor seal; 9-electric motor; 10-shaft, 11- frame) is generally described in Figure 1.1B. The machine is composed of a rotating drum built with wire gauze wounded on a frame made of iron rods and flat bars in a longitudinal manner.

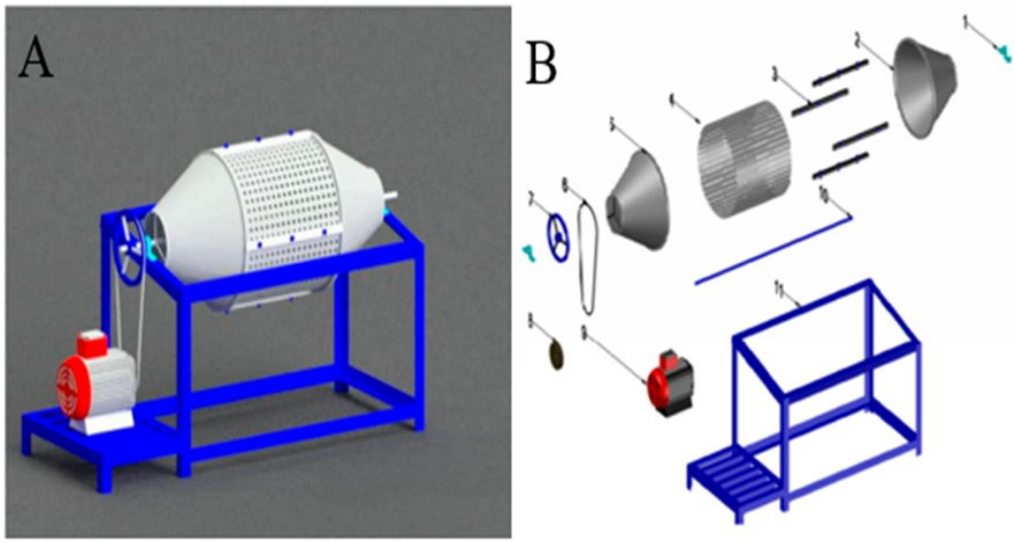

Figure 1. Existing yam peeling machine

A shaft is made to pass through the center of the drum supported at both ends with pillow bearings and at one end is mounted the pulley that enables the belt to be connected to the electric motor supported at the base with another frame. The entire component is placed on a frame support big enough to give the required rigidity. The drum has only one opening where the tubers are fed and discharged while the peels or wastes are passed through the perforated portion.

2.2. Modification of the existing design

In order to enhance the adaptability of the yam peeling machine, several factors were considered during its modification which include availability of locally sourced materials, cost of modification, safety, and selection of corrosion-resistant materials.

The modification carried out on the existing machine includes the introduction of a 0.5 horsepower pumping machine to keep the yam wet and wash away the peeled skin from the yams. The process was repeated until a cleaner peeled yam was obtained. Other modification includes the introduction of recycling water pipes (a 25 mm length pipe with a 1-inch diameter), strong stand to ensure good stability, and sprockets to get the optimum speed for the peeling machine.

2.3. Design analysis

The physical and mechanical variations between the various tuber kinds were taken into account when designing the yam peeling machine. The machine's capacity exceeds that of the human peeling technique, and the materials required to build it are easily accessible. The device drastically minimizes the amount of labor necessary for conventional peeling techniques and does away with the hassle of using a mono-tuber peeler. The peeling chamber, transmission unit, and cleaning mechanism for the redesigned machine are the main parts that need to be designed.

2.3.1. Determination of volume of peeling drum

Jimoh et al. (2012) recommended Equation 1 to determine the volume of the peeling drum.

$$v=\pi (D\text{^}2\ L)/4 \quad (1)$$

where, V is the volume of the peeling drum (mm^3), D is the diameter of the drum in m = 0.0155 m, L is the length of the drum in m= 0.43 m. Therefore volume = 81148.04 ⟦mm⟧^3

2.3.2. Power requirement for peeling tubers

The torque of the peeling machine and the power required to operate the peeling drum, also known as the power to peel the tubers, were calculated using Equations 2 and 3 as recommended by Khurmi and Gupta (2005).

$$T=60P/2\pi N \quad (2)$$

Where T is the torque in Nm, p is the power in kW = 2.238 kW and N is the speed of rotation in rpm. Three different speeds of rotation for the drum were assumed (40 rpm, 50 rpm, and 60 rpm), Therefore torques of 534.21 Nm, 427.37 Nm and 3561.14 Nm were obtained respectively.

$$p=T\ 2\pi N/60 \quad (3)$$

Where, P is the power required to turn the peeling drum in kW = 2.238 kW, T1 is the torque on the drum in Nm = 534.21 Nm at the speed of rotation of 60 rpm, T2 is the torque on the drum in Nm = 427.37 Nm at the speed of rotation of 50 rpm and T3 is the torque on the drum in Nm = 3561.14 Nm at the speed of rotation of 40 rpm respectively.

The torque was determined using Equation 4 as recommended by Khurmi and Gupta (2005).

$$T=Fr \quad (4)$$

Where; T is the torque, F is the force acting on the inner drum wall = mg = mass of yam in kg = 2.89 kg, g is the acceleration due to gravity in 9.81 ms^{-2} and r is the radius of the peeling drum in m = 0.35m. Therefore, torque = 9.92 Nm.

The power requirement was determined using Equation 5 as recommended by Khurmi and Gupta (2005).

$$P=(T \times \omega)/(1000 \times \eta) \quad (5)$$

Where; p is the power in kW, T is the torque in Nm, ω is the angular velocity in rad/s and η is the efficiency in % = 92.74% = 0.9274.

The angular velocity was determined using Equation 6 as recommended by Khurmi and Gupta (2005).

$$\omega=2\pi N/60 \quad (6)$$

Where ω is the angular velocity, and N is the speed of the machine in rpm, which are 40, 50, and 60 rpm. Therefore, angular velocities for the three speeds are 4.18 rad/s, 5.24 rad/s and 6.28 rad/s. Power requirements for the machine at speed 40, 50, and 60 rpm using Equation 5 are 0.045 kW, 0.056 kW and 0.067 kW.

2.4. Component parts of the machine

A general breakdown of the modified machine's parts is shown in Figure 2. The machine consists of a revolving drum made of material that resembles wire mesh and is coiled longitudinally on a frame made of flat bars and iron rods. The drum's center is made out of a shaft that is supported at both ends by pillow bearings. To link the chain to the electric motor, which is supported on a different frame at the base, a pulley is installed at one end of the shaft. To guarantee enough stiffness, the complete unit is placed on a robust frame. Tubers are fed into and removed from the drum by a single entrance, while peels or other waste materials are released through the perforated area.

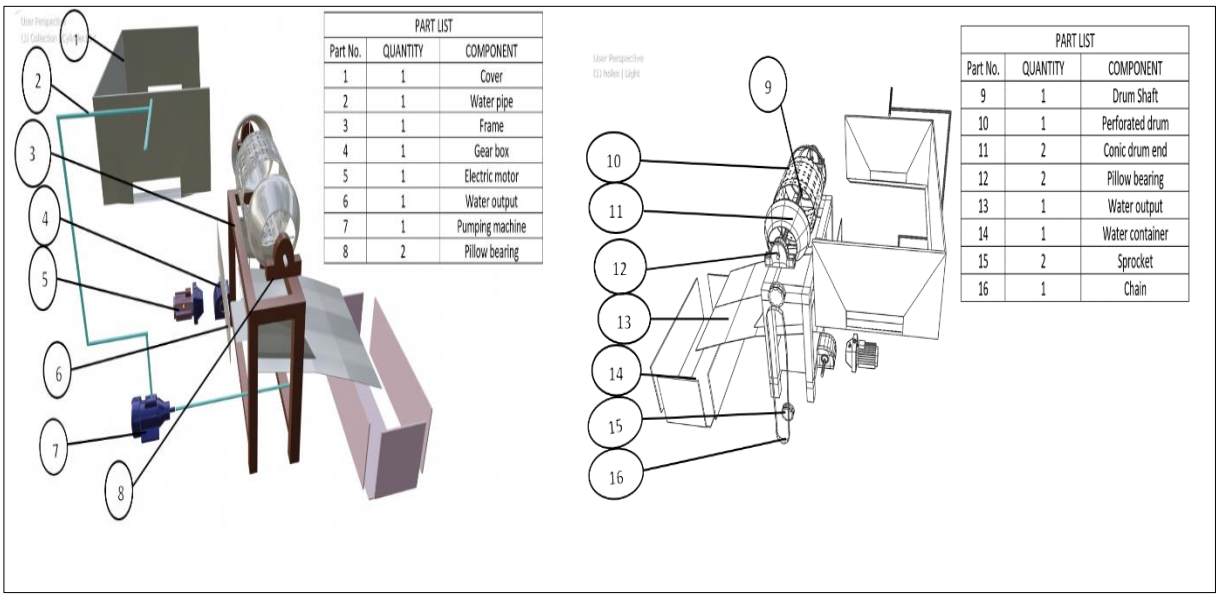

Figure 2. Exploded view of the modified yam peeling machine

2.4.1. Assembly of the new components

The peeling machine utilized various bought-out components, including a sprocket, pumping machine, water container, water pipe, elbow, t-joint, and angle bar iron. To accommodate the water container, angle bar iron was welded onto the four legs of the frame to increase the machine's height. Additionally, a sprocket was affixed to the machine to enable power transmission, and a pumping machine was installed to facilitate the cleaning of yams during the peeling process.

2.5. Construction detailed drawing

The peeling machine's isometric and orthographic projections can be seen in Figure 3, which provides a comprehensive view of the machine's construction.

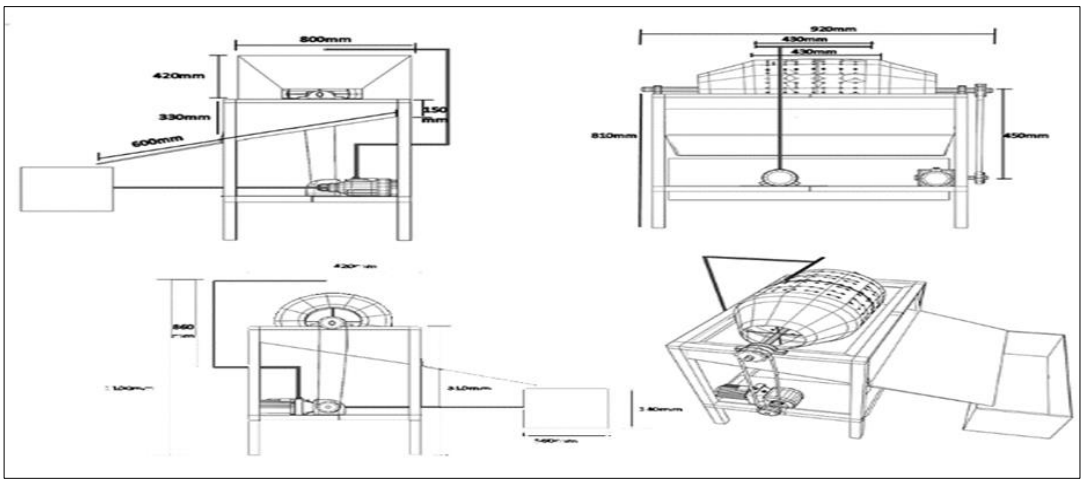

Figure 3. Orthographic view of the modified yam peeling machine

2.6. Performance evaluation of the modified machine

Three varieties of yam - white yam (*Dioscorea rotundata*), white guinea yam (*Dioscorea rotundata*), and water yam (also known as *Dioscorea alata*) - were procured based on their morphological aspect. Straight, fairly cylindrical yams were obtained, sorted by size, and fed into the yam peeling machine to ensure even tuber clearance. The weight of each yam tuber was recorded before and after mechanized peeling, along with the duration of peeling, the mass of peel removed by the machine, and the mass of the tuber after manual peeling. The experiment was conducted in triplicates, with three yam tubers of each variety tested at three different drum speeds (40, 50, and 60 rpm). Moisture content, tuber length, and size were measured following standard procedures, taking into account both transverse and longitudinal sections. The oven-dried technique was used to calculate the moisture content. The performance of the machine was determined based on varieties of yam, moisture content, the speed of the machine and the rate at which it cleans. The following performance indicators were statistically studied which are; capacity (kg/h), efficiency (%), peel loss (%), and time efficiency (kg/h). The peeling duration was set at 10 min, and the peeling speed was varied at 40 rpm, 50 rpm, and 60 rpm. The tests were repeated thrice, and the outcome was measured in terms of the machine's time peeling efficiency in kilograms per hour (kg/h).

2.7. Determination of performance evaluation parameters

2.7.1. Determination of peeling efficiency

The peeling efficiency of the yam peeling machine was calculated as the ratio of its throughput capacity to its theoretical capacity and expressed as a percentage using Equation 7 as recommended by Balami et al. (2012).

$$\varepsilon = \frac{M_{po}}{M_{pr}} \times 100 \quad (7)$$

Where, ε is the peeling efficiency (%), M_{po} is the weight of yam peeled (kg), M_{pr} is the weight of yam fed into the machine (kg).

2.7.2. Determination of peeling capacity

The peeling capacity was determined using Equation 8 as described by Agrawal (1987).

$$W_p = \frac{M_p}{M_o} \times 100 \quad (8)$$

Where, W_p is the peeling capacity (kg/h), M_o is the time taken (h) and M_p is the total weight of tuber (kg).

2.7.3. Determination of flesh loss percentage

The flesh loss percentage refers to the amount of yam that is lost during the peeling process and it was calculated using Equation 9 as described by Agrawal (1987).

$$FL = \frac{M_o - M_f}{M_o} \times 100 \quad (9)$$

Where FL is the flesh loss percentage (%), M_f is the weight of flesh removed (kg) and M_o is the total weight of tuber (kg).

2.7.4. Moisture content determination

The machine was run under different operational parameters, including machine speeds of 40 rpm, 50 rpm, and 60 rpm. The moisture content of each tuber was determined by weighing the initial and final weight using a digital weighing scale. The moisture content of the yam was removed using the oven drying method set at 20°C and the time was set to 10 min at every interval until the value became constant. Equation 10 was used to determine the moisture content.

$$M_c = \frac{W_w - W_D}{W_w}\% \quad (10)$$

Where, M_c is the moisture content, W_w is the weight of tuber, W_D is the weight of dried yam.

2.8. Statistical analysis

The data obtained from the experiment was analyzed using graphical method and with the use of SPSS. In SPSS, one-way ANOVA was used to investigate the effect of speed on peeling efficiency, flesh loss, and capacity for the three varieties of yam.

3. Results and discussion

3.1. Effect of speed and varieties on yam peeling efficiency

Figure 4 illustrates the effect of peeling speed on the peeling efficiency of the machine. The results indicate an inverse relationship between speed and peeling efficiency, whereby as the speed increases, the peeling efficiency decreases. The highest peeling efficiencies were obtained at the lowest speed (40 rpm) with mean values of 92.73%, 82.51%, and 91.00% for white yam (*Dioscorea rotundata*), water yam (*Dioscorea alata*), and white guinea yam (*Dioscorea rotundata*), respectively. At a speed of 50 rpm, the mean peeling efficiency dropped to 84.83%, 73.51%, and 83.67% for the respective yam varieties. The lowest mean peeling efficiencies of 74.90%, 63.26%, and 72.04% were recorded at the highest speed (60 rpm) for all moisture content levels (64.93%, 75.76%, and 66.95%). The highest peeling efficiency was at the highest with water yam (*Dioscorea alata*) having 75.76% and white yam (*Dioscorea rotundata*) having the moisture content of 64.93% while white guinea yam (*Dioscorea rotundata*) has 66.9% this shows that moisture content of yam tuber has influence on the peeling efficiency of the machine. These findings suggest that to achieve optimal peeling efficiency, yam peeling requires low machine speeds, which is consistent with the observations made in previous studies (Olukunle, 2012; Jimoh et al., 2014; Isa and Olukunle, 2021) regarding the correlation between increased mechanical damage and loss of yam ground tissue at higher machine speeds. According to Ojolo et al. (2016), the mass of the tubers is one more factor that could influence peeling efficiency in addition to speed. Ukatu (2005) reported that the diameter of the tuber has no bearing on peeling efficiency, Adetoro (2012) proposed that peeling efficiency is based on the size of the tuber. This disparity may be due to the various methods used in designing the yam peeling machine. The modified machine used in this study exhibited higher peeling efficiency compared to the initial machine developed.

The current research achieved a peeling efficiency range of 63.27-92.74%. The variation in peeling efficiency could be attributed to differences in the design and operating conditions of the yam peeling machine used in each study, as well as the yam varieties and properties used. However, the current research's peeling efficiency is relatively consistent with the results reported in other recent studies. Therefore, the yam peeling machine used in this study can be considered efficient in terms of peeling performance. The peeling efficiency mean ranged from 72.04 %-92.7%, with the White yam (*Dioscorea rotundata*) variety achieving the highest peeling efficiency of 92.7% at a machine speed of 40 rpm and moisture content of 64.93% respectively. This is because White yam (*Dioscorea rotundata*) has a fairly rough skin. The water yam (*Dioscorea alata*) variety recorded the lowest peeling efficiency of 63.26% at a machine speed of 60 rpm with a moisture content of 75.76%.

The soft skin and high moisture content of water yam (*Dioscorea alata*) result in the lowest peeling efficiency. The graph illustrates that the peeling efficiency of the machine increases at a machine speed of 40 rpm but declines as the speed increases to 50 rpm and 60 rpm. The results obtained in this study is higher than the ones obtained in the previous machine.

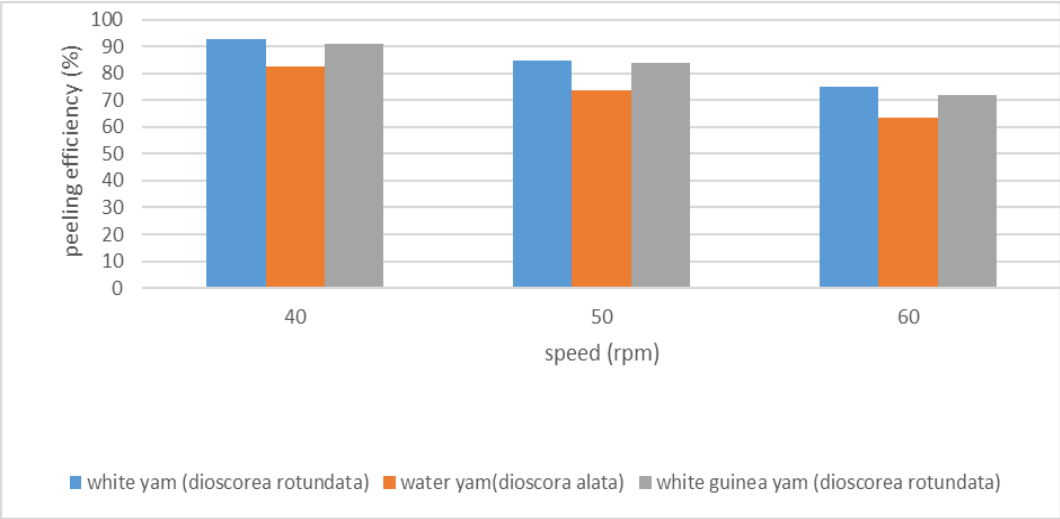

Figure 4. Effect of speed and varieties on yam peeling efficiency

3.2. Effect of speed on the flesh loss of yam

Figure 5 illustrates the impact of yam peeling machine speed on the flesh loss of different yam varieties. Overall, there is a direct relationship between the speed of the machine and the amount of flesh loss during peeling. The highest flesh loss was observed at the highest speed (60 rpm), with mean values of 25.09%, 36.73%, and 27.96% for white yam (*Dioscorea rotundata*), water yam (*Dioscorea alata*), and white guinea yam (*Dioscorea rotundata*), respectively. At a speed of 50 rpm, the flesh loss decreased to 15.16%, 26.48%, and 16.32% for the respective yam varieties. The lowest flesh loss of 7.26%, 17.48%, and 9.00% were recorded at the lowest speed (40 rpm) for all moisture content levels (64.93 %, 75.76%, and 66.95%). The findings suggest that to minimize flesh loss during yam peeling, it is preferable to use lower machine speeds. This finding is consistent with previous studies conducted by Isa and Olukunle (2021), which indicated that tuber loss increases with higher brush speeds and abrasive strength. Ojolo et al. (2016) found no clear correlation between the diameter or mass of the tubers and peeling loss.

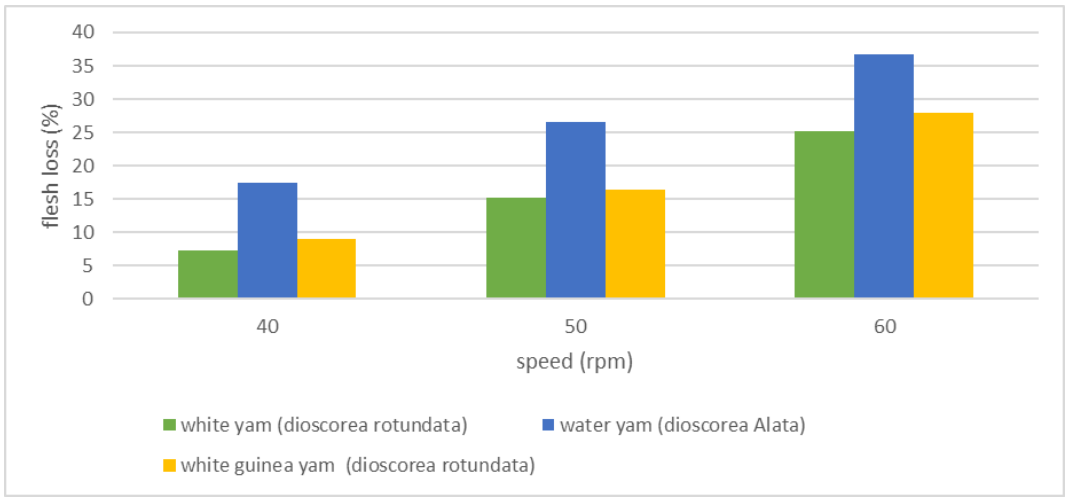

Figure 5. Effect of speed on the flesh loss of yam

Isa and Olukunle (2021) confirmed a direct relationship between abrasive force, speed of the peeling brush, and tuber loss. The current research showed a range of 7.26-36.73% for flesh loss. The variation in flesh loss across all studies can be attributed to differences in yam varieties and the methodology employed in designing the yam peeling machines.

The flesh loss ranged from 7.26-36.73%, with the Water yam (*Dioscorea alata*) variety recording the highest flesh loss of 36.73% at a machine speed of 60 rpm and 75.76% moisture content. The morphology of water yam (*Dioscorea alata*) might have a direct effect on the high flesh loss and the soft skin of the yam. In contrast, the white yam (*Dioscorea rotundata*) variety recorded the lowest flesh loss of 7.26% at a machine speed of 40 rpm. The graph illustrates an increase in flesh loss with higher machine speeds of 60 rpm, followed by a decline as the speed reduces to 50 rpm and 40 rpm. These findings suggest that yam peeling should be carried out at lower machine speeds to achieve lower flesh loss, which is consistent with previous studies by Isa and Olukunle (2021) who found that tuber loss increases with abrasive strength at higher brush speeds. The results obtained in the modified machine is higher than the ones obtained in the previous machine.

3.3. The effect of speed on the time efficiency

The graph in Figure 6 shows the relationship between the speed of the machine and the meantime efficiency. It can be seen that the highest time efficiency was recorded at the highest speed (60 rpm), with values of 8.49 kg/h, 2.13 kg/h, and 4.80 kg/h for white yam (*Dioscorea rotundata*), water yam (*Dioscorea alata*), and white guinea yam (*Dioscorea rotundata*), respectively. At a speed of 50 rpm, the mean peeling efficiency dropped to 7.38 kg/h, 1.68 kg/h, and 5.37 kg/h for the respective yam varieties, with Water yam (*Dioscorea alata*) having the lowest time efficiency. On the other hand, at speed 40 rpm, the results show 8.07 kg/h, 3.39 kg/h, and 5.73 kg/h for all moisture content levels (64.93%, 75.76%, and 66.95%).

The results suggest that the time efficiency of the yam peeling machine varies significantly with the peeling speed. Water yam (*Dioscorea alata*) has the lowest time efficiency due to the soft skin and high moisture content which made it easy for the machine to peel it much faster following white guinea yam (*Dioscorea rotundata*) and white yam (*Dioscorea rotundata*). Ojolo et al. (2016) found that a motorized yam peeling machine significantly reduced the time required for peeling compared to manual peeling. At the lowest speed of 40 rpm, the machine achieved a relatively consistent peeling efficiency, while at higher speeds of 50 rpm and 60 rpm, the efficiency was more variable. These findings indicate the importance of selecting an appropriate peeling speed to optimize the performance of the yam peeling machine. The graph illustrates that the peeling efficiency of the machine varies as the machine speed changes from 60 rpm to 50 rpm and 40 rpm. The results obtained for the time efficiency in the modified machine is higher than the ones obtained in the previous machine.

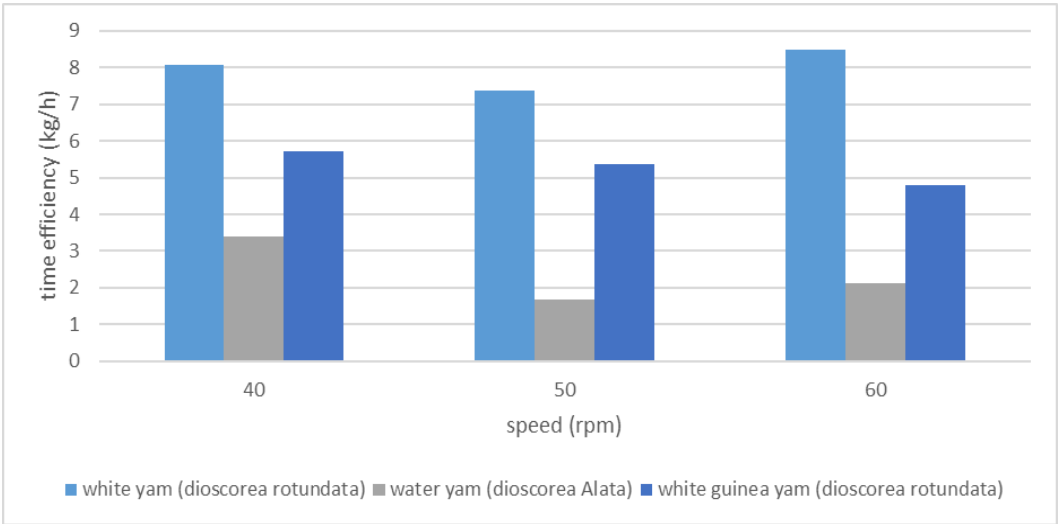

Figure 6. The effect of speed on the time efficiency

3.4. Impact of speed on yam peeling capacity

From Figure 7 it can be observed that the peeling capacity of the yam peeling machine is affected by the peeling speed. The peeling capacity is the highest at the highest speed (60 rpm) and decreases as the speed decreases (50 rpm and 40 rpm). For example, at the highest speed, the mean peeling capacity ranges from 3.36 kg/h to 11.34 kg/h for the different yam varieties, while at a speed of 50 rpm, it ranges from 2.28 kg/h to 8.70 kg/h, with water yam (*Dioscorea alata*) having the lowest peeling capacity. At a speed of 40 rpm, the mean peeling capacity was 8.70 kg/h, 4.11 kg/h, and 6.30 kg/h for all moisture content levels (64.93%, 75.76%, and 66.95%). The results indicate that the peeling capacity of yam peeling machines has indeed varied widely in previous research studies since 2012. The peeling capacity of the machines ranged from 38.88 kg/h to 920.00 kg/h, depending on the specific study. The current research achieved a peeling capacity ranging from 2.28 kg/h to 11.34 kg/h. The findings also highlight that the peeling capacity significantly differs among the studies, which can be attributed to various factors such as the design of the machine, the type of yam being peeled, and the specific operating conditions used in each study. Therefore, when designing or selecting a yam peeling machine, it is crucial to carefully consider the optimal peeling capacity required to meet the desired processing capacity for the specific type of yam being utilized.

Therefore, it can be concluded that the higher the peeling speed, the higher the peeling capacity, as more yam can be peeled per hour at a faster speed. However, it is important to note that the peeling capacity also depends on other factors, such as the moisture content, skin toughness, shape, and varieties of the yam. The graph illustrates that the peeling capacity of the machine varies at a machine speed of 60 rpm to 50 rpm and 40 rpm.

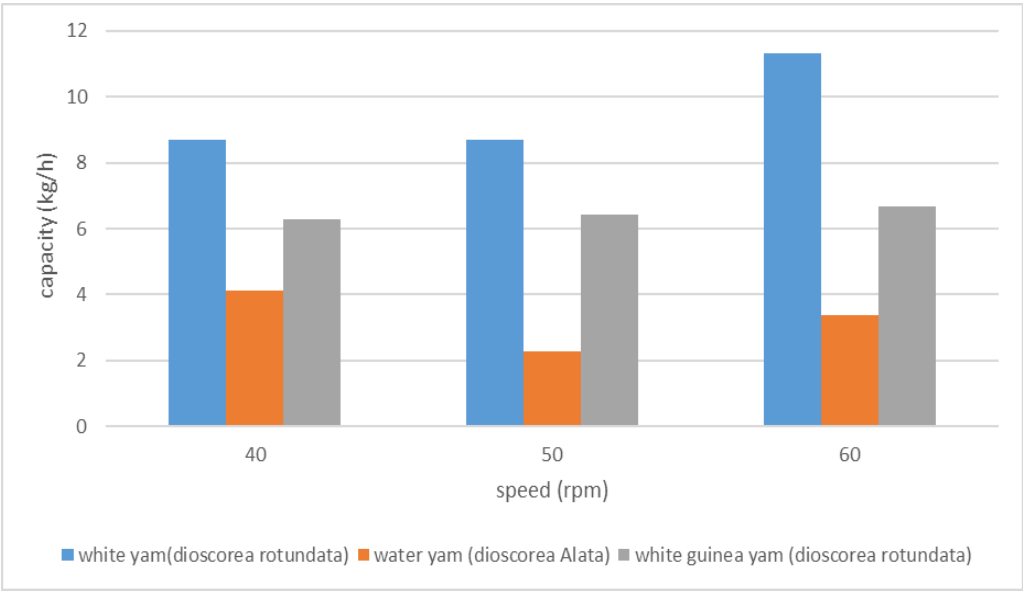

Figure 7. Impact of Speed on Yam Peeling Capacity

4. Conclusions

The peeling efficiency and capacity of the yam peeling machine are influenced by the operating speed and the variety of yam being peeled. The study found that the lowest speed of 40 rpm achieved relatively consistent peeling efficiency while higher speeds of 50 rpm and 60 rpm resulted in more variable efficiency. The highest peeling capacity was recorded at the highest speed (60 rpm), with mean values ranging from 3.36 kg/h to 11.34 kg/h, while the lowest capacity was recorded at a speed of 50 rpm for water yam (*Dioscorea alata*). In addition, the operating speed and yam varieties had a significant impact on the peeling efficiency and capacity with a p-value less than 0.05 which suggests that it is important to select an appropriate peeling speed and yam variety to optimize the performance of the yam peeling machine.

5. Conclusion and recommendations

However, further research could be conducted to optimize the design and operating conditions of the yam peeling machine to achieve even higher peeling efficiency. Additionally, it is essential to consider factors such as cost-effectiveness, ease of use, and maintenance requirements when designing yam peeling machines for commercial use.

Compliance with Ethical Standards

Conflict of Interest

As the author of article declare that there are no conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' Contributions

Olufemi Adeyemi ADETOLA: Conceptualization, writing original draft, review, and editing and validation.

Idris Ajibola MUSTAPHA: Investigation, methodology, formal analysis, writing original draft and data curation.

Ethical approval

Not applicable.

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Data availability

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Consent for publication

We humbly give consent for this article to be published.

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Research Article

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Effects of different types of dried fruit on sensory and texture properties of white cheese

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A B S T R A C T

In the study, five kinds of white cheese produced, one of which was a control sample (CC). According to the amount of curd used in the production of cheese, black mulberry (C1), blackberry (C2), black grape (C3) and raspberry (C4) dried fruit added to the curd at a rate of 2%. The cheeses packed with a vacuum packaging machine and left to ripening for 90 days at 7±1°C. During the ripening period (3rd, 30th, 60th, 90th days) samples were taken from cheeses, sensory analyses were performed, and textural parameters including resilience, hardness, springiness, gumminess, cohesiveness, adhesiveness, and chewiness were determined using texture profile analyses (TPA). It was observed that neither cohesiveness, adhesiveness and springiness parameters of texture profile differed in terms of cheese type, nor important statistical difference was identified ($p>0.05$), differences regarding the ripening process found out though ($p<0.05$).

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1. Introduction

With their high antioxidant capacity, unique taste, color and smell, raspberries, blackberries, black grapes and black mulberries have a wide range of uses in the field of food. In the last few decades, there has been a constant increase of rich sources of biochemical compounds with health benefits popularity and interest regarding research of all fruit species. Fruits have a special importance among other fruits due to their unique color, taste and smell, rich vitamin and mineral contents and various usage possibilities in the food industry (Güneşli et al., 2019). Dried fruits are a concentrated form of fresh fruits, although they have lower moisture content than their fresh counterparts, as most of their moisture content has removed through various modern drying techniques such as sun drying or mechanical devices (Chang et al., 2016). The fruit pineapple, peach and pear are added to fresh cheese to obtain cheese desserts of different flavor variations and thus offer a product that would approach consumers, especially children, who are eating more and more unhealthy foods at the time (Brčina et al. 2017). When we look at the fruit types, berries such as black mulberries, raspberries, blackberries, and black grapes distinguished from other fruits by the organic and inorganic components in their chemical composition, and thanks to their high antioxidant structures, they reduce the damage caused by oxidative stress in the body (Tosun and Yüksel, 2003). The phenolic compounds contained in black mulberry increase body of resistance against diseases with their high antioxidant properties. Its high antioxidant content is due to the anthocyanins found in the composition of black mulberry. Anthocyanins give black mulberry its red color. When consumed, black grapes, which contain fruit sugars used as an energy source in the body, help the body to recover the energy it spends during the day in a short time. It contains color pigments, phenolic substances, flavonoids, flavones and vitamins, and its fibrous structure is higher than other fruit types. Anthocyanins constitute most of the phenolic compounds found in blackberries. They used in the food industry to produce functional products with high antioxidant capacity and to increase the shelf life of products (İstek et al., 2021). Raspberry, which has sweet, red fruits that ripen in summer and autumn, belongs to the Rosacea family in the berry group. The aim of this study is to create a new cheese variety with the addition of dried black mulberry, blackberry, black grape and raspberry and to determine the nutritional, sensory, functional, aroma and structural properties of these cheeses.

2. Materials and methods

Cow milk obtained from a local dairy plant in Ordu, Türkiye. Commercial rennet (1/16000) obtained from Mayasan Company®, Istanbul. All dried fruit samples were products of Bağdat Baharat Company, Kahramanmaraş, Türkiye.

2.1. Cheesemaking

Raw milk pasteurized at 75 °C for 30 s and cooled to 32 °C. Then milk coagulated with rennet for 75 min. After coagulation, the curd cut into 8-10 mm cubes with a wire knife and pressed for 120 min. According to the amount of curd used in the production of cheese, black mulberry, blackberry, black grape and raspberry dried fruit added to the curd at a rate of 2%. Five kinds of white cheese produced, one of which was a control sample (CC), black mulberry (C1), blackberry (C2), black grape (C3) and raspberry (C4) dried fruit added. After the pressing process was completed, the cheeses were removed from the cloth and dry salted to 4% salt by weight. The cheeses packed with a vacuum packaging machine and left to mature for 90 days at 7±1°C. During the ripening period (3^{rd}, 30^{th}, 60^{th}, 90^{th} days) samples were taken from cheeses, sensory analyses were performed, and textural parameters including resilience, hardness, springiness, gumminess, cohesiveness, adhesiveness, and chewiness were determined using texture profile analyses (TPA). Two replicates of cheese samples were prepared for each cheese type.

2.2. Texture profile analysis

For the texture analysis, the temperature of the cheeses adjusted to 20±2 °C. The cheeses cut into cubes with the dimensions of 20x20x20 mm with a cutting wire. Texture profile analyses (hardness, springiness, gumminess, cohesiveness, adhesiveness, resilience and chewiness) of cheese samples performed using TA-XT2 (Stable Micro Systems Ltd., Surrey, UK). Analysis conditions: P/36 aluminum cylinder probe (36 mm diameter, AACC) and cell strength 25 kg weight, test speed 0.4 mm/s, initial test speed 1.0 mm/sec, print 40 %, hold time 5 s (Everard et al., 2006).

2.3. Sensory analysis

In the study, five kinds of white cheese produced, one of which was a control sample, other four samples, black mulberry, blackberry, black grape and raspberry dried fruit added to cheese curd. Sensory evaluations of cheese with dried fruit made by a panel of ten graduate students, experienced in the sensory evaluation of cheeses. Before evaluation, each cheese cut into 20 g cubes, left at room temperature (20 °C) for 2 h, and randomly served to the panelists. Overall sensory quality was assessed using a hedonic scale method (1-10 points), with 1 being unacceptable and 10 being very good for color and appearance, smell, structure and texture, taste and flavor. The panelists given a glass of water to rinse their mouths between cheese samples (Gezmiş and Tarakçı, 2020). Panelists also asked to report any flaws in color and appearance, texture, odor, taste, and overall acceptability.

2.4. Statistical analysis

All analyses performed in duplicate. Minitab 16.0 Statistical Software (Minitab Inc.) used for all statistical calculations, and the results presented as mean ± standard deviation. Analysis of variance (ANOVA) used to determine significance, followed by Tukey's multiple range tests. The significance level of $p<0.05$ was used for statistical differences.

3. Results and discussion

3.1. Texture profile analysis (TPA) in cheese samples

Texture profile analysis (TPA) used to obtain information about the physical and sensory properties of cheeses. With texture profile analysis, properties of cheeses such as flexibility, hardness, springiness, gumminess, cohesiveness, adhesiveness, stability, and chewiness are examined (Günasekaran et al., 2003). In the other method, cheeses placed on the device probes with the help of devices and information about their texture obtained by applying pressure on them in various ways. In texture analysis, properties vary depending on the type of cheese, and changes occur in sensory properties (Hort and Grysç, 2001).

3.2. Hardness values in cheese samples

Hardness defined as the pressure that applied between the teeth to shatter and deform the cheese (Baysal, 2019). To determine the hardness level of the cheese, a sample placed in the device and the first compression process is applied. This compressive force applied on the cheese is an indicator of the hardness level of the cheese. Hardness varies depending on the salt, water, pH and acidity values in the cheese and the ripening time (Özcan and Delikanlı, 2011). The change in hardness values of cheeses with various dried fruits added to the curd during their ripening periods is shown in Table 1. In the study, the hardness values of cheeses with added dried fruits, analyzed during their ripening periods, were found to be between 15.28 and 28.52. The highest hardness degree was measured in the C4 sample on the 5^{th} d, and the lowest hardness degree was measured in the control sample on the 90^{th} d. When the hardness values analyzed during the ripening periods of cheeses with added dried fruit are evaluated statistically; In the variation analysis, variety and period factors affected the hardness values of the cheeses significantly ($p<0.05$) independently of each other. When variety and period factors were evaluated together, their effects on the hardness values of cheeses were found to be significant ($p<0.05$). It is thought that the decrease in the hardness values of cheeses is affected by factors such as ripening time, ripening conditions, type of milk used and cheese production technique. It is also thought that proteolysis reactions during cheese ripening will affect the hardness values (Okumuş, 2016, 2019).

3.3. Adhesiveness values in cheese samples

Adhesiveness defined as the degree to which food deforms in the mouth without falling apart or breaking (Altuğ and Demirbağ, 1993). The change in the adhesiveness values analyses during the ripening period of cheeses produced by adding dried fruit to curd shown in Table 1. The adhesiveness degrees of the cheeses varied between -1.29 and -26.74 during ripening periods. When the stickiness values of cheeses with added dried fruits are evaluated statistically during their ripening period; In the variation analysis, the effect of type and period factors on the adhesiveness values of cheeses was not found to be significant ($p<0.05$), regardless of each other.

When variety and period factors were evaluated together, their effects on the adhesiveness values of cheeses were found to be significant ($p<0.05$). It thought that the increase in stickiness values in the samples is due to the high acidity rates of dried fruit varieties affecting the cheese and decreasing the pH values. Studies show that cohesiveness decreases, and adhesiveness increases at the end of the ripening period in cheeses. Proteolysis during ripening periods, increased lipolysis levels and moisture content of the cheese are factors that affect stickiness.

3.4. Cohesiveness values in cheese samples

Cohesiveness, it refers to the pressure that must be applied to break down a semi-soft food product (Akan and Kinik, 2018). The change in the cohesiveness values of cheeses with added dried fruits analyses during their ripening periods, shown in Table 1. In the statistical study, the consistency values during the ripening times of cheeses with added dried fruits varied between 0.25 and 0.83. While the highest cohesiveness measured in the control sample and the C2 sample on the 5th d, the lowest cohesiveness value measured in the C4 sample on the 90th d. When the cohesiveness values of dried fruit added cheeses analyses during their ripening periods are evaluated statistically; In the variation analysis, variety and period factors significantly ($p<0.05$) affected the cohesiveness values of the cheeses independently of each other. When variety and period factors were evaluated together, their effects on the cohesiveness values of cheeses were found to be significant ($p<0.05$).

Cohesiveness, it is an indicator of the strength and durability of the bonds in the internal structure of the cheese. There is a positive relationship between the moisture content of cheeses and their cohesiveness values. As the ripening time of cheese samples increases, the cohesiveness values decrease. This decrease is due to the increase in the levels of proteolysis and lipolysis that occur during the ripening process and the decrease in the rates of casein and peptide, which are breakdown products (Çelebi and Şimşek, 2020).

3.5. Springiness values in cheese samples

Springiness stated as the rate at which the cheese returns to its original form after the first compression (Tarakçı and Bayram, 2020). Akan and Kinik (2018) stated that the amount of salt added to cheese reduces the springiness of cheese samples during the storage process.

The change in the springiness values analyses during the ripening period of cheeses with added dried fruit shown in Table 1. Elasticity values analyses during ripening periods of cheeses with added dried fruits varied between 0.66 and 0.94. The highest springiness value measured in the control sample on the 5th d, and the lowest value measured in the C2 sample on the 90th d. When the springiness values during the ripening periods of cheeses with added dried fruits are evaluated statistically; In the variation analysis, variety and period factors affected the springiness values of the cheeses significantly ($p<0.05$) independently of each other. When variety and period factors were evaluated together, the effects of cheeses on springiness values were found to be significant ($p<0.05$).

3.6. Gumminess values in cheese samples

It defined as the energy required breaking down a semi-solid food into swallow able sizes (Kahyaoglu et al., 2005). The change in the gumminess values of cheeses with added dried fruit analyses during their ripening periods, shown in Table 1. The gumminess values of cheeses with added dried fruits, measured in textural analysis, varied between 9.86-28.85. The highest gumminess value measured in the C2 sample on the 5th d, and the lowest value measured in the control sample on the 3rd d. When the gumminess values of cheeses with added dried fruits, analyses during their ripening periods, are evaluated statistically; In the variation analysis, variety and period factors independently affected the gumminess values of the cheeses significantly ($p<0.05$). When variety and period factors were evaluated together, their effects on the gumminess values of cheeses were found to be significant ($p<0.05$). The gumminess values of the control sample were lower than the values of cheeses with added dried fruit. The gumminess values of cheeses are affected by factors affecting hardness, internal and external stickiness. The gumminess values of the cheeses generally increased until the 60th d of ripening and decreased in the following period.

3.7. Chewiness values in cheese samples

The degree of chewiness defined, as the pressure-energy required deforming the product and breaking it into

pieces and putting the disintegrated product into a suitable shape for swallowing. It has observed that the degree of chewiness in some cheeses increases as the protein content increases (Erbay et al., 2010). The change in the chewiness values of cheeses with added dried fruit analyses during their ripening periods, shown in Table 1. Chewiness degrees of cheeses with added dried fruit varied between 10.36 and 26.60. The highest chewiness value measured in the C2 sample on the 3rd d, and the lowest chewiness value measured in the control sample on the 3rd d. The most important factor in chewiness is the moisture content of the cheese. The fat and protein ratio of cheese also affects its chewiness values. When the chewiness values of cheeses with dried fruit added, analyses during their ripening periods, are evaluated statistically; In the variation analysis, while the variety factor significantly affected the chewiness values of the cheeses ($p<0.05$), the period factor did not have a significant effect on the chewiness values of the cheeses ($p>0.05$). When the variety and period factors were evaluated, their effects on the chewiness values of cheeses were found to be significant ($p<0.05$).

Table 1. Changes texture profile values during the ripening of white cheeses

	Cheese Types	Ripening Times (Days)			
		3	30	60	90
Hardness	CC	24.17±0.54$^{c, B}$	23.20±0.057$^{b, C}$	23.70±1.20$^{b, C}$	15.28±0.32$^{a, A}$
	C1	26.95±0.18$^{c, C}$	16.61±0.83$^{a, A}$	19.63±0.31$^{b, A}$	17.26±0.68$^{a, A}$
	C2	22.24±0.53$^{c, A}$	26.43±0.56$^{d, D}$	21.27±0.40$^{b, A}$	19.06±1.09$^{a, B}$
	C3	24.30±0.49$^{b, B}$	20.35±0.43$^{a, B}$	27.20±0.06$^{c, D}$	20.01±0.67$^{a, C}$
	C4	28.52±0.42$^{d, D}$	24.95±0.23$^{b, C}$	22.16±1.14$^{a, B}$	27.15±0.10$^{c, D}$
Adhesiveness	CC	-1.85±0.16$^{d, BC}$	-16.87±0.36$^{a, B}$	-15.13±0.34$^{b, BC}$	-12.01±0.12$^{c, C}$
	C1	-3.97±0.18$^{b, A}$	-18.96 ±1.17$^{a, AB}$	-22.14±2.34$^{a, AB}$	-17.03±0.25$^{a, B}$
	C2	-3.24±0.33$^{b, AB}$	-22.41±0.77$^{a, C}$	-25.46±2.88$^{a, A}$	-21.86±0.41$^{a, A}$
	C3	-1.29±0.26$^{b, C}$	-26.74±4.41$^{a, A}$	-18.41±1.66$^{a, AB}$	-18.32±0.42$^{a, B}$
	C4	-4.39±0.64$^{c, A}$	-15.09±0.99$^{a, B}$	-10.79±0.01$^{b, C}$	12.33±0.40$^{ab, C}$
Cohesiveness	CC	0.83±0.005$^{c, B}$	0.45±0.1$^{a, B}$	0.61±0.002$^{b, A}$	0.51±0.01$^{ab, BC}$
	C1	0.80±0.01$^{c, B}$	0.40 ±0.01$^{a, B}$	0.74±0.09$^{bc, C}$	0.58±0.00$^{b, C}$
	C2	0.83±0.02$^{d, B}$	0.28±0.00$^{a, A}$	0.62±0.00$^{c, AB}$	0.44±0.02$^{b, BC}$
	C3	0.80±0.03$^{d, B}$	0.47±0.00$^{b, B}$	0.69±0.04$^{c, ABC}$	0.38±0.07$^{a, AB}$
	C4	0.51±0.06$^{b, A}$	0.61±0.02^{c,C}	0.73±0.02$^{d, BC}$	0.25±0.00^{a,A}
Springiness	CC	0.94±0.002$^{b, C}$	0.90±0.07$^{ab, A}$	0.89±0.007$^{ab, A}$	0.88±0.021$^{a, B}$
	C1	0.84±0.004$^{b, A}$	0.81±0.012$^{b, A}$	0.82±0.06$^{b, A}$	0.66±0.08$^{a, A}$
	C2	0.92±0.008$^{a, C}$	0.90±0,.57$^{a, A}$	0.85±0.043$^{a, A}$	0.86±0.006$^{a, B}$
	C3	0.89±0.001$^{a, B}$	0.86 ±0.021$^{a, A}$	0.83±0.027$^{a, A}$	0.84±0.038$^{a, B}$
	C4	0.92±0.007$^{b, C}$	0.86±0.021$^{b, A}$	0.88±0.000$^{b, A}$	0.75±0.023$^{a, AB}$
Gumminess	CC	9.86±0.53$^{a, A}$	12.49±0.08$^{b, A}$	11.64±0.24$^{b, A}$	11.61±0.39$^{b, A}$
	C1	13.1±0.86$^{a, B}$	17.5 ±0.35$^{b, B}$	27.01±0.11$^{d, D}$	24.8 ±0.35$^{c, D}$
	C2	28.85±0.12$^{b, D}$	24.17±0.45$^{a, C}$	24.70±0.99$^{a, C}$	21.64±1.63$^{a, CD}$
	C3	19.15±0.70$^{a, C}$	19.13±0.93$^{a, B}$	18.26±0.60$^{a, B}$	18.36±0.73$^{a, BC}$
	C4	14.07±0.29$^{b, B}$	11.33±0.12$^{a, A}$	13.55±0.46$^{b, A}$	15.68±0.18$^{c, B}$
Chewiness	CC	10.36±0.167$^{a, A}$	17.99±0,793$^{b, B}$	25.21±0.80$^{c, C}$	22.11±1.10$^{c, C}$
	C1	19.18±0.16$^{b, C}$	19.15±0,31$^{b, B}$	22.08±0.72$^{c, B}$	17.76±0.14$^{a, B}$
	C2	26.60±0.15$^{b, D}$	22.01±0,89$^{a, C}$	21.82±1.09$^{a, B}$	25.44±2.07$^{b, C}$
	C3	19.15±0.70$^{a, C}$	19.13±0,93$^{a, B}$	18.26±0.60$^{a, A}$	18.36±0.73$^{a, B}$
	C4	14.07±0.29$^{b, B}$	11.33±0,12$^{a, A}$	18.36±0.73$^{c, A}$	15.68±0.18$^{b, A}$
Resilience	CC	0.50±0.007$^{c, C}$	0.41±0.006$^{b, C}$	0.43±0.007$^{b, C}$	0.34±0.21$^{a, C}$
	C1	0.23±0.004$^{d, A}$	0.18±0.005$^{c, A}$	0.15±0.009$^{b, A}$	0.10±0.00$^{a, A}$
	C2	0.30±0.034$^{a, B}$	0.30±0.026$^{a, B}$	0.30±0.007$^{a, B}$	0.33±0.023$^{a, C}$
	C3	0.33±0.02$^{b, B}$	0.31±0.01$^{b, B}$	0.34±0.04$^{b, B}$	0.21±0.03$^{a, B}$
	C4	0.30±0.045$^{d, B}$	0.19±0.001$^{c, A}$	0.16±0.012$^{b, A}$	0.10±0.004$^{a, A}$

a–d indicate differences ($p<0.05$) between columns. A–C indicate differences ($p<0.05$) between rows. Mean values ± standard deviation of two trials.

3.8. Resilience values in cheese samples

The change in the resilience values of cheeses with added dried fruits during their ripening periods shown in Table 1. Resilience values of cheeses with added dried fruits, analyses during their ripening periods, found to be between 0.10-0.50. The highest resilience value measured in the control sample on the 3rd day, while the lowest value measured in the C1 and C4 samples on the 90th day. When the elasticity values of cheeses with

added dried fruits, analyses during their ripening periods, are evaluated statistically; In the variation analysis, the effect of variety and period factors on the resilience values of cheeses was found to be significant ($p<0.05$), independently of each other. When variety and period factors were evaluated together, their effects on the resilience values of cheeses were found to be significant ($p<0.05$).

3.9. Sensory scores in the cheese samples storage ripening

Sensory analyses carried out by 10 different panelists who previously informed about the subject, according to the criteria specified in the sensory evaluation form, throughout the ripening period of the cheeses. Color and appearance scores of the white cheese samples we produce during storage shown in Table 2.

3.10. Color and appearance scores of cheese samples

The color and appearance scores given by the panelists in the sensory analyses carried out during the ripening periods of cheeses with dried fruits shown in Table 2. In sensory analysis, color and appearance evaluation, the C3 sample received the highest score from the panelists on the 90th d, and the C1 sample received the lowest score on the 30th d. When the changes in color and appearance values of cheeses with added dried fruit during their ripening period are evaluated statistically; In the variation analysis, while the variety factor significantly affected the color and appearance values of the cheeses ($p<0.05$), the period factor did not significantly affect the color and appearance values of the cheeses ($p>0.05$). When the variety and period factors were evaluated together, their effects on the color and appearance values of the cheeses were not found to be significant ($p>0.05$). The color and appearance scores of cheeses with added dried fruits found to be lower than the color and appearance scores of Tarakçı and Küçüköner (2006) herby cheeses samples, and higher than the color and appearance scores of Gezmiş and Tarakçı (2019) for traditional Circassian cheeses. Dried fruit types added to curd affected the sensory properties of cheeses such as taste, smell and aroma during ripening. While the control sample group was the most liked cheese in terms of color and appearance, the cheeses with black mulberries and blackberries less appreciated. It thought that the reason for this is that dried black mulberries and blackberries completely cover the unique color of the cheese. Among the cheeses, cheeses with dried raspberries received the highest scores from the panelists. It is that the panelists appreciated dried raspberries more because they affect the characteristics of the cheese less than other dried fruits.

3.11. Odor scores of cheese samples

According to the sensory evaluation results carried out by the panelists, the change in the odor value of the cheeses during their ripening periods shown in Table 2. In the sensory analysis of cheeses produced by adding dried fruit, the odor values found to be close to each other. While the panelists gave the highest score to the control sample at the 3rd, 60th and 90th d maturation periods, the C1 sample received the lowest score at the 30th and 90th d.

When the odor values given by the panelists during the ripening period of cheeses with added dried fruits are evaluated statistically; In the variation analysis, while the variety factor significantly affected the odor values of the cheeses ($p<0.05$), the effect of the period factor on the odor values of the cheeses was not found to be significant $p>0.05$). When the variety and period factors were evaluated together, their effects on the odor values of cheeses were not found to be significant ($p>0.05$). The odor scores of cheeses produced by adding dried fruit received from the panelists in sensory analyses are lower than the odor scores of Tarakçı and Deveci (2019) in spicy cheeses.

3.12. Structure and texture values of cheese samples

The structure and change in texture values of cheeses produced by adding dried fruit to curd during their ripening period shown in Table 2. In the structure and texture evaluation of the cheeses during ripening, C3 sample received the highest score from the panelists, and C1 sample received the lowest score. Black mulberry, black grape and dried blackberry used in C1, C2 and C4 samples negatively affected the texture and structure values of the cheeses. When the structure and texture values of cheeses with added dried fruits are evaluated statistically during their ripening period; In the variation analysis, while the variety factor significantly ($p<0.05$) affected the taste and aroma values of the cheeses, the period factor did not significantly ($p>0.05$) affect the structure and texture value of the cheeses. When variety and period factors were evaluated together, their effects on the structure and texture values of cheeses were found to be significant ($p<0.05$). The

Table 2. Sensory scores for the cheese added dried fruit

	Cheese Types	Ripening Times (Days)			
		3	30	60	90
Color and appearance	CC	7.00±0.66$^{a, B}$	7.00±0.66$^{a, B}$	7.00±0,66$^{a, B}$	7.00±0.65$^{a, B}$
	C1	6.00±0.81$^{a, A}$	5.60±1.07$^{a, A}$	6.70±0,67$^{a, AB}$	5.90±1.10$^{a, A}$
	C2	6.30±0.67$^{a, AB}$	5.80±0.63$^{a, A}$	5.80±0,63$^{a, A}$	5.80±0.63$^{a, A}$
	C3	7.10±0.56$^{a, B}$	7.10±0.73$^{a, B}$	7.10±0,73$^{a, B}$	7.40±0.51$^{a, B}$
	C4	6.50±0.52$^{a, ABC}$	6.80±0.63$^{a, B}$	6.90±0,56$^{a, AB}$	6.60±0.51$^{a, BC}$
Odor	CC	6.90±0.31$^{a, A}$	6.60±0.96$^{a, A}$	6.90±0.31$^{a, A}$	6.90±0.31$^{a, C}$
	C1	6.22±0.66$^{a, A}$	6.00±0.70$^{a, A}$	6.33±0.86$^{a, A}$	6.00±0.70$^{a, A}$
	C2	6.63±0.80$^{a, A}$	6.18±0.40$^{a, AA}$	6.27±0.46$^{a, A}$	6.18±0.40$^{a, AB}$
	C3	6.80±0.63$^{a, A}$	6.80±0.63$^{a, A}$	6.70±0.67$^{a, A}$	6.70±0.67$^{a, BC}$
	C4	6.70±0.82$^{a, ABC}$	6.60±0.96$^{a, AB}$	6.90±0.56$^{a, AB}$	6.80±0.42$^{a, BC}$
Structure and texture	CC	7.00±0.66$^{a, B}$	7.00±0.66$^{b, B}$	7.00±0.66$^{b, B}$	7.00±0.66$^{b, BC}$
	C1	5.56±1.13$^{a, A}$	5.88±0.78$^{b, A}$	6.55±1.13$^{b, B}$	6.44±0.72$^{c, ABC}$
	C2	6.45±0.68$^{a, AB}$	6.36±0.67$^{a, AB}$	6.45±0.82$^{a, B}$	6.36±0.67$^{a, AB}$
	C3	7.10±0.56$^{a, B}$	7.10±0.56$^{ab, B}$	7.00±0.66$^{b, B}$	7.20±0.42$^{b, C}$
	C4	6.9±1.19$^{a, B}$	6.90±1.19$^{b, AB}$	6.35±0.82$^{bc, A}$	5.90±0.73$^{c, A}$
Taste and flavor	CC	6.60±0.96$^{a, A}$	6.90±0.31$^{a, A}$	7.00±0.81$^{a, A}$	6.60±0.96$^{a, A}$
	C1	6.00±0.70$^{a, A}$	6.11±0.78$^{a, A}$	6.33±0.70$^{a, A}$	6.33±1.00$^{a, A}$
	C2	5.81±0.75$^{a, A}$	6.63±0.80$^{b, A}$	6.63±0.80$^{b, A}$	6.63±0.80$^{b, A}$
	C3	6.80±0.63$^{a, A}$	6.80±0.63$^{a, A}$	6.80±0.63$^{a, B}$	6.80±0.63$^{a, A}$
	C4	6.20±0.78$^{a, A}$	6.30±0.67$^{a, A}$	6.20±0.42$^{a, A}$	6.50±0.52$^{a, A}$
General acceptability	CC	7.00±0.66$^{a, C}$	7.10±0.56$^{a, B}$	7.10±0.56$^{a, B}$	7.00±0.66$^{a, A}$
	C1	6.00±0.70$^{a, A}$	6.00±0.70$^{a, A}$	6.22±0.83$^{a, A}$	6.44±0.52$^{a, A}$
	C2	6.18±0.40$^{a, AB}$	6.36±0.67$^{a, AB}$	6.45±0.68$^{a, AB}$	6.36±0.67$^{a, A}$
	C3	6.80±0.42$^{a, BC}$	7.00±0.47$^{a, B}$	6.80±0.63$^{a, AB}$	7.00±0.47$^{a, A}$
	C4	6.60±0.51$^{a, ABC}$	6.70±1.05$^{a, AB}$	6.50±0.52$^{a, AB}$	6.60±0.51$^{a, A}$

a–d indicate differences ($p<0.05$) between columns. A–C indicate differences ($p<0.05$) between rows. Mean values ± standard deviation of two trials.

structure-texture scores of cheeses with added dried fruits in sensory evaluations are lower than the structure and texture scores of Tarakçı et al. (2005) herby cheese, Tarakçı, and Deveci (2019) spicy cheeses, but higher than the structure and texture scores of Sekban and Tarakçı (2021) for golot cheeses.

3.13. Taste and aroma values of cheese samples

The change in taste and aroma values of cheeses produced by adding dried fruit to curd during their ripening period shown in Table 2. In the sensory analyses carried out on the taste and aroma of dried fruit cheeses, the control sample received the highest score and the C2 sample received the lowest score by the panelists. Dried fruit seems negatively affect the taste-aroma scores of the cheeses to which they added. It said that the reason for the negativity is that the unique taste, smell, aroma and color of the cheese have changed greatly.

3.14. General acceptability values of cheese samples

The change in acceptability scores of cheeses produced by adding dried fruit to curd during their ripening period shown in Table 2. In the sensory evaluations of cheeses with added dried fruit during their ripening period, the panelists to the control sample gave the highest general acceptability score, while the C1 sample received the lowest score during the 30th and 60th d ripening periods. When the general acceptability values of cheeses with added dried fruits are evaluated statistically during their ripening periods; In the variation analysis, while the variety factor significantly ($p<0.05$) affected the general acceptability values of the cheeses, the period factor did not significantly ($p>0.05$) affect the general acceptability values of the cheeses. When the variety and period factors were evaluated together, their effects on the general acceptability values of cheeses were found to be significant ($p<0.05$). The general acceptability scores of cheeses with added dried fruits were lower than the general acceptability scores of Tarakçı (2004) for herby cheeses, Tarakçı, and Deveci (2019) for spicy cheeses.

4. Conclusion

In the study, five types of cheese produced, one of which was a control sample, 2.0% of dried forms of different fruits added to the curd cheese. Sensory and textural analyses carried out on the cheeses during ripening periods. Textural values of cheeses with added dried fruit examined during their ripening period. When the values obtained in the texture profile analyses are evaluated statistically; In the variation analysis, variety and period factors affected the hardness, adhesiveness, cohesiveness, springiness, chewiness, chewiness, and resilience values of the cheeses significantly ($p<0.05$), independently of each other, and the variety and period factors together. The sensorial analyses carried out in this study, it has significant effects on color-appearance, smell, taste, aroma, structure, texture in addition, general acceptability values of cheeses in sensory evaluations. Cheeses containing dried fruit varieties less appreciated in sensory analysis than the control sample. Textural analyses showed that they negatively affected the cheese texture. Future studies expanded to investigate the effects of antioxidants found in the chemical structure of dried fruit on cheese. By combining the anthocyanin contained in dried fruits with cheese as a natural colorant, healthy cheeses with colors that will appeal to the consumer produced.

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Zekai TARAKÇI: Methodology, Investigation, Conceptualization, and Writing - original draft, Visualization. **Murat YOLAŞAN**: Formal analysis, Data curation, Statistical analysis

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

We humbly give consent for this article to be published.

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Comparison of nut losses and foreign material separation efficiencies of hazelnut harvesting machines under different orchard yield conditions

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A B S T R A C T

In this study, the possibilities of using the prototype manufactured hazelnut harvesting machine with a mechanical efficient harvesting unit and hazelnut harvesting machine with a pneumatic efficient harvesting unit in the mechanical harvesting of hazelnuts grown in flat and near flat land conditions were examined and the nut losses and the efficiency values of separating foreign materials were determined to reveal how these machines affect the hazelnut harvesting system. For this purpose, the trials were carried out under five different orchard yield conditions (71.74, 143.48, 215.23, 286.97, and 358.72 kg ha^{-1}). As a result of the experiments, the nut loss and foreign material removal efficiency obtained by hazelnut harvesting with a hazelnut harvesting machine with a mechanical effective harvesting unit were determined between 34.39-37.92% and 96.91-95.62%, respectively. The nut losses and foreign material removal efficiency values obtained by hazelnut harvesting machine with a pneumatic effective harvesting unit were determined between 6.84-5.07% and 93.33-86.73%, respectively. The data to be obtained as a result of the study, in addition to examining the mechanical harvesting of hazelnuts, will enable the reasons for the changes that may occur in the performance characteristics of existing machines to be explained and suggestions can be made for improvement.

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1. Introduction

Türkiye has 74.50% of the world's hazelnut planting areas with an area of approximately 700.000 t ha. Hazelnuts in shell production are approximately 665.000 t and supply about 76% of the world's production. Hazelnut exports are approximately 500.000 t and 75% of the world's exports (TÜİK, 2023). Although Türkiye is the world's leading producer and exporter of hazelnuts and the sole source of livelihood for around 500.000 farmers, harvest mechanization has not reached the same level with competing countries. Hazelnut harvesting, which has an important place in the farm of our country, is done manually, but it is also done mechanically with the widespread use of hazelnut harvesting machines developed by local manufacturers recently (Beyhan and Sauk, 2018). The most prominent difference that distinguishes our hazelnuts from the varieties of other countries is that in Turkish varieties, the mature long, and tightly wrapped kernel. For this reason, ripe nuts do not fall as kernels. But, in almost all cultivars, mature fall to the ground spontaneously (Ayfer et al., 1986; Beyhan, 1992).

The healthiest way of harvesting hazelnuts is to shake the mature husked to the ground and harvest the hazelnuts from the ground. However, due to the prolongation of the harvest until the rainy periods and the drying problem, the harvest method applied in the Black Sea Region is the hand-harvesting of hazelnuts from the branch. When hazelnuts are collected by hand on branch, the buds and twigs that will form the next year's crop are also damaged. On the other hand, if the hazelnuts are harvested after being dropped to the ground by shaking, these damages are prevented, labor is saved and high yield and quality nuts are obtained since the hazelnuts are harvested at maturity (Beyhan, 1992; Beyhan and Yıldız, 1996; Bozoğlu, 1999).

Mechanical harvesting of seed kernels includes ground preparation, dropping the kernels, barreling the dropped kernels, harvesting, and cleaning. The purpose of ground preparation is to obtain a flat and compacted ground for sweeping the dropped kernels. To increase the work success of the harvesting machines, the dropped kernels should be swept to form a barrel between the rows before the harvesting process. Beyhan (1992), In his study in which he designed and manufactured an aspiration harvesting machine suitable for Türkiye conditions, tested the machine at 3 different orchard yields of 113.4, 226.8, and 340.2 kg ha^{-1} and determined that the nut loss of the machine was 4.27%, 4.87%, and 7.57%, respectively. It was reported that 2.67% of these nut losses were due to hazelnuts spilled into the brush (“ocak” in Turkish). According to orchard yields, the losses of 1.6%, 2.2%, and 4.9%, respectively, were caused by the hazelnuts that the machine could not harvest. Emphasized that the hazelnuts that could not be harvested were found in densely weeded areas and soil crevices, and it was observed that the majority of these were kernels. Ghiotti (1989), designed a hazelnut harvesting machine with a harvesting system based on the principle that chains mounted on a drum rotating against the direction of movement strike the kernels. Stated that the machine removes soil and plant residues from the harvested kernels with an airflow generated by a fan. During the harvesting, a total of 11-14% nut loss occurred due to hazelnuts left at the inside of the trees and in places where the machine could not reach, hazelnuts thrown from the cleaning unit, hazelnuts that the machine could not harvest and hazelnuts damaged while passing through the machine, and the rates of these losses were 6-7%, 3.7-5%, 0.9-1.1% and 0.33-0.51%, respectively. Pagano et al. (2010), in their study to determine the performance values of a hazelnut harvesting machine with a mechanical harvesting unit, found that the amount of foreign material in the sack constituted 10.25% of the total amount.

The possibilities of using the hazelnut harvesting machine with pneumatic effective harvesting unit (PHM) and the hazelnut harvesting machine with mechanical effective harvesting unit (MHM) manufactured by local manufacturers in the mechanical harvesting of hazelnuts grown in flat and near flat terrain conditions will be examined and the efficiency of hazelnut harvesting machines in separating nut losses and foreign materials will be determined to reveal how these machines affect the hazelnut harvesting system. The data to be obtained as a result of the study, besides examining the mechanical harvesting of hazelnuts, will enable us to explain the reasons for the changes that may occur in the performance characteristics of the existing machines and to make suggestions for improvement.

2. Materials and methods

2.1. Experimental design

Harvesting experiments, it was carried out in a farmer's orchard in the Kızılot neighborhood of Çarşamba

town of Samsun city. The hazelnut orchard where the trials were carried out has Çakıldak hazelnut variety (*Corylus avellana*), which is widely grown in the region. In five ocak to determine the distribution of naturally fallen hazelnuts around the ocak, squares of 1x1 m were formed from the inside of the ocak to the outermost branch. The hazelnuts (kernel+husked hazelnut) were harvested. The harvested hazelnuts were weighed and the hazelnut weight (10% moisture(w.b.)) was determined. During the experiments, no intervention was made regarding the setting levels of MHM and PHM. During the trials, the PHM was operated at 2500 min^{-1} engine shaft speed, 3750 min^{-1} ventilator shaft speed, and 41.19 m s^{-1} delivery airspeed, while the MHM was operated at 1.60 m s^{-1} working velocity.

2.2. Determination of nuts losses

Nut losses were analyzed under 3 main groups: hazelnuts that remained in places that were not in the area of influence of the machine (inside the ocak, the base of the ocak, and above the row), hazelnuts that could not be harvested in the area swept by the machine, hazelnuts that were damaged while passing through the machine. Nut losses of hazelnut harvesting machines in each orchard yield are given as percentages (%).

The hazelnuts remaining inside the ocak, at the base of the ocak, above the row, and between the rows of five hazelnut ocak were harvested by hand and weighed. The weight of hazelnuts harvested in each area was proportioned to the yield of the ocak the percentage of hazelnuts remaining inside the ocak, at the base of the ocak, above the row, and between the rows was determined. The weight of hazelnuts that could not be harvested in the area swept by the machine and the weight of hazelnuts damaged while passing through the machine were proportioned to the total hazelnut weight to determine the percentage of hazelnuts remaining and the percentage of damaged hazelnuts.

Since the MHM could not reach the inside of the ocak, the ocak that barrel the hazelnuts with kernel+husked hazelnuts was operated in such a way that 25 cm was left to the inside of the ocak and the harvesting trials were carried out in this way. In addition, since the MHM could only harvest the hazelnuts between the rows, the hazelnuts on the rows could not be harvested because they could not reach the hazelnuts on the rows. When working with this machine, the nut losses occurring in this area are given in the places that are not in the affected area of the machine.

Since hazelnuts are harvested from the ground using the transmission hose manually moved with PHM hazelnuts on the inside of the ocak the rows can be harvested. The nut losses in these areas are indicated within the hazelnuts that the machine couldn't gather in the swept area.

The determination of the areas (inside the ocak, base of the ocak, and above the row) where MHM couldn't harvest in a standard plot (1 ha) utilized the following equation (Yıldız, 2000).

$$M_{KA}\,(\%) = [[(n-1).\ (OG + MYM.2).\ OAM.m]/10000]\,.100 \quad (1)$$

where;

M_{KA}: The area that MHM cannot harvest in a standard plot (%),

n : Number of ocak on rows in a standard plot (number),

m: Number of ocak between rows in a standard plot (number),

OG: The average width of ocak (m),

OAM: Distance between ocak centers (m),

MYM: Approach the distance of the machine to the inside of the ocak (m),

The following equation was used to determine the area (inside the ocak and base of the ocak) that PHM could not harvest in a standard parcel (1 ha).

$$P_{KA}\,(\%) = [[n.m.OG.OU]/10000]\,.100 \quad (2)$$

where;

P_{KA}: The area that PHM cannot harvest in a standard plot (%),

OU: The average length of ocak (m),

2.3. Determination of the efficiency of separation of foreign materials

The hazelnuts harvested with MHM and PHM were subjected to a separation process where dust, soil, dry twig pieces, husk crumbs, leaf particles, and weeds were removed and individually weighed. The weight of each component forming foreign materials was determined as a percentage (%) of the total material quantity. Additionally, a size analysis was conducted to evaluate foreign materials that came along with the hazelnuts in sacks, considering their types and diameter groups.

All the material thrown under the PHM and the outlet of the aspirator was harvested by spreading a cloth underneath. Similarly, for the MHM all the material thrown was captured by attaching a cloth under the conveyor and oscillating screens.

2.4. Damaged hazelnut rate

It was determined by proportioning the number of hazelnuts with partially broken shells or hazelnut kernels to the total number of hazelnut kernels taken per unit time from all output channels of the machine.

3. Results and discussion

3.1. Nuts losses

In the harvesting trials conducted with MHM and PHM, the harvesting efficiency values obtained after the hazelnuts remained in the areas not in the machine's area of influence (inside the ocak, base of the ocak, and above the row), the hazelnuts that could not be harvested in the area swept by the machine and the hazelnuts damaged while passing through the machine, and the nut losses in these areas are given in Table 1.

Table 1. Harvesting efficiency values and nut loss rate in machine harvesting trials (%)

Harvesting efficiency values and Nut loss, (%)		Orchard yield (kg da^{-1})				
		71.74	143.48	215.23	286.97	358.72
MHM	Harvesting efficiency	63.48	62.40	62.08	63.90	65.61
	Nut loss	36.52	37.60	37.92	36.10	34.39
PHM	Harvesting efficiency	93.16	94.26	94.87	94.87	94.83
	Nut loss	6.84	5.74	5.13	5.13	5.17

MHM can only harvest 77.54% of the standard parcel. The remaining 22.46% is composed of the areas that are not under the influence of the machine; inside the ocak, base of the ocak, and above the rows. PHM, on the other hand, can harvest 98.15% of the standard plot, and the remaining 1.85% are the areas inside the ocak that are not under the influence of the machine.

As can be seen from Table 1, in the harvesting of hazelnuts with MHM, the harvesting efficiency was at the lowest level at 62.08% when the orchard yield was 215.23 kg ha^{-1}, while the nut loss was at the highest level with 37.92% at this yield. When the orchard yield is 358.72 kg ha^{-1}, the harvesting efficiency is the highest at 65.61% and the nut loss is the lowest at 34.39% in this yield. Accordingly, when the orchard yields are 71.74, 143.48, 215.23, 286.97, and 358.72 kg ha^{-1}, the harvesting efficiency and nut loss rates of MHM are 63.48% and 36.52%; 62.40%, and 37.60%; 62.08% and 37.92%; 63.90% and 36.10%; 65.61% and 34.39%, respectively.

Again, as can be seen from Table 1, when hazelnut is harvested with PHM, an increase in the harvesting efficiency of the machine is observed with an increase in the garden yield from 71.74 kg ha^{-1} to 286.97 kg ha^{-1}, while nut loss decreases. With the increase in orchard yield from 286.97 kg ha^{-1} to 358.72 kg ha^{-1}, harvesting efficiency decreases, and nut loss increases.

When the orchard yield was 71.74 kg ha^{-1}, the harvesting efficiency was at the lowest level at 93.16%, while the nut loss was at the highest level at 6.84% at this orchard yield. At 215.23 kg ha^{-1} and 286.97 kg ha^{-1} orchard yields, harvesting efficiency was at the highest level at 94.87%, while nut loss was at the lowest level at 5.13% at these orchard yields. Accordingly, when the orchard yields are 71.74, 143.48, 215.23, 286.97, and 358.72 kg ha^{-1}, the change in harvesting efficiency and nut loss values of PHM are 93.16% and 6.84%; 94.26% and 5.74%; 94.87% and 5.13%; 94.87% and 5.13%; 94.83% and 5.17%, respectively.

The changes in the nut loss during the harvesting trials with MHM and PHM depended on the orchard yield of the hazelnuts that were left in the places that were not in the area of influence of the machine (inside

Table 2. Distribution of nut losses during the harvesting trials with machines (%)

	Distribution of nut losses, (%)	Orchard yield (kg da^{-1})				
		71.74	143.48	215.23	286.97	358.72
MHM	Remaining hazelnuts in areas not affected by the machine	24.78	24.78	24.78	24.78	24.78
	The machine could not be harvested in the swept area	6.90	5.57	3.89	3.76	3.76
	Hazelnuts are damaged as they pass through the machine	4.84	7.25	9.25	7.56	5.85
	Total nut loss	36.52	37.60	37.92	36.10	34.39
PHM	Remaining hazelnuts in areas not affected by the machine	3,68	3,68	3,68	3,68	3,68
	The machine could not be harvested in the swept area	2.21	1.12	0.99	0.59	0.61
	Hazelnuts are damaged as they pass through the machine	0.95	0.94	0.46	0.86	0.88
	Total nut loss	6.84	5.74	5.13	5.13	5.17

the ocak, base of the ocak, and above the row), the hazelnuts that could not be harvested in the area swept by the machine and the hazelnuts that were damaged while passing through the machine are given in Table 2.

As can be seen from Table 2, in the harvesting of hazelnuts with MHM under all orchard yield conditions, the nut loss caused by the hazelnuts remaining in the ocak, inside of the ocak above the row, which were not under the influence of the machine, was 3.68%, 4.75%, and 16.35%, respectively. Again, as seen in Table 2, the proportion of hazelnuts that could not be harvested in the area swept by the machine decreases with the increase in orchard yield. However, the hazelnuts that the machine could not harvest include hazelnut kernels and hazelnuts with partially broken shells. These are the hazelnuts that fall from the lower auger to the floor after being damaged while passing through the machine. At 71.74 kg da^{-1} orchard yield, the nut loss caused by the hazelnuts that the machine could not harvest in the area swept by the machine is 6.90% (hazelnuts with kernel+shelled hazelnuts 4.74%; hazelnuts with kernel and damaged hazelnuts 1.96%). Depending on the orchard yield (143.48, 215.23, 286.97, and 358.72 kg h^{-1}), the ratios of hazelnut kernel+husked hazelnut and hazelnut kernel+damaged hazelnut in the nut losses caused by the hazelnuts that the machine could not harvest in the swept area were 3.23% and 2.34%; 1.92% and 1.97%; 1.62% and 2.14%; 2.29% and 1.47%, respectively.

Again, as can be seen from Table 2, the ratio of hazelnuts damaged while passing through the machine increases up to 215.23 kg ha^{-1} orchard yield depending on the increase in orchard yield, while the ratio of hazelnuts damaged decreases after this orchard yield. When the orchard yield was 215.23 kg ha^{-1}, the rate of hazelnuts damaged while passing through the machine was at the highest level at 9.25%, while when the orchard yield was 71.74 kg ha^{-1}, it was at the lowest level with 4.84%.

Again, as can be seen from Table 2, when hazelnuts were harvested with PHM under all orchard yield conditions, all of the nut loss (3.68%) caused by the hazelnuts remaining in the places not under the influence of the machine consisted of the hazelnuts remaining in the ocak. The proportion of hazelnuts that could not be harvested in the area swept by the machine decreased with the increase in orchard yield from 71.74 kg ha^{-1} to 286.97 kg ha^{-1}, while it increased with the increase in orchard yield from 286.97 kg ha^{-1} to 358.72 kg ha^{-1}.

In the case of 286.97 kg ha^{-1} yield, the ratio of hazelnuts that could not be harvested in the area swept by the machine was at the lowest level with 0.59%, while it was at the highest level at 2.21% in 71.74 kg ha^{-1} orchard yield. Accordingly, depending on the orchard yield, the change in the proportion of hazelnuts that could not be harvested in the area swept by the machine is 2.12%, 1.12%, 0.99%, 0.59%, and 0.61%, respectively.

Again, as seen in Table 2, the proportion of hazelnuts damaged while passing through the machine decreased with the increase in orchard yield from 71.74 kg ha^{-1} to 215.23 kg ha^{-1}, while it increased with the increase in orchard yield from 215.23 kg ha^{-1} to 358.72 kg ha^{-1}. Accordingly, depending on the orchard yield, the change in the proportion of hazelnuts damaged while passing through the machine is 0.95%, 0.94%, 0.46%, 0.86%, and 0.88%, respectively.

3.2. Foreign material separation efficiencies

The average percentage of the foreign materials separated from the separator units of the machine and the average percentage of the foreign materials separated from the separator units of the machine and the average percentage of the foreign materials in the sack during the harvesting of the hazelnuts from the orchard ground by MHM and PHM depending on the yield of the orchard is given in Table 3.

Table 3. In harvesting trials with machines, the distribution of average foreign material in the sack (%)

Distribution of the foreign material (%)		Orchard yield (kg da^{-1})				
		71.74	143.48	215.23	286.97	358.72
MHM	in the sacks	3.09	2.38	4.79	3.22	4.38
	Separated from the separator	96.91	97.62	95.21	96.78	95.62
PHM	in the sacks	6.67	9.22	8.19	8.65	13.27
	Separated from the separator	93.33	90.78	91.81	91.35	86.73

As can be seen in Table 3, the rate of material separated from the separator units of MHM was the highest with 97.62% at 143.48 kg ha^{-1} orchard yield, while the amount of foreign material in the sack was the lowest with 2.38%. With an orchard yield of 215.23 kg ha^{-1}, the amount of foreign material separated from the separator was the lowest at 95.21%, while the amount of foreign material in the sack was the highest at 4.79%. Again, as seen in Table 3, the amount of foreign material separated from the separator units of PHM was the highest at 93.33% with an orchard yield of 71.74 kg ha^{-1}, while the amount of foreign material in the sack was the lowest at 6.67%. With an orchard yield of 358.72 kg ha^{-1}, the amount of material separated from the separator was the lowest at 86.73%, while the amount of foreign material in the sack was the highest at 13.27%.

The foreign materials separated and placed in the sack consist of soil, branch parts, dry grass, dry leaves, coarse dust, and mature parts. The distribution of the soil particles harvested by the machines under the same conditions and placed in the sacks together with the hazelnuts with kernel+shelled nuts according to their sizes is given in Table 4, and the distribution of the branch parts according to their sizes is given in Table 5 and the distribution ratios of the foreign materials harvested from the orchard ground are given in Table 6.

Table 4. Harvested by machines, kernel+husked in a sack together with the hazelnuts in the sack, distribution of soil particles according to their size (%)

Sieves Diameter (mm)	Soil Rate (%)	
	MHM	PHM
2	3.67	8.91
4	6.38	16.84
8	8.87	35.09
20	25.78	17.73
40	40.96	11.40
>40	14.34	10.03

As can be seen from Table 4, the proportions of soil classified in sieves with a diameter of 2-20 mm (as a percentage of total weight) were 44.70% for MHM and 78.57% for PHM. The proportion of soil with 40 mm and larger values is 55.30 % for MHM and 21.43 % for PHM. Soil parts with a diameter of 8-20 mm are hazelnut-sized lumps and approximately spherical soils. In the diameter group of 20 mm and above, it consists of walnut-sized lumps of soil that are approximately spherical.

Table 5. Harvested by the machines, kernel+husked in a sack together with the hazelnuts in the sack, distribution of branch particles according to their size (%)

Branch length (mm)	Rate (%)	
	MHM	PHM
20	14.99	13.48
40	46.98	32.57
60	17.82	35.12
80	12.89	14.91
>80	7.32	3.92

Table 6. Harvested by the machines, kernel+husked in a sack together with the hazelnuts in the sack, distribution of foreign materials (%)

Materials	Foreign materials rate (%)	
	MHM	PHM
Dry grass	1.67	2.01
Soil	24.39	26.47
Branch part	44.21	39.81
Husk part	10.70	12.73
Dry leaf	11.40	9.41
Coarse powder	7.63	9.57

As shown in Table 5, the proportion of branches 20-40 mm in length (as a percentage of total weight) was 61.97% for MHM and 46.05% for PHM. The proportion of 60 mm and larger branches was 38.03% for MHM and 53.95% for PHM.

As seen in Table 6, the majority of the harvested foreign materials, 68.60% for MHM and 66.28% for PHM, consisted of branch parts and soil. The majority of the soil was harvested from the weedless area inside of the ocak. The majority of the grass, branch parts, leaves, and coarse dust are materials accumulated from previous years. It is clear that if the harvesting process is carried out continuously by machine, the amount of materials such as grass, branch parts, leaves, and coarse dust remaining outside the soil will decrease.

As seen in Table 6, most of the harvested foreign material, 68.60% for MHM and 66.28% for PHM, consisted of branches and soil. Most of the soil was harvested from the weedless area at the inside of the ocak. Most of the grass, branches, leaves, and coarse dust are the materials accumulated from previous years. It is clear that if the harvesting process is carried out continuously by machine, the amount of materials such as grass, branches, leaves and coarse dust other than soil will decrease.

4. Conclusion

The total nut loss obtained from the orchard trials with MHM ranged between 34.39% and 37.92%. About 24.78% of the total nut loss is attributed to the hazelnuts remaining in areas not affected by the machine (inside the ocak, the base of the ocak, and above the rows). However, it is possible to reduce nut losses significantly in these areas by using a backpack blower, rake, or broom before harvesting, or by attaching a radial ventilator to the MHM to sweep the hazelnuts remaining inside the ocak, the base of the ocak, and above the rows into the machine's effective area. This way, it can be stated that the machine's performance will increase, and significant reductions in nut losses in these areas will occur. The hazelnuts damaged when passing through the machine were observed to have internal kernel and shell cracks. Some of the damaged hazelnuts were spilled into the area swept by the machine as whole kernels, while the majority were conveyed into the sack as internal kernels and damaged hazelnuts with shell cracks. The hazelnuts that the machine couldn't pick up in the swept area consisted of hazelnuts escaping from both sides of the chain finger-picking system and hazelnuts spilled from the machine's lower auger as internal kernels and damaged hazelnuts.

The total nut loss obtained from orchard trials with PHM varies between 6.84% and 5.07%. About 3.68% of the total nut loss is attributed to hazelnuts left in areas unaffected by the machine (above the rows). It has been observed that the machine can easily harvest hazelnuts left in the row ends and between rows using the manually operated suction tube. This situation increases the harvesting efficiency of PHM and reduces nut losses. Hazelnuts in this area should also be swept into the machine's area of influence. The hazelnuts damaged in the PHM are also in the form of grains and most of them are in the form of shell cracking. The hazelnuts that the machine could not harvest were found in weedy areas and soil crevices, and most of them were in the form of hazelnuts.

The highest separation efficiency of foreign materials (twigs, stones, soil, leaves, etc.) of MHM and PHM during the harvesting of kernel+husked hazelnuts was 97.62% (143.48 kg ha^{-1} orchard yield) and 93.33% (71.74 kg ha^{-1} orchard yield), respectively. On the other hand, the amount of foreign material coming into the sack with kernel+husked hazelnuts in these orchard yield conditions of MHM and PHM is 2.38% and 6.67%, respectively. The excessive amount of foreign material in the sack will increase the sack tying-unloading time and will cause a decrease in the work success of the hazelnut harvesting machines.

Moreover, this may result in a change in the threshing time of the husker, as more material will enter the husker. As a result, when the performance characteristics of the machines are compared, the MHM developed as a prototype will be able to provide economic and agronomic benefits for hazelnut producers such as reducing the harvesting cost and the demand for labor, the other hand, preventing damage to the trees as a result of hazelnut harvesting. Thus, by manufacturing a machine suitable for our orchard structure and hazelnut varieties, an important step will be taken for the mechanization of hazelnuts, which is one of the most important problems in our country.

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Hüseyin SAUK: investigation, methodology, data curation, validation, writing - original draft. **Mehmet Arif BEYHAN**; investigation, conceptualization, data curation, review, and editing.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

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Relationships between anthocyanin content and some pomological and colour characteristics of black mulberry (*Morus nigra*) fruit

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ABSTRACT

The analytical method used to determine the total monomeric anthocyanin content of fruits is costly and labour intensive. Researchers are endeavouring to develop prediction models to determine anthocyanin content in a simpler and more accurate way. The aim of this study was to investigate whether there is a relationship between anthocyanin and some fruit characteristics (width, length, weight, L*, a*, b*, chroma, hue) in black mulberry (*Morus nigra*) fruit. With the outputs of the study, it is aimed to provide preliminary information for the models to be developed for anthocyanin estimation in future studies. The study material, black mulberry fruits, was collected from a single black mulberry tree in Kemalpaşa village of Tokat province in July 2022. Harvesting of the fruits continued for two weeks as raw, semi-ripe and ripe. A total of 586 fruits were individually evaluated and the weight, width, length, colour parameters (L*, a*, b*, chroma, and hue) and total monomeric anthocyanin contents of each fruit were determined. Then, Pearson correlation coefficients between the variables were determined. Stepwise regression analysis was used to find the appropriate model to explain the change in the dependent variable anthocyanin with independent variables (length, width, weight, L*, a*, b*, chroma, hue). After the multiple regression model was established, residual analysis was performed to see the outliers in the full model and to check the accuracy of the model. As a result of the study, it was observed that anthocyanin content could be predicted by colour parameters up to a certain maturity stage. This relationship was found to weaken at the ripeness stage when the fruit colour turns black.

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1. Introduction

Scientific studies have reported the positive effects of phytochemicals found in fruits and vegetables on human health through their antioxidant capacities (Koca and Karadeniz, 2009; Veberic et al., 2009; Arozarena et al., 2012; Aman and Masood, 2020; Naja and Hamadeh, 2020; de Faria Coelho-Ravagnani et al., 2021). The obtained findings emphasize the importance of fruit consumption in the daily diet. Many researchers have concluded that the amount of fruits consumed should be considered as well as the ratio of beneficial compounds in them. Researchers believe that dark red and purple coloured fruits such as black mulberry, raspberry, blackberry, pomegranate, strawberry, sour cherry, cherry, plum and grape, which are especially rich in anthocyanins, are very effective in preventing the emergence of some diseases that cause premature death such as some types of cancer, diabetes vascular and heart diseases (Zafra Stone et al., 2007; Castaneda-Ovando et al., 2009; Castro-Acosta et al., 2016; Feng et al., 2016). Colour is therefore one of the first perceived property of fruit and is a characteristic that greatly influences the choice of consumers, as it leads to an idea of the taste, smell and composition of the fruit.

Plants with different colours often reflect the presence and distribution of essential compounds such as flavonoids, carotenoids and chlorophyll that contribute to plant biochemistry and physiology (Manetas, 2006; Li et al., 2024). For example, the pigment responsible for the blue, red or purple hues in plants is the water-soluble anthocyanins. They are part of flavonoids of plant origin and are transported to the vacuole as glutathione conjugates via glutathione transporters and stored there. In the plant kingdom, with the exception of betalain producers, anthocyanins are present as glycosidic compounds in various of plant tissues, mainly flowers and fruits, but also leaves, stems, tubers and roots. Anthocyanins are aglycone (anthocyanidin) glycosides containing one or more hexose sugar groups (glucose, galactose, rhamnose, arabinose, rutinose, sambubiose and soforose), usually linked to the -OH group of the pyrylium ring. Aglycone is a polyphenolic ring structure based on diphenylpropane. The most common anthocyanin aglycones found in plants are pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin (Kong et al., 2003; Hou et al., 2004; Deroles, 2009; Heldt and Piechulla, 2015; Yang et al., 2023; Chu et al., 2024).

The number and localisation of hydroxyl and methoxyl groups in the flavium cation and the presence of glycosyl and acyl groups attached to the aglycone affect the stability or reactivity of anthocyanins (Guidi et al., 2015). As a result of these events, anthocyanidins and their related structures make fruits and vegetables appear in many different colours and hues. The stability of anthocyanins is very sensitive and depends on the pH level of the environment, the type of anthocyanin pigment, copigments, enzymes, antioxidants, temperature, light, metal ions and oxygen (Khoo et al., 2017).

The method used to determine the total anthocyanin content in fruits is both laborious and costly because it consists of many steps. Researchers are in search of models to predict the amount of anthocyanin instead of the current method. For this purpose, it is investigated whether there is a relationship between anthocyanin and other fruit characteristics. Some researchers have reported a high correlation between colour parameters (L, a, b, chroma, hue) and colour pigments (Itle and Kabelka, 2009; Shibghatallah et al., 2013; Yan et al., 2023). Based on this relationship, it is thought that anthocyanin amounts can be estimated by determining the colour values for species in the berry fruit group, which do not have large differences between skin colour and flesh colour. The extraordinary colour and rich anthocyanin content of "Black Mulberry" fruit known as Morus nigra (Özgen et al., 2009) indicates that there will be a high correlation between colour values and anthocyanin values.

The aim of this study was to investigate whether there is a relationship between anthocyanin and some fruit characteristics (width, length, weight, L*, a*, b*, chroma, hue) in black mulberry fruit. The outputs obtained from the study will provide preliminary information for the models to be developed for predicting anthocyanin in further studies.

2. Materials and methods

2.1. Plant materials

The fruits were picked from a single black mulberry tree in Kemalpaşa village of Tokat province in July 2022.

The harvesting and analysis process of raw, semi-ripe and ripe black mulberry fruits continued for one month. A total of 586 fruits were individually evaluated and the weight, width, length, colour parameters and total monomeric anthocyanin contents of each fruit were examined.

2.2. Measurement and analyses

Each fruit was weighed using a balance with a precision of 0.01 and the length and width were measured using a compass. Fruit colour parameters (L*, a*, b*) were measured with a colorimeter (Minolta, model CR-400, Tokyo, Japan) from the outside of the fruit (4 measurements for each fruit). L* represents values from black (0) to white (100), a* from green (-) to red (+) and b* from blue (-) to yellow (+). The hue angle is defined as a colour circle, with red-purple at 0° and 360°, yellow at 90°, bluish green at 180° and blue at 270°. Chroma values indicate the saturation of the colour. Chroma values decrease in dull colours and increase in vivid colours. Hue angle (h^o) and chroma values were calculated according to Equations 1 and 2 (McGuire, 1992). Total monomeric anthocyanins were analysed using the pH differential method and the results were given as μg cyanidin-3-glucoside equivalent (μg cy-3-glu g^{-1} fw) per g fresh weight (Giusti and Wrolstad 2001; Özgen et al., 2009).

$$h^\circ = \tan^{-1}\left(\frac{b}{a}\right) \quad \text{.....(1)} \qquad C = (a^2 + b^2)^{1/2} \quad \text{..... (2)}$$

2.3. Statistical analysis

First, Pearson correlation coefficients between variables were determined. Stepwise regression analysis was used to find the appropriate model to explain the change in the dependent variable, anthocyanin, by independent variables (length, width, weight, L, a, b, chroma, hue). After the multiple regression model was created, residual analysis was performed to see the outliers in the full model and to check the accuracy of the model. Finally, the simple regression line of the independent variable hue value, which has the highest R value in the model, and the anthocyanin content of the dependent variable, and the distribution of errors on this line are given. All statistical analyses were performed using SAS version 9.0 (SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. Pearson correlation analysis

The correlations between the variables and anthocyanin are shown in Table 1. As seen in the table, high, negative and significant correlations were found between anthocyanin and colour parameters (L*, a*, b*, hue and chroma) ($p<0.0001$). It has also been reported in some previous studies that L* value decreases with the advancement of maturity in mulberry fruit, indicating darkening of fruit colour (Lin and Lay, 2013; do Lago et al., 2020). The negative correlation between L* and anthocyanin content indicates that anthocyanin content increases as fruit colour darkens. In agreement with the findings of this study, it was also reported by do Lago et al. (2020) and Smrke et al. (2023) that the intensity of colour pigments increased as the fruit ripened, while the brightness (L*) and vividness (C*) of the colour decreased and the fruit gained a dull appearance.

It is known that while the red colour intensity (a*) is high until the semi-ripe stage of the fruits, this colour intensity and the shades of yellow colour (b*) decrease with ripening. The purplish-blackish colour intensity that occurs with the ripening of the black mulberry fruit is located close to the 0 and 360° angles on the colour angle. This indicates that the hue value decreases with the advancement of ripening (Ercisli and Orhan, 2007; Lin and Lay, 2013; do Lago et al., 2020). The negative correlations determined between colour parameters and anthocyanin content in this study are consistent with the above literature information. Significant correlations between colour values and anthocyanin content of the fruit were also reported for other fruits other than mulberry. For example; Karaat et al. (2019) reported that there was a high negative correlation between anthocyanin content and Chroma values (r = - 0.63).

In a similar study with strawberry fruit, in parallel with our findings, Hernanz et al. (2008) reported negative correlations between colour values and colour pigments, with the best correlations found between pelargonidin-3-rutinoside and outer a* (r = -0.87), followed by pelargonidin-3-glucoside and inner L* (r = -0.72).

In another study, Yan et al. (2023) determined the total amount of 25 anthocyanins and 14 flavonols in 20 blueberry genotypes and examined their relationship with the external colour of the fruit. As a result of the study, it was reported that L*, C* and h° values showed weak correlations (r < 0.4) with anthocyanin concentrations measured at harvest and during post-harvest storage, but colour changes (Δ colour) after four weeks of storage showed a strong correlation (r > 0.7) with changes in anthocyanin concentrations. Vieira et al. (2018) reported that the correlation between L* value and anthocyanin content was higher for extracts produced with 70% ethanol and diluted 20 times (r = - 0.95). Although our findings are consistent with the literature, they also contain inconsistencies. This inconsistency may be due to different solvent and dilution ratios as well as different coloured fruits and different molecular structures of the anthocyanins they contain (Han et al., 2008). Positive and significant correlations were found between length, width and weight parameters and anthocyanin (p<0.0001). This is due to the increase in the size and chemical content of the fruit as it passes from raw, semi-ripe and full-ripe levels.

Table 1. Pearson correlation analysis, N=586

	Anthocyanin	Length	Width	Weight	L*	a*	b*	Chroma
Length	**0.25** *0.0001*							
Width	**0.56** *0.0001*	0.61 *0.0001*						
Weight	**0.39** *0.0001*	0.84 *0.0001*	0.72 *0.0001*					
L*	**-0.79** *0.0001*	-0.36 *0.0001*	-0.59 *0.0001*	-0.54 *0.0001*				
a*	**-0.85** *0.0001*	-0.42 *0.0001*	-0.63 *0.0001*	-0.55 *0.0001*	0.73 *0.0001*			
b*	**-0.82** *0.0001*	-0.39 *0.0001*	-0.66 *0.0001*	-0.55 *0.0001*	0.86 *0.0001*	0.894 *0.0001*		
Chroma	**-0.86** *0.0001*	-0.42 *0.0001*	-0.66 *0.0001*	-0.56 *0.0001*	0.79 *0.0001*	0.98 *0.0001*	0.96 *0.0001*	
Hue	**-0.86** *0.0001*	-0.38 *0.0001*	-0.65 *0.0001*	-0.55 *0.0001*	0.83 *0.0001*	0.90 *0.0001*	0.96 *0.0001*	0.93 *0.0001*

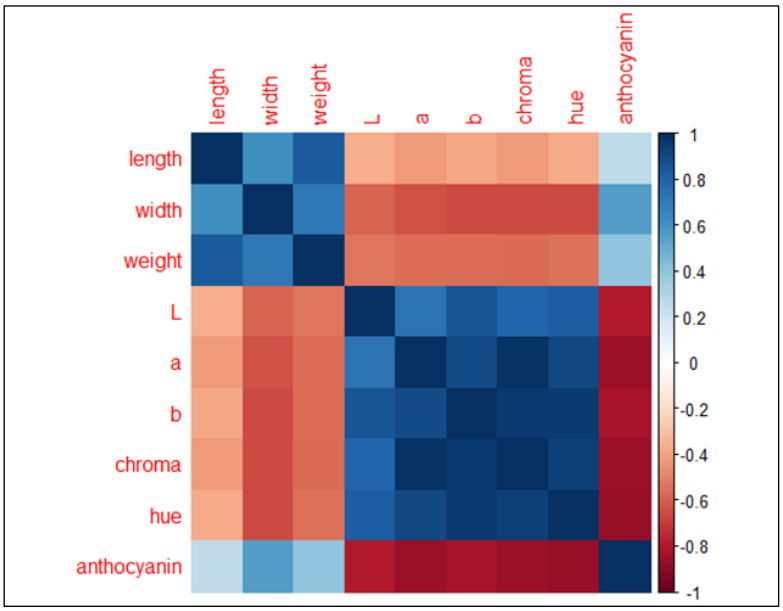

Figure 1. Colour representation of correlations between variables (Blue areas indicate positive correlation, red areas indicate negative correlation. As the darkness of the colours increases, the correlation between the variables increases)

3.2. Stepwise analysis

Stepwise analysis was performed to determine the appropriate model and the results of the analysis are given in Table 2. In Stepwise analysis, variables up to a significance level of 0.15 are included in the model. Variables with a significance level above 0.15 are excluded from the model and cannot enter the model (Hosmer and Lemeshow, 1999). 'Hue' entered the model first and 'hue' alone explained 74% of the variation in anthocyanin. In the presence of 'hue', the parameter 'a' explained 03% of the change in anthocyanin. Both together explained 77%. In total, when all variables were evaluated together (8 variables), it was determined that 85% of the change in anthocyanin was explained. In a previous study, Vieira et al. (2018) developed prediction models for the anthocyanin content of 13 different fresh fruits and vegetables using the stepwise method. For this purpose, L*, a*, b*, C* and h* values of colorimetry were used. As a result of the study, it was reported that linear equations with R2 values ranging from 0.80 to 0.99 were obtained. In this respect, our findings were in agreement with the literature.

Table 2. Stepwise analysis

Independent variable	Number of variables in the model	Partial R^2	Cumulative R^2	F value	P (significant)
Hue	1	0.7415	0.7415	1675.61	0.0001
a*	2	0.0314	0.7729	80.59	0.0001
L*	3	0.0239	0.7969	68.53	0.0001
Weight	4	0.0246	0.8215	80.05	0.0001
b*	5	0.0170	0.8385	61.17	0.0001
Chroma	6	0.0101	0.8486	38.51	0.0001
Width	7	0.0029	0.8515	11.34	0.0008
Length	8	0.0006	0.8521	2.44	0.1192

3.3. Multiple regression analysis

The multiple regression equation obtained as a result of stepwise regression analysis was as follows. This equation expresses that: 1 unit increase in fruit length corresponds to 1.65 unit increase in anthocyanin content while other variables are constant. Similarly, a unit increase in hue value corresponds to a decrease of 7.95 units in anthocyanin content.

Anthocyanin = 841.93 – (1.65 x **Length**) + (6.52 x **Width**) – (20.87 x **Weight**) – (11.2 x **L***) + (30.29 x **a***) + (49.43 x **b***) – (57.07 x **chroma**) – (7.95 x **hue**)

In order to test the accuracy of this regression equation and to identify outliers, standardised residual analysis was performed. When the errors shown in Figure 2 are examined, it is seen that the model is compatible and there are few outliers in the range of 0 to 300 μg cy-3-glu g^{-1} anthocyanin, while the predictive power of the model decreases and the number of outliers increases when the anthocyanin amount exceeds 300 μg cy-3-glu g^{-1}. These deviations are seen especially in black mulberry fruits that have reached full ripeness. Black mulberry fruits gain a darker appearance when they reach full ripeness. At this stage, L*, a*, b*, C* and h^o values of the fruit decrease and anthocyanin values increase. A similar situation was observed in blueberry fruit (Smrke et al., 2023). These colour parameters and anthocyanin content, which change with ripeness, weaken the predictive power of the model.

The weakening relationship between colour parameters and anthocyanin content at high anthocyanin values was also observed in the simple regression analysis between hue values and anthocyanin content (Figure 3). At hue values between 50 and 0, there is a linear relationship between anthocyanin content and hue value, while this relationship breaks down at lower hue values.

4. Conclusion

In this study, which was carried out to develop a model for easy estimation of anthocyanin content in fruits as an alternative to labour-intensive and costly analysis methods to determine this content, it was seen that anthocyanin content up to a certain maturity period can be predicted by colour parameters. This relationship was found to be weakened at the ripeness stage when the fruit colour turns black. Other criteria are needed for accurate estimation of anthocyanin content at this stage.

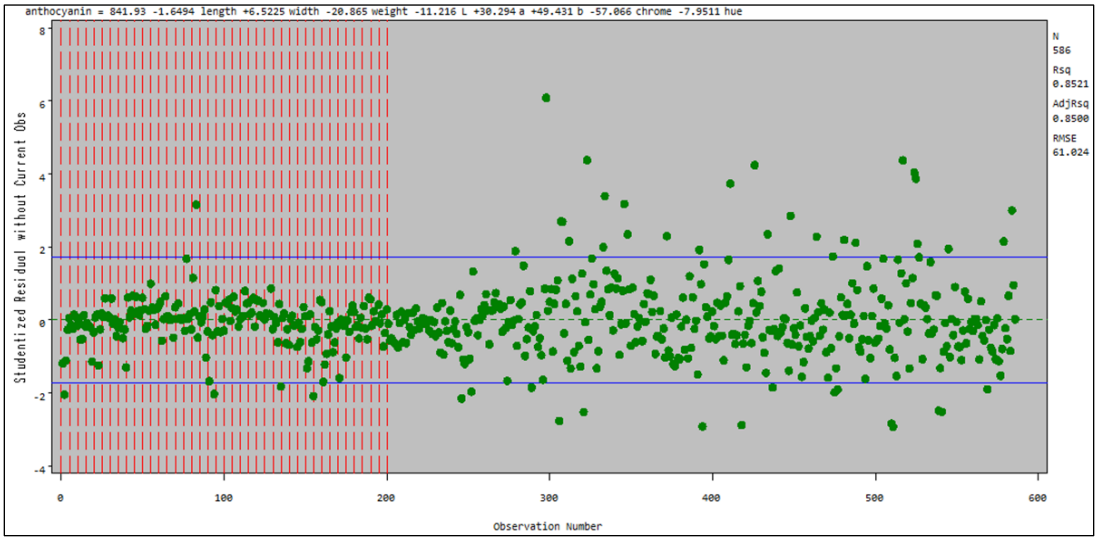

Figure 2. Scatterplot of errors in multiple models

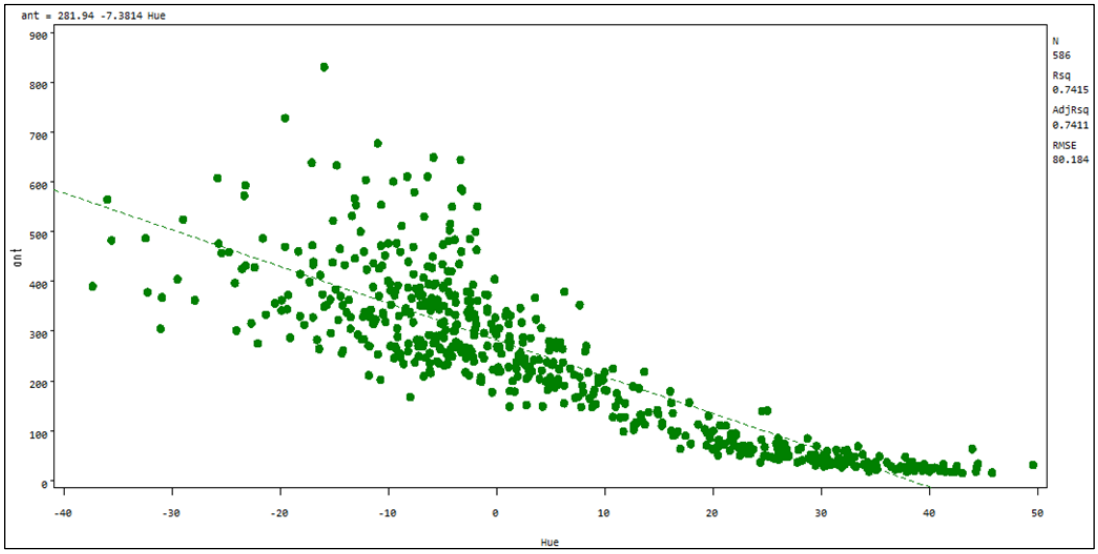

Figure 3. Scatterplot of errors when only the hue parameter is included in the model

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Osman Nuri ÖCALAN: Investigation, statistical analysis and writing. **Onur SARAÇOĞLU**: Writing, review and editing.

Ethical approval

Not applicable.

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Comparison of physical, chemical and sensory analyzes of tarhana containing black carrot extract and classical tarhana

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A B S T R A C T

Tarhana, which is very rich in nutritional value, has been the crown of our tables for centuries and is one of our common values wherever we live. It is obtained with black carrot extract to give this special food an even richer form. Black carrot extract was used to increase the anthocyanin content of the tarhana. In this study, physical and chemical analyzes, total phenolic content and total flavonoid content, antioxidant activities, colour measurement, sensory analysis of tarhana prepared with black carrot extract were determined. At the same time, the total amount of monomeric anthocyanin was determined. All these values were compared with classical tarhana and it was determined that tarhana containing black carrot extract had high DPPH activity (4.21±1.78 mg/mL) and high anthocyanin content (19.14±2.02 mg cyn3-glu/kg sample). According to the sensory analysis, the acceptability of tarhana with black carrot extract was determined to be high.

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1. Introduction

In the realm of traditional foods, few can rival the unique and rich history of tarhana. This ancient staple, originating from the Middle East and Mediterranean regions, has been a beloved dish for centuries (Sormaz et al., 2019). Tarhana is a fermented grain and yogurt-based soup mix that not only carries a distinctive taste but also boasts remarkable health benefits. Tarhana offers a host of nutritional benefits. Being a fermented food, it contains beneficial probiotics that promote a healthy gut flora, aiding digestion and supporting the immune system (Gok and Vatandost, 2021). Additionally, it is rich in dietary fiber, vitamins, and minerals derived from its various ingredients. The combination of wheat and yogurt provides a good source of protein, essential amino acids, and calcium (Atasoy and Ertop, 2021). Tarhana is also relatively low in fat and can be a valuable addition to a balanced diet.

Black carrot (*Daucus carota* ssp. sativus var. atrorubens Alef) is a vegetable originating from Türkiye, Middle and Far East. It has a bluish-purple color due to its high anthocyanin level (Karatas et al., 2014). Black carrot is a product rich in phenolics and carotenoids with antioxidant activity. Anthocyanins found in black carrots are used as natural food colorants due to their high light, heat and pH stability. The effects of foods rich in anthocyanins on health (such as anti-inflammatory, anti-diabetic, anti-tumor, anti-ulcer, antioxidant, anti-carcinogenic) have been revealed in literature studies (Kammerer et al., 2004).

The majority of the red, blue and purple colors in flowers, fruits, vegetables and other plant tissues are due to anthocyanins (Mazza, 2007). In addition to the attractive red, orange and purple colors of anthocyanins, their solubility in water has provided the opportunity to use these compounds as natural colorants (Bakowska-Barczak, 2005). Food colorants are used to give foods an original appearance, to standardize the color or to indicate the quality of the color. For these purposes, the use of synthetic colorants in the food industry is common. However, the demand for anthocyanins, which is an alternative natural source, has increased because the use of artificial colorants has legal limits and creates consumer concerns (Giusti and Wrolstad, 2003).

An antioxidant can be defined as a substance that prevents reactions with oxygen or peroxides and resists oxidation. Many of the antioxidants are used as preservatives in various products. Antioxidants have a very wide area in the food industry. The most important factor determining the place of antioxidants on human health is their structure-activity relationships, their solubility, their chemical structures and their ability to be obtained from natural sources (Huang et al., 2005; Nacak, 2014). The antioxidant capacities of flavonoids differ depending on their structure (Bronze et al., 2012). Flavonoids; It is generally responsible for color, taste, inhibition of fat oxidation, protection of vitamins and enzymes in foods (Yao et al., 2004).

In this study, it is aimed to compare classic tarhana with a new tarhana product, modified by adding black carrot extract, regarding physical, chemical and sensory properties. It is thought that the nutritional value of tarhana will increase with the addition of black carrot extract. Physical and chemical analyses of classical tarhana and tarhana containing black carrots were performed and their protein contents were determined. Total phenolic compounds were extracted from classical tarhana and black carrot tarhana. ABTS and DPPH activities as antioxidant futures were investigated. In addition, total flavonoid and total anthocyanin amounts were also examined. The color parameters of the tarhana obtained were compared.

2. Materials and methods

2.1. Materials

All chemicals and solvents used in analysis were bought from Sigma Aldrich (Sternheim, Germany) and Merck (Darmstadt, Germany). Onions, garlic, capia pepper, green pepper, tomato paste, flour, yogurt, salt and black carrot were purchased from the local market. Yeast was provided from a local bakery.

2.2. Extraction of black carrot

10 g black carrot was treated with 100 mL distilled water for 1 h at room temperature. The mixture was filtered under vacuum on a Buchner funnel using Whatman No. 1 paper (Whatman Inc., Clifton, N.J.) to use making tarhana.

2.3. Tarhana production

Classical tarhana and black carrot tarhana productions are given in Figure 1. Black carrot tarhana productions: Onions (120 g), garlic (50 g), capia pepper (150 g), green pepper (120 g) and tomato paste (120 g) are cooked over medium heat for 10 min. The cooled mixture is passed through the blender and all other ingredients [wheat flour (1000 g), yogurt (400 g), black carrot extract (100 g), salt (80 g), fresh baker's yeast (20 g)] are added and kneaded until a homogeneous dough is obtained. The resulting dough is left to ferment for 5 days at 30 °C. The fermented product is placed on stainless steel trays with a thickness of 1–1.5 cm, dried in the oven at 50 °C for 48 h, ground and sieved.

Classic tarhana productions: Same method with black carrot tarhana was used for producing classical tarhana. Only in this production, instead of not used black carrot extract, the amount of yoghurt was increased to a total of 500 g.

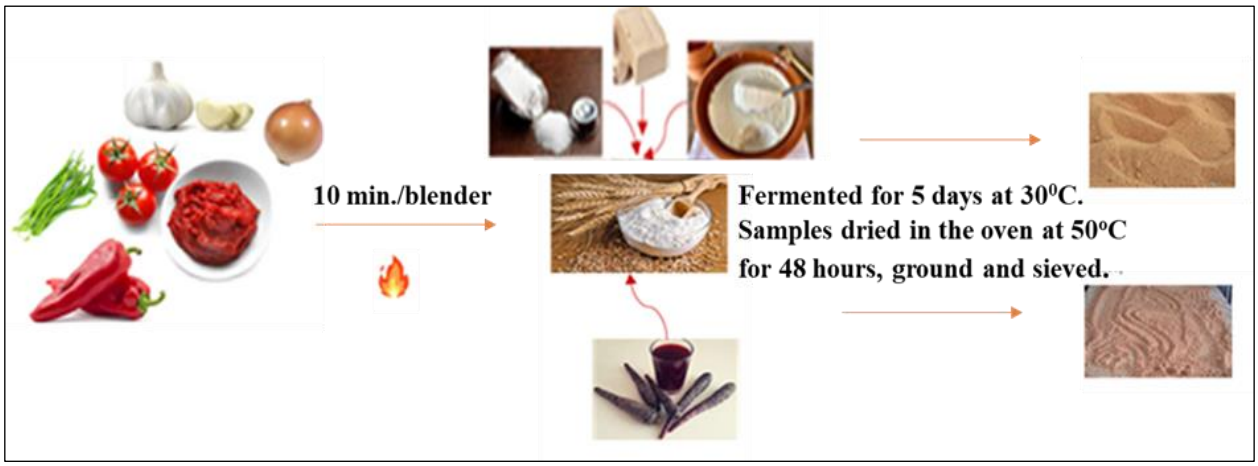

Figure 1. Tarhana Containing Black Carrot Extract and Classical Tarhana productions

2.4. Physical analyses

10 g of sample was mixed with 100 ml of distilled water at room conditions for 30 min and the mixture was filtered through filter paper. The pH of the solution was then measured using a digital pH meter. The percent humidity of tarhana samples was made according to the AACC 44-01.01 method (AACC, 2010).

2.5. Chemical analyses

The acidity of the fermentation products was determined by titration using 0.1 M NaOH. The results were calculated as lactic acid (Hendek and Atasoy, 2019). AACC 08-01.01 International methods were used for the determination of ash. The protein contents of the samples were determined by the Kjeldahl method and were determined from the crude nitrogen content of the samples (AOAC 2000, methods 992.23).

2.6. Tarhana extraction

After the tarhana samples were thoroughly mixed with methanol at a ratio of 1:5, the methanolic extracts were filtered under vacuum on a Buchner funnel using Whatman No. 1 paper (Whatman Inc., Clifton, N.J.). The filtrates were evaporated under vacuum at 400C on a rotary (HEI-VAP Value G1, Schwabach, Germany). This evaporated extract, prepared as stock, was stored at 40 °C to be used for all analyses. Samples were prepared fresh at the desired concentration and used.

2.7. Total phenolic content (TPC)

Total phenolic content was determined according to Sonmez and Sahin (2022). Folin-Ciocalteu was diluted 1:10 with water. 0.1 ml of methanolic tarhana extracts and 0.2 ml of diluted Folin-Ciocalteu reagent were mixed and incubated for 3 min. Aqueous sodium carbonate solution (20% w/v) was added followed by incubation in the dark for 60 min. Gallic acid (GAE) was used as a standard for the calibration curve.

All measurements were determined using UV-vis spectrophotometer at 765 nm. The results of total phenolic content (TPC) was given as mg GAE equivalent/g sample.

2.8. DPPH radical scavenging activity assay

DPPH radical scavenging activities of tarhana extracts were measured according to Cadi et al. (2020). Tarhana samples prepared at different concentrations of 0.2 mL were mixed with 0.05 mM DPPH. After the mixture was incubated at room temperature for 30 min, the absorbance was measured at 517 nm. A graph was drawn with % inhibition-absorbance values. The extract concentration providing 50% inhibition (IC50) was calculated from the graph of scavenging effect percentage against the extract concentration.

$$\%\, I = \frac{(A_{control} - A_{\,sample})}{A_{control}} * 100$$

2.9. ABTS radical cation decolorization assay

ABTS scavenging activities of the extracts were measured according method of Sonmez et al. (2019). ABTS and $K_2S_2O_3$ were dissolved in distilled water to prepare ABTS radical solution. This mixture was kept in the dark for 18-24 h at room temperature and the absorbance of the solution was adjusted at 734 nm. Tarhana samples were prepared at different concentrations. Initial absorbance of ABTS radical and absorbance values after 6 min were measured at 734 nm. The results were expressed as IC50.

$$\%\, I = \frac{(A_{initial} - A_{\,expiration})}{A_{initial}} * 100$$

2.10. Total monomeric anthocyanin

Total monomeric anthocyanin contents were applied with reference to Giusti and Wrolstad, (2001). The absorbance of tarhana samples in buffers at pH 1.0 and 4.5 were measured at 520 nm (λmax) and 700 nm using an UV-Vis spectrophotometer. Results were calculated as mg cyn3-glu/kg sample.

2.11. Total flavonoid content (TFC)

Total flavonoid content was determined by a colorimetric method (Chlopicka et al., 2012). Tarhana extracts were diluted with distilled water. Then $NaNO_2$ solution was added to the mixture, and after 5 min $AlCl_3.6H_2O$ solution was added. After the mixture was left for incubation, 1 M NaOH was added and made up to a total of 10 mL with distilled water. The absorbance values of these mixtures were measured with a UV-vis spectrophotometer at a wavelength of 510 nm. The results were stated as mg catechin eq./g sample.

2.12. Colour measurement

Colour values of redness/ greenness (a*), yellowness/ blueness (b*) and lightness (L*) of samples were measured using a colourimeter (CR-10; Konica Minolta, Japan). Measurements were made in 5 repetitions. The results were given as an average value and statistical analysis was performed.

2.13. Sensory analysis

Sensory evaluation, color, taste, smell, consistency and general acceptability of tarhana soup were evaluated. Evaluations for this purpose were determined by 10 untrained panelists consisting of students and staff of Pamukova Vocational School. Within the scope of the study, 100 g of tarhana sample, 1.5 L of distilled water, 40 g of oil, 10 g of salt are mixed over medium heat and cooked for 10 min. The prepared tarhana samples were presented to the panelists in porcelain bowls at 60°C (Tarakci and Ogurlu, 2023). Scoring was made by the panelists between 1 and 5 (1; very bad, 5; excellent).

2.14. Statistical analysis

Statistical evaluations were analysed by Minitab Statistical Software using ANOVA with a 95% confidence interval. Differences among samples were determined by Tukey's test ($p < 0.05$). Also, the results of statistical analysis were checked and corrected by Fisher's test ($p < 0.05$).

3. Results and discussion

The physical and chemical analyzes, total phenolic content, total flavonoid content, DPPH, ABTS antioxidant activity and total monomeric anthocyanin values of tarhana containing black carrot extract and classical tarhana are given in Table 1.

While pH values of tarhana extracts were determined as 5.05±0.005 in classical tarhana and 4.15±0.01 in tarhana containing black carrot extract, titration acidity in terms of lactic acid was calculated as 0.15±0.01 and 0.24±0.01, respectively ($p<0.05$). While the pH of tarhana containing black carrot extract is lower than that of classical tarhana, its titratable acidity is higher. Since black carrot extract is acidic, it decreased the pH value of tarhana and increased its acidity (Table 1). As a result, it was discovered that adding black carrot extract to Tarhana increased its acidity value. When the tarhana were compared, a statistically significant difference was found between the samples in terms of titratable acidity ($p<0.05$). In the research conducted by Cankurtaran et al. (2020) on tarhana obtained with taro and yam flours, pH values (4.23-5.21) were determined to be higher than tarhana containing black carrot extract. Titratable acidity values of tarhana samples containing 0%, 5%, 10%, 15%, 20%, 25% and 30% hazelnut pulp were examined by Ogurlu and Tarakcı (2023). Calculated between 0.58-0.74 values in terms of lactic acid per 100g sample. The titratable acidity value of tarhana containing black carrot extract is lower than that of tarhana containing hazelnut pulp. %dry matter, ash and protein content of classical tarhana is 13.66±0.15, 1.46±0.45 and 12.52±0.24 respectively. At the same time, the %dry matter, ash and protein values of tarhana containing black carrot extract were determined as 12.66±0.05, 1.61±0.41 and 12.72±0.61, respectively. In the study conducted by Aktaş and Akın (2020), tarhana with rice bran and corn bran was examined. While the % moisture and %ash values of tarhana containing black carrot extract were determined to be higher than the tarhana containing rice bran and corn bran, the % protein was determined to be lower. Tarhana containing different amounts of almond pulp was examined by Sensoy and Tarakcı (2023). It was reported that the % ash content of these samples were between 1.41 and 1.65, and the % protein content between 13.02 and 16.11. While the % ash content of tarhana containing black carrot extract had a similar effect, it was observed that the % protein content was lower.

In the data obtained, the total phenolic content of classical tarhana is 1.04±0.008 mg GAE/g sample, while tarhana with black carrot extract is 1.08±0.08 mg GAE/g sample ($p<0.05$). DPPH and ABTS activated IC50 values of classical tarhana as antioxidant activity were determined as 6.01±1.25 mg/mL and 0.83±0.03 mg/mL, respectively. For tarhana containing black carrot extract, antioxidant activity values were determined as 4.21±1.78 mg/mL and 0.93±0.01 mg/mL, respectively. While the DPPH activity of tarhana containing black carrot extract was higher than that of classical tarhana, ABTS activity had a lower effect ($p<0.05$). When the total flavonoid content is considered, it is seen that there is 1.17±0.06 mg catechin/g sample for classical tarhana and 0.75±0.05 mg catechin/g sample for tarhana containing black carrot extract ($p<0.05$). While both tarhana have similar phenolic content, classical tarhana contains more flavonoids. When the total monomeric anthocyanin content of tarhana are examined, it is seen that it is 9.96±1.27 mg cyn3-glu/kg sample for classical tarhana and 19.14±2.02 mg cyn3-glu/kg sample for tarhana containing black carrot extract ($p<0.05$). Tarhana, which contains black carrot extract, contains a significant amount of anthocyanins. Tarhana containing shalgam residuals was examined by Tanguler and Tatlısoy (2022) and the anthocyanin content of the obtained products was determined as 4.13 to 13.72 mg/L. It appears that tarhana containing black carrot extract has a higher anthocyanin content. Ghafoor et al. (2021) examined the total phenolic and total flavonoid values of tarhana samples containing different concentrations of pickling herb (PHET; E. tenuifolia subspp. sibthorpiana L.) (2-18%). Total phenolic content changed from 78.26 to 336.88 mg GAE/L in tarhana containing 18% PHET (in free form). In the same study, the highest total flavonoid content (1371.33 mg/L and 364.67 mg/L) was detected in tarhana, which contains 18% PHET.

As mentioned above, various tarhana productions modified by adding different natural products or extracts have been reported in the literature. The physical and chemical properties of the modified tarhana products can increase or decrease depending on the structural characteristics of the used additives.

The products of tarhana with black carrot extract and classic tarhana are given Figure 2. Graphical representation of colour measurement of tarhana with black carrot extract and classical tarhana extracts are given in Figure 3.

Table 1. Physical and chemical analyzes, total phenolic content (TPC), total flavonoid content (TFC), DPPH activity, ABTS activity and total monomeric anthocyanin values of tarhana with black carrot extract and classical tarhana.

Component	Classic tarhana	Tarhana with black carrot
pH	5.05±0.005[a]	4.15±0.01[b]
Titration acidity (lactic acid g/100g)	0.15±0.01[b]	0.24±0.01[a]
% Moisture	13.66±0.15[a]	12.72±0.05[b]
% Ash	1.46±0.45[a]	1.61±0.41[a]
% Crude protein	12.52±0.24[a]	12.72±0.61[a]
TPC (mg GAE/g sample)	1.04±0.008[b]	1.08±0.08[a]
DPPH assay (IC_{50}, mg/mL)	6.01±1.25[a]	4.21±1.78[b]
ABTS assay (IC_{50}, mg/mL)	0.83±0.03[b]	0.93±0.01[a]
TFC (mg catechin/g sample)	1.17±0.06[a]	0.75±0.05[b]
Total monomeric anthocyanin (mg cyn3-glu/kg sample)	9.96±1.27[b]	19.14±2.02[a]

Results are expressed as means ± SD (standard deviation) (n=3). 'a-b' refers the significant differences between the values in the same row ($p < 0.05$).

Lightness (L*), redness (a*) and yellowness (b*) values of tarhana containing black carrot extract were measured and the corresponding values were found to be in the range of 82.90±0.54, 7.63±0.13 and 21.97±0.17. The L*, a* and b* values of classic tarhana were found to be in the range of 84.66±0.24, 7.76±0.12 and 17.49±0.15, respectively. It was observed that the L* value decreased with the addition of black carrots and it was determined to be statistically significant ($p<0.05$). When the results are examined, it is understood that the effect of black carrot extract on the a* value of tarhana is statistically significant ($p>0.05$). Additionally, the effect of black carrot extract on the b* value of tarhana was found to be statistically significant ($p<0.05$). L* values of tarhana found in the studies of Tarakçı et al. (2013) and Kose and Cagindi (2002) are lower compared to our values. a* and b values of tarhana found in the studies of Tarakçı (2013) are higher compared to our values. Additionally a* and b values of tarhana found in the studies of Kose and Cagindi (2002) are higher compared to our values.

Figure 2. Tarhana with black carrot extract and classic tarhana

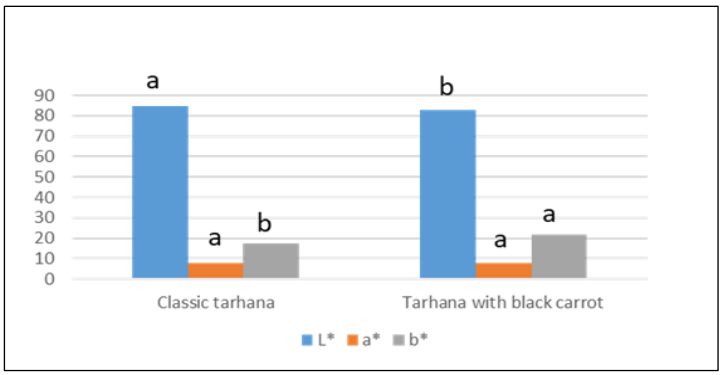

Figure 3. Colour measurement of tarhana with black carrot extract and classical tarhana

The graphical representation of the sensory analysis results of tarhana with black carrot extract and classic tarhana is given in Figure 4. Tarhana produced in the same way were presented to the panelists and evaluated in terms of color, taste, smell, consistency and general acceptability. Sensory analysis results of classic tarhana and tarhana with black carrot extract were determined as color (3.8±0.63 and 4.2±0.78, respectively), taste (3.6±0.69 and 4.3±0.67, respectively), smell (3.7±0.82 and 4.3±0.82, respectively), consistency (3.4±0.96 and 4.2±0.76, respectively) and general acceptability (4±0.66 and 4.4±0.69, respectively).

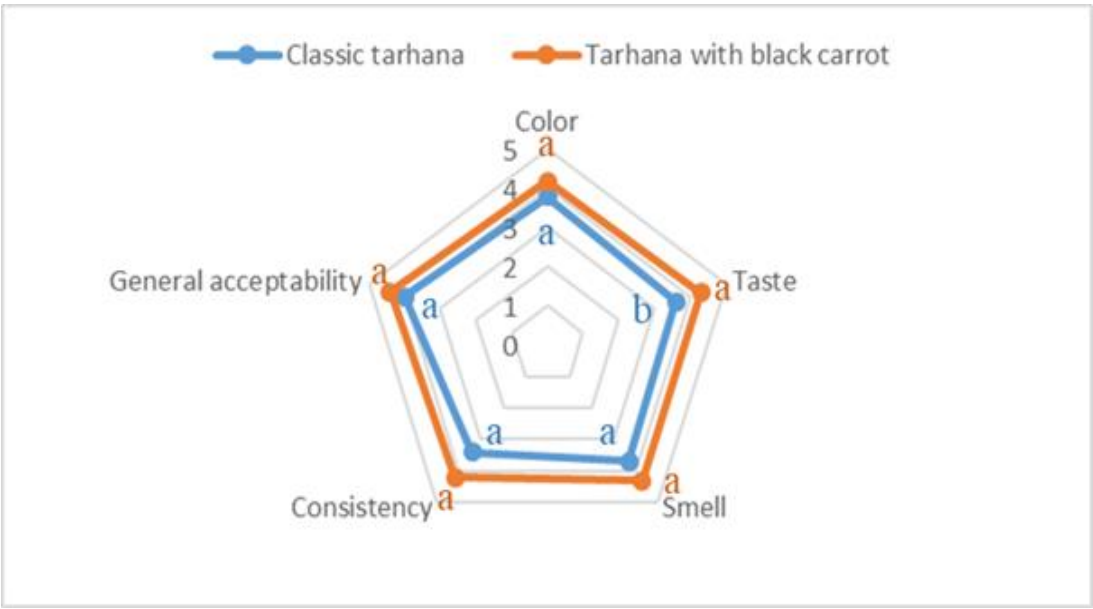

Figure 4. Sensory evaluation of tarhana with black carrot extract and classic tarhana.

4. Conclusions

This study shows that tarhana prepared with black carrot extract has a lower pH value and higher acidity in terms of lactic acid. Tarhana containing black carrot extract, which has a higher phenolic content than classical tarhana, shows a very high DPPH activity (4.21±1.78 mg/mL). At the same time black carrot extract contains a significant amount of anthocyanins (19.14±2.02 mg cyn3-glu/kg sample). According to color measurement and sensory analysis evaluations, the acceptability of tarhana containing black carrot extract was determined to be very high. These results lead to further studies on the importance of tarhana consumption containing black.

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Zuhal SAHIN: Validation, Writing - original draft., Methodology, Investigation, Conceptualization, Review and editing, Visualization, Formal analysis, Data curation. **Fatih SONMEZ**: Validation, Review and editing, Formal analysis, Data curation. Validation, Review and editing, Formal analysis, Data curation.

Ethical approval

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Evaluating the response of different synthetic and organic fertilizers on Carrot vegetative and reproductive characteristics in Gothgaun, Nepal

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ABSTRACT

This research aimed to assess the influence of various chemical and organic fertilizers on the vegetative and reproductive characteristics of the 'New Kuroda' variety of carrots (*Daucus carota*). The study included the recommended dose (RD) of NPK (80:60:60 kg ha^{-1}), individual components of N (80 kg ha^{-1}), P (60 kg ha^{-1}), and K (60 kg ha^{-1}), as well as organic sources such as goat manure (15 tons ha^{-1}), Farmyard manure (FYM) (20 tons ha^{-1}), and a control group without any fertilizer. Growth and reproductive traits were measured at 45, 55, 65, 75, and 85 days after sowing. The results indicated that the recommended NPK dose consistently outperformed other fertilizer sources, enhancing both vegetative and reproductive parameters. The highest yield of 10.94 tons ha^{-1} was achieved with NPK fertilizer, whereas organic sources such as goat manure demonstrated the second-highest growth and development traits. The control group exhibited the lowest growth and development parameters. These results show that these fertilizer sources considerably influence the vegetative and reproductive development of the 'New Kuroda' carrot variety. The outcomes indicate how various fertilizer sources may significantly improve the vegetative and reproductive growth of the 'New Kuroda' carrot; the recommended dosage of NPK in conjunction with the supply of goat manure stands out as an appropriate technique for producing carrots. This illustrates how applying a balanced dosage of these fertilizers may lead to increased carrot yield.

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1. Introduction

The carrot, formally known as *Daucus carota* L., is an Apiaceae family member with a chromosome number of 2n=18 (Yadav et al., 2021). This widely planted cool-season root vegetable is usually grown in temperate areas in the spring and summer months, wheras it is grown in tropical locations during the winter (Ahmed et al., 2014). Carrots are a worldwide popular crop, praised for their nutritionally dense roots, which are particularly high in fiber and beta-carotene a necessary precursor to vitamin A (Valšíková-Frey et al., 2021). A diversity of hues may be observed in carrot roots, including white, yellow, orange, red, purple, and dark purple. The flesh of the cultivated types was originally purple and yellow (da Silva Dias, 2014). Rich in nutrients such as carotene, thiamin, riboflavin, iron, calcium, and phosphorus, carrots may be used in a variety of culinary applications, including salads, soups, stews, and curries, as well as pickles, jams, and sweets (Afrin et al., 2019). Carrots have several therapeutic uses in addition to being used in food; they are a diuretic, intestinal cleanser, remineralizer, anti-diarrhea, tonic, and anti-anemic agent (Bahadur et al., 2015). Rich in alkaline components, carrots cleanse and revitalize the blood, providing antioxidants that promote heart health, stave against heart disease, and have anti-tumor effects (Agbede et al., 2021).

In Nepal, during 2078/79, carrot cultivation covered 3,354 hectares, yielding 37724.9 metric tons at a productivity of 11.18 metric tons per hectare (MoALD, 2022). However, the country faces lower yields compared with other carrot-producing nations, attributed to insufficient agro-technical knowledge concerning irrigation intervals and judicious fertilizer application (Kiraci et al., 2018). The overuse of inorganic fertilizers in modern agriculture has harmed the ecology and soil (Kiran et al., 2022). Lately, there has been a growing trend in utilizing organic fertilizers such as farmyard manure (FYM), vermicompost, poultry manure, neem cake, and goat manure to improve crop yield and maintain soil fertility, as noted by Yadav et al. (2023a). The quantity of soil organic carbon (C) is affected differently over the long term by the utilization of nitrogen (N), phosphorus (P), and potassium (K), which are affected by cropping patterns, soil variances, and environmental conditions (Ahmad et al., 2016). NPK, with a particular emphasis on nitrogen, is a crucial nutrient for plant growth, significantly impacting crop development and yield, as highlighted by Mandal et al. (2023). Global research efforts are focused on finding alternatives such as green manures, legumes, and organic materials to generate food that is on par with that produced using inorganic fertilizers (Zakir et al., 2012). Katel et al. (2023) found that excessive use of NPK can lead to a decrease in crop productivity. Additionally, Yadav et al. (2022a) stated that overuse of manure like poultry manure can result in contamination of crops, soil, or water. Numerous research studies have demonstrated how well farm manure and other organic nutrient supplies may increase soil fertility, crop yields, and soil water-holding capacity (Suswadi et al., 2022).

A significant movement toward organic farming is underway worldwide to lessen the harmful effects of synthetic pesticides and fertilizers have on the environment and human health (Shakeel et al., 2021). When it comes to growing vegetables, the use of vermicompost or organic manure in nutrient management systems has recently gained (Ahmed et al., 2014). Organic manure is essential for improving soil health over the long term and reducing crop production costs, which makes it important in both Nepalese and global settings (Agbede et al., 2021). Additionally, according to Yadav et al. (2023b), the importance of soil biota in improving soil quality, supporting plant health, and enhancing soil resilience is significant. Furthermore, the presence of beneficial microorganisms is essential for maintaining soil fertility, enhancing plant resilience, and promoting overall crop health (Yadav et al., 2023c). While inorganic fertilizers quickly provide nutrients to fulfil crop demands, organic fertilizers gradually release minerals to promote vigorous plant development (Lamichhane et al., 2022). Carrot yields increase when inorganic fertilizers are mixed with organic manures (Chen et al., 2020). One important aspect of managing soil is applying fertilizer, which has a major impact on increasing soil fertility in agricultural activities (Afrin et al., 2019). Consumers of vegetables like organic farming because it improves the quality of their product and worries about the harmful effects of inorganic fertilizers on health are driving this trend (Dawuda et al., 2011). Consequently, many farmers have shifted to organic farming, motivated by the greater market value of organic goods in addition to health concerns (Havlin and Heiniger et al., 2020). The goal of this research is to investigate the effects of different chemical and organic fertilizer sources on carrots' vegetative and reproductive growth of carrots. In addition, identified the best source of organic fertilizer that might increase carrot productivity and associated characteristics.

2. Materials and methods

2.1. Experimental site

The field experiment, which was conducted at G.P Koirala College of Agriculture and Research Centre in Sundarharaicha, Morang, Nepal, from August to November 2023, sought to evaluate the yield of the 'New Kuroda' carrot variety. The tropical climate in the region is characterized by an average annual temperature ranging from 18.81 to 33.46 °C and an annual precipitation of 858.75 mm. The geographical coordinates are 26^0 40' 49.9" N latitude, 87^0 21' 16.7" E longitude, and an elevation of 149 m. A soil test kit was used for soil analysis; Table 1 contains more information about the product. The highest and lowest average temperatures reported during the research period were 36.74 °C and 20.44 °C, respectively, with an average precipitation of 211.09 mm (Figure 1). This study concentrated on assessing the vegetative and reproductive characteristics of the 'New Kuroda' carrot variety, which matures 85–100 days after planting and is well-known for its wonderful sweetness and ultra-fine texture.

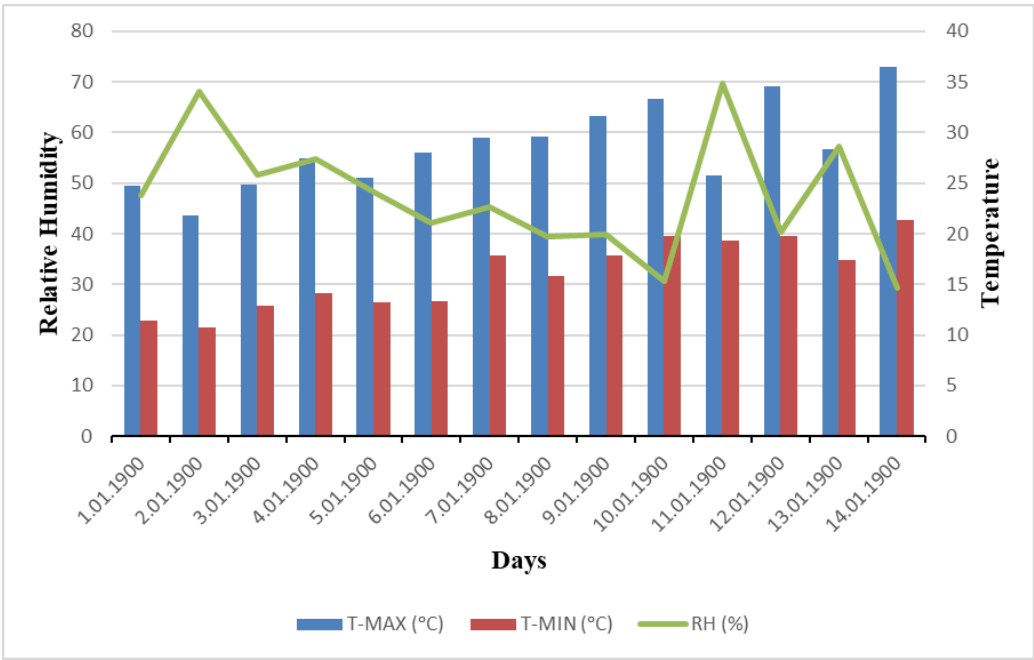

Figure 1. Meteorological data of the study site during the research period

Table 1. Soil Characteristics of Experimental site

Serial Number	Soil Characteristics	Properties
1	Nitrogen	Slightly High
2	Phosphorous	Medium
3	Potassium	Medium
4	Soil pH	6.2
5	Soil texture	Loamy soil

2.2. Research design and cultural practices

The study was designed as a Randomized Complete Block Design (RCBD), with seven treatments replicated three times for 21 plots (Yadav et al., 2022b). Each plot, measuring 3 m2 (2 m*1.5 m), contributed to a total area of 150 m2. A 1 m gap separated the two replications, but the distance between treatments remained constant at 0.5 m. Each plot comprised 100 plants with 30 cm row-to-row spacing and 10 cm plant-to-plant spacing. Organic fertilizers were administered during field preparation, whereas chemical fertilizers were added during seed sowing.

The complete phosphorus and potassium doses, along with half of the nitrogen dose, were administered during the sowing period, while the other half of the nitrogen dose was applied after the initial weeding. Manual weeding was conducted to manage weed growth, followed by irrigation. Irrigation was implemented during the early growth stage, flowering, and fruit development phases. The details of all treatments and their respective doses employed in our research are outlined in Table 2.

Table 2. List of treatments and their doses

Serial Number	Treatments	Symbol	Doses
1	RD of NPK	T1	80: 60: 60 kg ha^{-1}
2	N	T2	80 kg ha^{-1}
3	P	T3	60 kg ha^{-1}
4	K	T4	60 kg ha^{-1}
5	FYM	T5	20 t ha^{-1}
6	Goat manure	T6	15 t ha^{-1}
7	Control	T7	untreated

Note; RD; recommended dose, N; Nitrogen, P; Phosphorous, K; Potassium, FYM; farmyard manure

2.3. Data collection

Twelve plants were chosen randomly from each experimental plot to acquire the relevant data. The data were obtained at 10-day intervals. Plant height, leaf number, root length, fresh shoot weight, root diameter, dry shoot weight, and yield were all measured for both vegetative and reproductive growth.

2.4. Statistical analysis

For both replication and treatment blocks, raw data were input using MS Excel 2021 (Microsoft Corporation, Washington, USA) chronologically. Then, using statistical software (R Studio, Version 4.2.2, Boston, Massachusetts, USA), analysis of variance (ANOVA) was performed. Duncan's Multiple Range Test (DMRT) was used to compare mean values among different treatments at a significance level of 5%. Regression analysis was also performed.

3. Results and discussions

3.1. Effects of different fertilizer sources on vegetative growth parameters

The variety 'New Kuroda' showed the significant variation in vegetative growth among different fertilizer sources after application, which is clearly noticeable from the results presented in Table 3. The results revealed that among the several treatments used in the study, the recommended dose of NPK exhibited the highest value for plant height, which is 20.41 cm initially at 45 days after sowing, which follows the positive growth trends and attains a maximum height of 44.53 cm at 85 days after sowing. The study conducted by Afrin et al. (2019) reported that inorganic fertilizer (NPK) in combination with organic fertilizer yielded the highest plant height of 47.58 cm at harvest. This result is found to be almost similar to our findings. This can be due to the recommended dose of NPK supplying the optimal amount of essential nutrients such as nitrogen, phosphorus, and potassium, thus fostering robust growth and maximizing carrot plant height. Similar results were also concluded in the study by Kiran et al. (2022). Katel et al. (2021) found that super combined fertilizer releases its active ingredients gradually, a characteristic that proves beneficial in the agricultural sector.

Likewise, goat manure and the recommended dose of Nitrogen and phosphorous recorded the second highest plant height and number of leaves after their application, which is 42.25 cm, 38.80 cm, 38.72 cm, and 10.90, 11.20, and 10.93, respectively. This result is very close to the findings given by the previous study carried out by Kiran et al. (2022) and Smoleń et al. (2014). After applying goat manure and the required amounts of nitrogen and phosphorus, carrot plants develop more vigorously because of increased soil fertility and nutrient availability, which results in increased plant height and leaf count. Farmyard manures exhibited the average plant height and leaf number as compared to other chemical fertilizers and goat manures. Initially, FYM records 15.01 cm plant height and 5.43 leaf numbers which increases and gives a final plant height of 38.53 cm and 10.56 leaves number as given in Table 4.

Table 3. Effects of various sources of fertilizers on plant heights of carrots

Treatments	Plant height (cm)					
	45DAS	55DAS	65DAS	75DAS	85DAS	Pooled
NPK	20.41^{a}	31.88^{a}	40.78^{a}	42.16^{a}	44.53^{a}	35.95^{a}
Goat manure	17.31^{bc}	29.57^{ab}	40.16^{a}	41.69^{a}	42.25^{ab}	34.19^{ab}
N	18.92^{ab}	29.73^{ab}	35.87^{ab}	38.26^{ab}	38.80^{bc}	32.32^{abc}
P	16.80^{bc}	26.68^{bc}	34.81^{b}	38.20^{ab}	38.72^{bc}	31.04^{bc}
K	14.33^{c}	22.72^{c}	31.34^{bc}	37.63^{ab}	37.89^{bc}	28.78^{c}
FYM	15.01^{c}	25.43^{c}	33.15^{bc}	38.05^{ab}	38.53^{bc}	30.02^{c}
Control	9.17^{d}	18.29^{d}	28.27^{c}	32.29^{b}	34.64^{c}	24.53^{d}
Grand mean	15.996	26.319	34.914	38.328	39.34	30.979
CV %	9.933	8.608	7.587	8.512	6.604	6.388
SEM (±)	0.823	1.110	1.078	0.946	0.846	0.894
F-test	***	***	***	*	*	***

* Significant at 5% level of significance. ** Significant at 1% level of significance. *** Significant at 0.1% level of significance. NS: Non-significant. SEM: Standard error of the mean. CV: Coefficient of difference. PH: Plant height.

According to Ahmed et al. (2014), they concluded that the average plant height was given by FYM which supports our findings. The slow release of nutrients from farmyard manure promotes consistent carrot development, as shown by the plants' increased height and leaf counts. The lowest results were recorded in the control in which no fertilizers were applied. The lowest plant height was 9.17 cm at 45 DAS which grew slowly and reached at maximum height of 34.64 cm. Similarly, the overall mean plant height and leaf number at harvest time were recorded as 39.34 cm and 10.64 respectively. Overall, the results showed that vegetative growth followed a positive growth trend in terms of both plant height and leaf number among the several treatments used in the study. The results were highly significant at 0.1% level of significance. These findings were also supported by the previous study by Kiran et al. (2022) and Kiraci et al. (2018). The lowest plant height and leaf number in the control may be due to insufficient nutrient supply, hindering overall growth.

Table 4. Effect of various sources of fertilizer on the number of leaves in carrot

Treatments	Leaf numbers (LN)					
	45DAS	55DAS	65DAS	75DAS	85DAS	Pooled
NPK	6.00^{a}	8.13^{a}	10.16^{a}	11.10^{a}	11.83^{a}	9.44^{a}
Goat manure	5.96^{a}	7.60^{ab}	9.23^{b}	9.73^{bc}	10.90^{ab}	8.68^{a}
N	5.86^{a}	7.13^{abc}	8.80^{bc}	9.33^{bc}	11.20^{ab}	8.46^{bc}
P	5.90^{a}	7.56^{ab}	8.73^{bc}	9.80^{b}	10.93^{ab}	8.58^{b}
K	5.33^{a}	6.93^{bc}	8.63^{bc}	9.26^{bc}	10.20^{b}	8.07^{d}
FYM	5.43^{a}	6.96^{bc}	8.36^{c}	9.23^{c}	10.56^{b}	8.11^{cd}
Control	4.03^{b}	6.10^{c}	7.33^{d}	8.20^{d}	8.86^{c}	6.90^{e}
Grand mean	5.504	7.204	8.752	9.523	10.642	8.325
CV %	6.772	8.047	4.687	2.979	5.277	2.400
SEM (±)	0.167	0.177	0.197	0.200	0.219	0.172
F-test	***	*	***	***	***	***

* Significant at 5% level of significance. ** Significant at 1% level of significance. *** Significant at 0.1% level of significance. NS: Non-significant. SEM: Standard error of the mean. CV: Coefficient of difference. LN: Leaf number

3.2. Effect of different fertilizer sources on reproductive growth parameters

Tables 5 and 6 illustrate the response of several reproductive parameters to the different fertilizer sources used in the study, such as fresh shoot weight, fresh dry weight, dry shoot weight, dry root weight, root length, root diameter, shoot diameter, and ultimate yield. The results recorded significant variation among these metrics, except for dry root weight and shoot diameter. The 'New Kuroda' variety of carrot treated with the recommended dose of synthetic fertilizer, NPK records the highest reproductive growth among all above mentioned parameters. The highest fresh and dry shoot weights were observed in the NPK treatment at 61.06 g and 9.03 g, respectively. Following closely were the weights from goat manure at 41.23 g and 8.40 g, and then the recommended nitrogen quantity at 44.26 g and 8.00 g.

Contrary to our findings, Kiran et al. (2022) reported the highest fresh plant weight (128.00 g) with the combined application of chemical (NPK) and organic fertilizers. Discrepancies between their results and ours could be attributed to variations in soil composition, climate conditions, and microbial activity, influencing nutrient availability. Additionally, differences in fertilizer application rates, timing, and sources may also contribute to variations in plant growth, underscoring the influence of environmental and fertilizer management practices. The second highest fresh shoot weight was recorded by the recommended dose of Nitrogen that is (44.26 g). This result is in parallel with the findings reported by Smoleń et al. (2014). This is due to the optimal nitrogen dose that boosts plant growth, enhancing proper development and biomass accumulation. The lowest value for fresh shoot weight was recorded in the control treatment by Kumar et al. (2023), and this result is similar to our findings with treatments without any fertilizer application. It may be because the absence of fertilizer leads to nutrient deficiency, hindering plant growth and reducing fresh shoot weight. Similarly, the highest Fresh & dry root weights were noticed in the recommended dose of NPK followed by the recommended dose of nitrogen and goat manures. This result is well supported by the previous study carried out by Kiran et al. (2022) and Colombari et al. (2018). This is because of the optimum application of NPK and goat manures which contain essential nutrients, promoting robust root development, and ensuring increased fresh and dry weight of roots. When subjected to farmyard manure application, this particular variety exhibits favorable responses, demonstrating significant improvements across various reproductive characteristics. The fresh and dry weights provided by farmyard manure closely resemble those from goat manure. This similarity arises from the fact that farmyard manure enhances soil with organic material, replicating the nutrient effects of goat manure and resulting in comparable fresh and dry weights in plant development. Findings from researchers Hussain and Kerketta et al. (2023) and Kiraci et al. (2018) align closely with our observations using farmyard manure.

Table 5. Effect of various sources of fertilizers on the reproductive growth of carrot

Treatments	FSW(g)	FRW(g)	DSW(g)	DRW(g)
NPK	61.06^{a}	89.06^{a}	9.03^{a}	11.56^{a}
Goat manure	41.23^{b}	68.98^{ab}	8.40^{a}	10.20^{ab}
N	44.26^{b}	69.06^{ab}	8.00^{a}	8.75^{abc}
P	35.50^{b}	60.33^{ab}	7.25^{ab}	8.93^{abc}
K	35.30^{b}	49.95^{bc}	6.68^{ab}	7.20^{bc}
FYM	35.73^{b}	66.66^{ab}	5.52^{bc}	7.51^{bc}
Control	31.13^{b}	28.79^{c}	3.82^{c}	5.48^{c}
Grand mean	40.604	61.836	6.958	8.520
CV %	20.736	27.283	18.240	24.404
SEM (±)	2.545	5.017	0.441	0.597
F-test	*	*	**	NS

* Significant at 5% level of significance. ** Significant at 1% level of significance. *** Significant at 0.1% level of significance. NS: Non-significant. SEM: Standard error of the mean. CV: Coefficient of difference. FSW: Fresh shoot weight. FRW: Fresh root weight. DSW: Dry shoot weight. DRW: Dry root weight.

Synthetic fertilizers provide precise nutrient control, boosting initial growth, while organic sources such as compost enhance soil structure, microbial activity, and nutrient retention, collectively promoting robust reproductive parameters in carrots Havlin and Heiniger et al. (2020), and Ahmad et al. (2016). Application of these fertilizers has a major impact on carrot yields in terms of root length, root diameter, shoot diameter, and overall yield. In terms of reproductive measures such as root length at a significance level of 5%, root diameter at a significance level of 1%, and yield at a significance level of 0.1%, the findings were highly significant across several fertilizers. This is consistent with the outcomes suggested by the previous study by Ige et al. (2019) and Kiran et al. (2022). The highest root length (21.52 cm), root diameter (2.63 cm), shoot diameter (0.41 cm), and yield (10.94 tons ha^{-1}) were observed on the recommended dose of fertilizers, followed by the recommended dose of nitrogen, goat manure which is clearly presented in table 6. The reasons behind this are because of timely available of essential nutrients as released by NPK and goat manure is highly rich in organic matter as well as essential nutrients, which further boosts initial growth ensuring rapid developments of reproductive traits in carrots resulting in highest root length, diameter, shoot diameter, and yields.

According to the Kiran et al. (2022), they concluded that the recommended dose of synthetic fertilizers along with organic amendments plays a significant role in the proper development of reproductive traits, which is also superior as compared to other fertilizer sources. This shows that their results agree with our findings suggesting that synthetic fertilizers along with organic amendments may be the suitable fertilizers sources for effective growth and developments of carrot crops. The yields recorded by farmyard manures and phosphorous were similar to the average yield of carrot among all treatments showing that using these fertilizers in combination might give better results. The lowest yield was recorded in the control group, in which no fertilizer was applied. This lowest yield is because of the lack of essential nutrients that plants require during growth and development, hindering plant overall growth and thus reducing the final yield in carrot. These results are also supported by several studies carried out by Hussain & Kerketta et al (2023), Afrin et al. (2019) & Kiran et al. (2022). Additionally, Adhikari et al. (2023) and Sangam et al. (2023) both noted that organic fertilizers have the potential to significantly enhance crop productivity. Therefore, Goat manure governed better yield production after NPK combined, which reflects partial agreement with the aforesaid findings.

Table 6. Effect of different sources of fertilizer on the reproductive parameters of carrot

Treatments	RL (cm)	RD (cm)	SD (cm)	Yields (ton^{-1}ha)
NPK	21.52^{a}	2.63^{a}	0.41^{a}	10.94^{a}
Goat manure	19.10^{abc}	2.46^{ab}	0.34^{ab}	9.53^{ab}
N	19.77^{ab}	2.40^{ab}	0.33^{ab}	9.37^{ab}
P	18.27^{bc}	2.35^{ab}	0.30^{ab}	7.95^{bc}
K	17.97^{bc}	2.11^{b}	0.25^{b}	4.93^{de}
FYM	18.84^{bc}	2.31^{ab}	0.26^{b}	6.13^{cd}
Control	17.00^{c}	1.74^{c}	0.23^{b}	3.80^{e}
Grand mean	18.926	2.288	0.302	7.523
CV %	6.916	9.148	22.744	14.327
SEM (±)	0.378	0.071	0.018	0.578
F-test	*	**	NS	***

* Significant at 5% level of significance. ** Significant at 1% level of significance. *** Significant at 0.1% level of significance. NS: Non-significant. SEM: Standard error of the mean. CV: Coefficient of difference. RL: Root length. RD: Root diameter. SD: Shoot diameter.

3.3. Regression analysis

The scatter diagram and linear regression equation are shown in Figure 2, along with the coefficient of determination (R2) and the fitted simple regression line of Y (Yield) on independent variables X (plant heights, root length, root diameter, leaf number, fresh root weight, fresh shoot weight). Carrot yields and all independent variables show substantial linear connections, as the findings demonstrate. The degree of correlation between the variables and the yield variance that can be accounted for by each variable is shown by the R2 values. Plant height (73%) followed by root length (49%), root diameter (57%), leaf number (63%), fresh root weight (53%), and fresh shoot weight (26%) were the parameters that contributed most to the overall carrot fruit output among those examined. The significance of length and fresh weight of root is emphasized as they are identified as the most important quality parameters for carrots, as indicated by the highest R2 values in this study. However, it is noted that despite these parameters being identified as significant contributors to carrot yield, the applications (presumably referring to the use of different fertilizers) did not show a significant impact compared to the control, as indicated by the lowest R2 values for these parameters. Other factors that may have contributed to the remaining amount include the study's fertilizer dosages, irrigation schedules, planting dates, and weed management strategies. A higher R2 value denotes a more accurate depiction of the link between the independent variables and carrot yields, showing a better fit of the regression line to the data. Overall, as Figure 2 makes evident, the most significant factors for increased carrot fruit output of the 'New Kuroda' variety were plant height, leaf count, and root diameter.

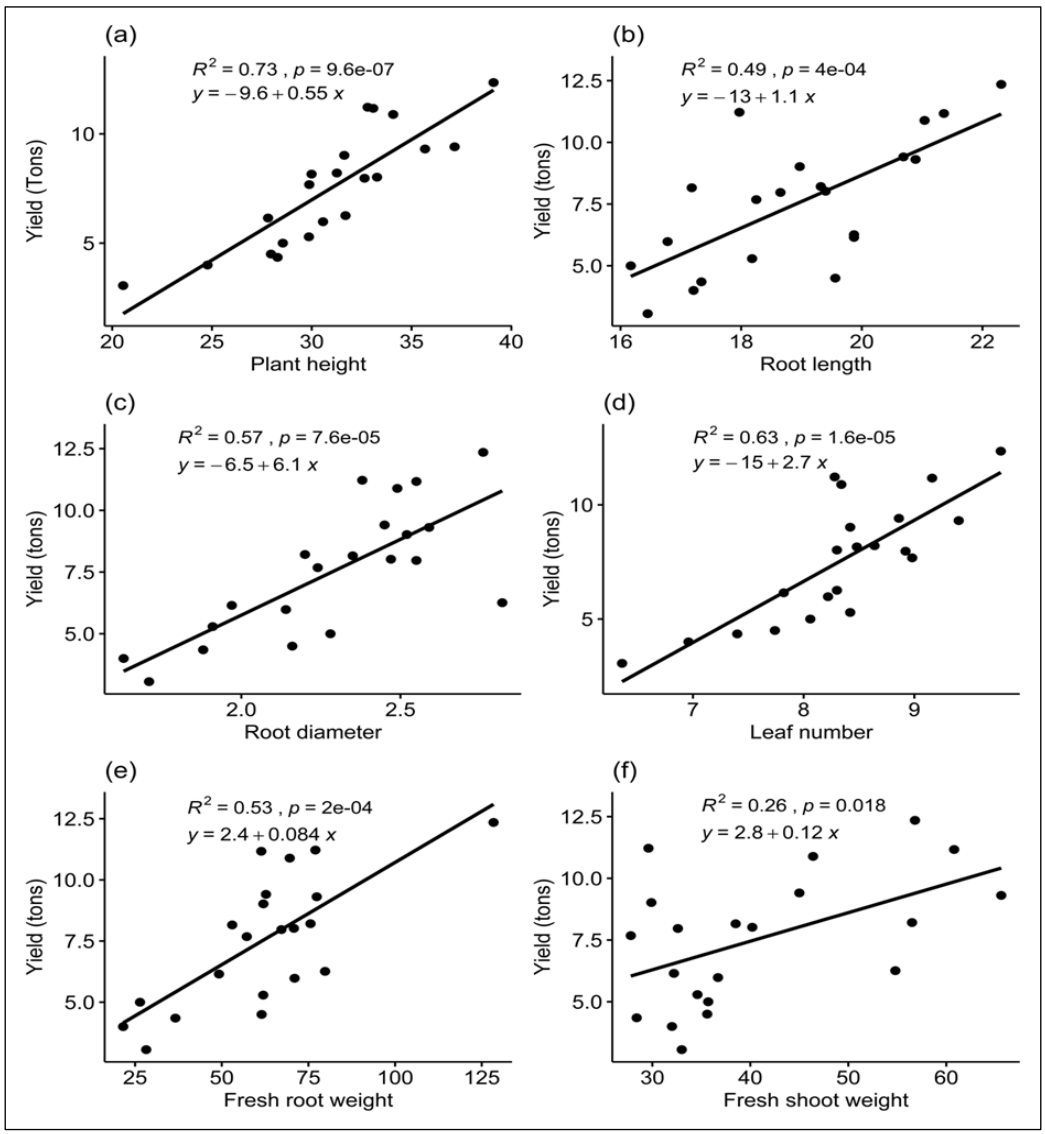

Figure 2. A scatter diagram, the linear regression equation, the coefficient of determination (R2), and the fitted simple regression line of Y (Yield) on X [(a)Plant heights (cm), (b)Root length (cm), (c)Root diameter (cm), (d)Leaf number, (e)Fresh root weight (g), (f)Fresh shoot weight (g)].

5. Conclusion

In conclusion, this study reveals a clear superiority of the recommended NPK dose in optimizing both vegetative and reproductive parameters, resulting in the highest yield of 10.94 tons ha^{-1} for the 'New Kuroda' carrot variety. Organic sources, particularly goat manure, demonstrated commendable growth and development traits, securing the second-highest performance. Notably, the control group exhibited the least favorable outcomes. These findings emphasize the substantial positive impact of NPK and organic fertilizers on the growth of 'New Kuroda' carrots. The study suggests using balanced synthetic fertilizer and organic additions, such as goat dung, in modern agricultural practices as an environmentally responsible way to boost carrot crop yields. This research emphasizes the effectiveness of using NPK in conjunction with goat dung, emphasizing these methods as essential strategies for obtaining strong carrot yields. This study highlights how a balanced fertilizer programme may greatly increase carrot output and offers important new information for sustainable farming methods.

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Dipesh Kumar MEHATA: Conceptualization, Funding acquisition, Validation, Visualization, Supervision, Investigation, Methodology, Resources, Software, Writing – review & editing: **Reema ISHWAR, Bina Kumari SAH, Sushma NEUPANE, Anish SUBEDİ, Sangita Puwar MAGAR**: Data curation. **Ravi ACHARYA**: Investigation, Supervision, Writing – review & editing. **Rupesh Raj YADAV, Jyoti KHATI, Abhishek Kumar SAH, Arzu CHAUDHARY**: Writing – original draft. All authors have read and agreed to the published version of the manuscript.

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Red LED light affects the physicochemical responses of strawberries during storage

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A B S T R A C T

This study aimed to evaluate the effect of the storage of strawberries under LED lights on 'Albion' strawberry quality. The treatments were applied as follows; (1) storage under continuous blue, red, and ultraviolet-A (UVA) LED light, (2) storage in the dark conditions (control), and (3) storage in the dark conditions after 1 h UVA (UVAh) LED lighting. Strawberries were stored at a temperature of 4±1°C with 85-90% relative humidity for 10 days. In the study, analyses were conducted on the total anthocyanin content, color (L*, hue angle, redness index), total soluble solids (TSS), fructose, glucose, total sugar content, titratable acidity (TA), fruit firmness (N), and weight loss at the start of the experiment and at 2-day intervals during storage. According to the results, the storage of strawberries under continuous red-LED light was successful in improving the anthocyanin and TSS contents, while preserving fruit firmness and reducing weight loss. Moreover, UVA treatment was effective in maintaining the L*, a*, and b* color values, whereas UVAh was effective on the hue angle and redness index. Furthermore, UVAh treatment caused a decrease in the glucose, fructose, and total sugar content and, in the titratable acidity of the strawberries.

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1. Introduction

Strawberry (*Fragaria x ananassa* (Weston) Dushesne ex crozier (pro sp.)) belongs to the *Fragaria* L. species in the Rosaceae family and Rosales order (USDA, 2024). Strawberry production in Türkiye amounted to 669.195 tons across 186.761 decares as of 2021 (TUIK, 2023). Strawberry is defined as a berry-like fruit. It is also named aggregate fruit because it comprises many individual tiny fruits embedded in a fleshy receptacle (Oğuz et al., 2022). It is a perishable fruit; therefore, it is important to ensure a cold chain process from harvest to consumer to maintain its quality. Losses are thus caused by water loss, fungal attacks, undesirable metabolic changes, and senescence, softening, structural changes, and color changes (Kuchi and Sharavani, 2019). Therefore, several studies have investigated the effects of modified atmosphere storage, gamma irradiation, ultraviolet irradiation, heat treatments, edible coating application, and recently light-emitting diode (LED) treatments for maintaining the postharvest quality of strawberries (Nassawara et al., 2021).

Previous studies have determined that LEDs affect secondary metabolites during postharvest storage of horticultural crops, the blue LED improves antioxidant enzyme activity in strawberries (Xu et al., 2014) and the white LED delays the breakdown of carotenoids in lettuces (Kasım and Kasım, 2017). Similarly, it was found that the red LED leads to an increase in the total carotenoids of Satsuma mandarins (Ma et al., 2012) and in the ascorbic acid of broccoli (Ma et al., 2014), whereas blue LEDs are more effective in increasing the vitamin C content of cabbages (Lee et al., 2014). LEDs improved the yield and biochemical quality of crops in both the pre-and postharvest periods and, increased the storage duration of horticultural crops via surface disinfection (Kasım and Kasım, 2017).

LED lights at 385, 470, 525, and 630 nm wavelengths increased the content of total soluble solids from 9.87% (both at harvest and during storage) to 12.77%. In addition, the vitamin C content (78.70 mg/100g), anthocyanin content (12.48 mg/100g), and total phenol content (172.75 mg/100 g) of strawberries treated with LED irradiation were higher than those in the control group (54.28 mg/100 g, 6.89 mg/100g, and 129.5 mg/100g, respectively) (Kim et al., 2011). Similarly, blue LED light treatment at a dose of 40 μmol/m2s increased in the total anthocyanin content of strawberries (Xu et al., 2014). However, it was stated that the white LED (300 μmol/m^2s) and blue LED (200 μmol/m^2s and 100 μmol/m^2s) treatments did not affect the vitamin C content, and the 100 μmol/m^2s white and blue light treatments caused weight loss in strawberries by increasing the transpiration of calyx (Li, 2016).

Ultraviolet A, B, and C irradiation have been commonly used in surface sterilization for years, and the effects of UV light on the quality of fresh produce have been well investigated and are currently being studied. However, studies on the effect of UVA-LED on the quality of fresh fruit and vegetables are limited. In a study investigating the use of UVA-LED for surface sterilization of fresh produce, it was concluded that UVA-LED treatment caused weight loss in cabbage, but no differences between UVA-LED and control were not found (Aihara et al., 2014). Therefore, the authors declared that UVA-LED treatment has great potential for surface sterilization of fresh produce (Aihara et al., 2014). Another study using red leaf lettuce detected that the different LEDs such as red and blue, blue and UVA LEDs, or white LEDs that were treated on lettuce three days before harvest did not affect leaf thickness and greenness, antioxidant capacity, and phytonutrient concentrations (Hooks et al., 2022). Lante et al. (2016) stated that UVA-LED (390 nm) is a technological alternative to the traditional approach for reducing the browning of fresh-cut apples and pears.

There is limited information on LED lighting applications for strawberry postharvest storage. To this end, there is a need to determine the effects of different LED light treatments such as continuous red, blue, and UVA and dark storage after one hour UVA (UVAh) light treatment on postharvest biochemical changes and the quality of strawberries during storage at a temperature of 4 ± 1°C.

2. Materials and methods

2.1. Plant material

In this study, the 'Albion' strawberry variety was used, which was grown under open field conditions in the town of Karamürsel located at coordinates 40.613365 north latitude and, 29.644316 east longitude in Kocaeli, as shown in Figure 1.

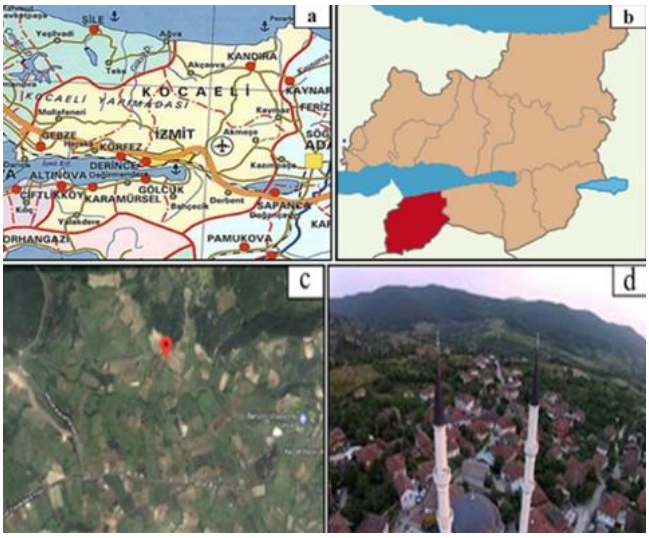


Figure 1. The location (a), region map (b), location (c) and visual status (d) of the field at which the strawberries are grown

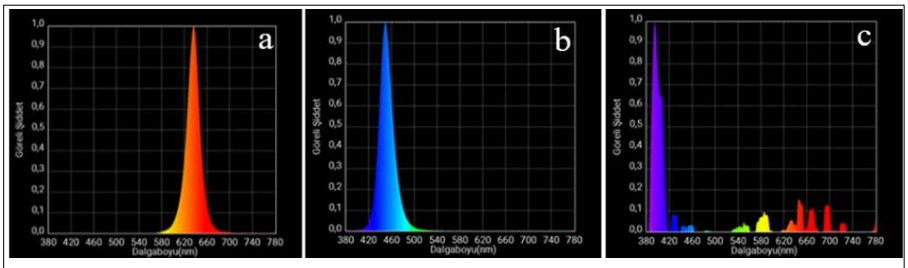
The strawberries were harvested at the 90% coloring stage, which was used as a commercial harvest stage, on July 15, 2017. They were immediately transported to the Postharvest Physiology Laboratory in the Arslanbey Vocational School of Kocaeli University within 1 h of harvest. The fruits were examined for uniformity and vigor, and those that were bruised, injured, etc., were separated. Only good looking fruits were used in the study.

2.2. LED light application apparatus

Three LED experiments were set up, which included red, blue, and ultraviolet-A (UVA) lights. These were set up using 1 m -1 m - 80 cm wooden frames on which a 5 m strip of LEDs was mounted for each light color. Strawberries were placed under LED lights, and the distance between the fruit and the light was 40 cm. The surroundings of the apparatus were covered with black polyethylene to inhibit light penetration into the strawberries from outside (Kasım and Kasım, 2017).

2.3. LED light treatments

The strawberries were divided into five groups. The first, second, and third groups of fruits were stored under continuous red (R), blue (B), and ultraviolet-A (UVA) LEDs, whereas the fourth control (C) group was stored in dark conditions. The fifth group was kept under dark storage conditions after 1 h of UVA-LED (UVAh) treatment. The wavelengths and properties of the LED lights were detected using the Asensetek Lighting Passport Essence and are given in Figure 2.



Red LED: 600-700 nm	Blue LED: 400-499 nm	UVA LED: 380-399 nm
PPFD: 2,0256 µmol/m²s	PPFD: 6,8933 µmol/m²s	PPFD: 0,0018 µmol/m²s
YPFD: 1,9631 µmol/m²s	YPFD: 5,0702 µmol/m²s	YPFD: 0,0011 µmol/m²s

Figure 2. The wavelengths and properties of the LED lights

2.4. Packaging and storage conditions

In the experiment, the strawberries were packaged into 15 x 25 x 5 cm transparent polyethylene boxes that included 350-400 g fruit. The packaged strawberries were stored at a temperature of 4 ± 1°C with 85-90% relative humidity for 10 days.

2.5. Total anthocyanin content (TAC)

For the calculation of the total anthocyanin content, 1 g of fruit sample was placed in a beaker, a 10 mL first buffer pH=1 (125 mL 0.2 M KCl + 375 mL 0.2 M HCl) was added to it, and the content was homogenized. The same process was repeated for the second buffer (pH=4.5) (400 mL 1 M sodium acetate + 240 mL 1 M HCl + 360 mL water). After homogenization, the samples for each buffer were centrifuged for 15 min at 5000 rpm, and the supernatant was collected and measured at 510 nm. The total anthocyanin content was calculated using the following formula by Şahin et al. (2021):

TAC (mg/kg TA) = (ABSpH:1,0 – ABSpH:4,5) x 484,82 x 1000 / 24825 x DF

In the formula: ABS expresses absorbance and DF: Dilution factor.

2.6. Color measurements

The color of the samples was measured using a colorimeter (Minolta CR 400 Chroma; Minolta Co., Osaka, Japan) at three different points on 10 strawberries. The L*, a*, and b* color values (CIELAB) were used in the expression of fruit color. From these values, the hue angle was calculated according to (Kasım and Kasım, 2016), and the redness index was calculated according to (Hobson, 1987).

2.7. Total soluble solids (TSS) content

The TSS was measured using a digital refractometer (Atago DR-A1) and expressed as a percentage.

2.8. Titratable acidity (TA)

Twenty milliliters of distilled water were added to 10 mL of filtered strawberry juice, and the pH was measured using a pH meter against 0.1 N NaOH. The TA content of the fruit was calculated from the NaOH amount used during titration as described by Karaçalı (2006), and the results were expressed as citric acid %.

2.9. TSS/TA values

It was calculated by the division of TSS values by TA values.

2.10. Sugar content

Three grams of strawberry fruit were placed in a beaker, and 10 mL distilled water was added, which was then homogenized and filtered. The filtrate was filtered from the injection filter (Nylon 66.25 μm) and, then injected into HPLC (Agilent, HP 1260 Hewlett Packard, CA/USA). The glucose, fructose, and total sugar content of the strawberries were calculated according to (Kasım and Kasım, 2015).

2.11. Fruit firmness

Fruit firmness was measured at three points on each strawberry using a digital penetrometer with a 7.9 mm plunger.

2.12. Weight loss

The three-box fruit samples were separated to measure weight loss, and the same samples were used during storage. The boxes were weighed at the start of the study and at 2-day intervals during storage. Weight loss was calculated using the following formula: WL (%) = (initial weight-final weight) x 100/initial weight.

2.13. Experimental design

The experiment was established, conducted, and evaluated using a Completely Randomized Design in three replicates, with three boxes of fruit (150 g fruit/box) for each replicate.

The data obtained were analyzed using the SPSS 16 software program, and the differences among the treatments were compared using Duncan's multiple range's test at 95% confidence interval.

3. Results and discussion

3.1. Anthocyanin content

The anthocyanin content of the strawberries was calculated as 18.2 mg/kg FW (fresh weight) before storage (Figure 3). It increased after 2 days of storage in all treatments and varied from 23.10 mg/kg FW in UVA to 31.90 mg/kg FW in B. This increase in anthocyanin content was attributed to the response of strawberries to low temperatures, which caused abiotic stress. The anthocyanin content of the strawberries in the R treatment was high compared with the other treatments during storage. The differences between the R treatment and the other treatments were found to be statistically significant ($p<0.05$). Anthocyanins are the most important phenolic compounds in colorful fruits and vegetables and are mostly found in their skin. It is also found in strawberries and account for up to 40% of total phenol (Warner et al., 2021). Besides, anthocyanin is important for human health because of its anticarcinogenic feature. Therefore, the maintenance of anthocyanin content or reduced losses with various applications is significant during postharvest storage of strawberries. The quality of light plays an important role in the accumulation of anthocyanins by plants.

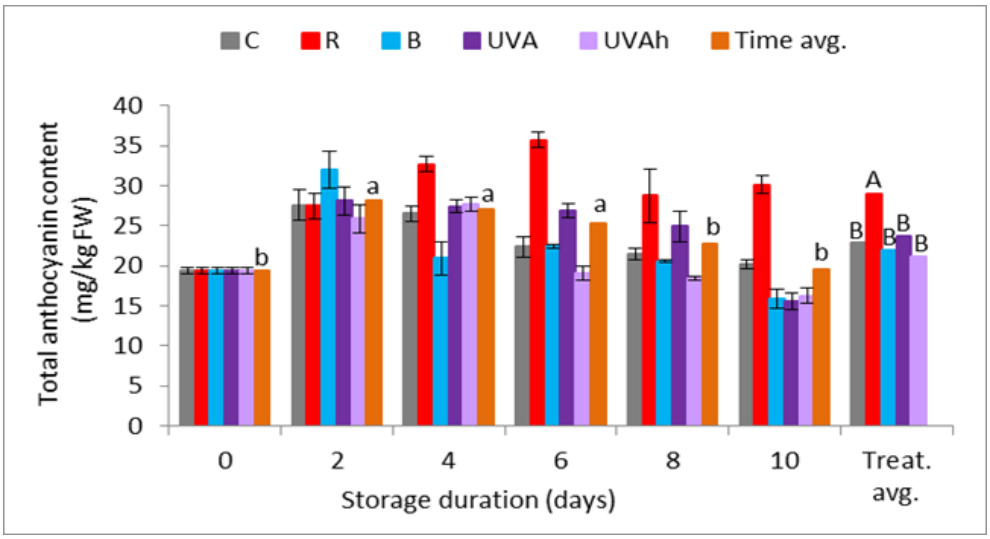


Figure 3. The changes in total anthocyanin content of the strawberries during 10 days storage at a temperature of 4±1oC. C: Control, R: Red LED, B: Blue LED, UVA: Storage under continuous UV LED, UVAh: Storage in dark conditions after one hour of UV LED treatment. Lower cases letters are shown differences among the storage time. Capital letters are shown differences among the treatments

Hobson (1987) also concluded that the total phenol and anthocyanin contents of strawberries were increased by 405-nm LED treatments. With LEDs of different colors, the color in which the plant is used more in the light spectrum is applied directly to the plant. Thus, the plant receives the required wavelength. In addition, because LED lamps are cold light emitters, long-term illumination can be provided without harmful effects on the plant. Nowadays, red and blue LEDs are also widely used to improve the postharvest quality of fruits and vegetables. In a study, it was shown that the content of total anthocyanins (TA), pelargonidin 3-glucoside (Pg3G), and pelargonidin 3-malonylglucoside (Pg3MG) significantly increased after blue and red light treatment. In addition, in a comparative transcriptome analysis that was conducted using six cDNA libraries from the treated strawberries, photoreceptors and light transduction components remained dynamic to upregulate the expression of regulatory factors and structural genes related to anthocyanin biosynthesis under red and white light, whereas most genes had low expression levels that were not consistent with the highest total anthocyanin content under blue light.

Therefore, the authors declared that light was an essential environmental factor for anthocyanin biosynthesis before the anthocyanin concentration reached saturation in strawberry fruits, and that blue light could quickly stimulate the accumulation of anthocyanin in the fruit. Moreover, it was suggested that red light not only elevated the anthocyanin content but also contributed to the synthesis of proanthocyanidin by inducing leucoanthocyanidin reductase and anthocyanidin reductase (Zhang et al., 2018). Thus, the total anthocynanin content increased by red light in this study was thought to be due to this proposed mechanism. In another study, blue LED and salicylic acid (2 mM) treatment increased the amount of anthocyanin in strawberries during storage at a temperature of 8 °C (Zhang et al., 2022). The blue LED light also increased the anthocyanin content of the strawberries, but it was not as effective as the red LED light in this study, contrary to the results of the study by (Zhang et al., 2022). (Wang, et al., 2022) found in their study that red LED light promotes the accumulation of pelargonidin-based anthocyanins in strawberries and that the expression of genes associated with anthocyanin biosynthesis is also upregulated by the red LED light.

3.2. Color values

The L* values of the strawberries declined after 2 days of storage in all treatments, then increased in UVA and C, and continued to decrease in R; however, a sharp decrease-increase in B and UVAh treatments was found after 8 days. There were, however, no significant differences among the treatments ($p<0.05$). Furthermore, in general, it can be said that UVAh promoted the brightness of the strawberries after the fourth day of storage because, the lowest decrease in the L* values occurred in UVAh (Table 1). The hue angle values of strawberries decreased on the second day of storage in all treatments. The lowest reduction was observed in R (32.19), whereas the highest reduction was observed in C (30.64). After this decrease, the hue angle values increased after the fourth day of storage in all treatments, and then changed to decrease-increase in all treatments except for C. The effect of the UVAh treatment was prominent in terms of raising the hue angle, but this increase was not significant (Table 1).

Table 1. The L*, hue angle and RI values of the strawberries treated with different wavelengths of LEDs

		Treatments					
	Storage duration (days)	**C**	**R**	**B**	**UVA**	**UVAh**	***Time average***
L*	0	37.67±2.83	37.67±2.83	37.67±2.83	37.67±2.83	37.67±2.83	**37.67 a**
	2	33.05±2.68	35.14±2.91	35.08±1.62	33.63±0.25	35.05±2.59	**34.39 b**
	4	34.05±2.58	34.82±1.81	35.08±0.17	34.40±0.09	37.22±1.16	**35.11 b**
	6	34.32±2.34	34.98±2.73	32.26±1.00	34.78±0.92	33.14±2.63	**33.90 b**
	8	33.85±2.62	33.27±1.48	34.16±0.37	33.92±0.74	35.15±1.06	**34.07 b**
	10	31.79±0.67	32.40±0.44	31.43±0.73	31.39±0.93	31.58±0.77	**31.72 c**
	Treatment average	**34.12**	**34.71**	**34.28**	**34.30**	**34.97**	
Hue	0	39.84±2.85	39.84±2.85	39.84±2.85	39.84±2.85	39.84±2.85	**39.84 a**
	2	30.64±1.29	32.19±0.49	30.97±0.37	31.22±1.91	31.46±1.92	**31.30 c**
	4	31.78±1.89	35.11±3.34	35.83±1.80	36.57±2.64	33.26±3.61	**34.51 b**
	6	33.12±2.01	34.99±3.79	31.94±1.40	33.85±3.01	32.11±2.82	**33.20 b**
	8	34.79±3.03	34.29±2.00	34.38±0.60	34.20±1.11	37.42±2.19	**35.02 b**
	10	34.32±2.32	33.83±2.58	33.87±2.41	34.86±1.22	36.66±4.30	**34.71 b**
	Treatment average	**34.08**	**35.04**	**34.47**	**35.09**	**35.12**	
RI	0	250.60±19.40	250.60±19.40	250.60±19.40	250.60±19.40	250.60±19.40	**250.60 d**
	2	299.69±8.42	286.02±13.10	289.69±7.44	294.84±6.84	288.44±10.92	**291.74 a**
	4	291.55±6.54	277.32±17.88	273.69±6.08	273.66±9.20	273.85±12.70	**278.01 c**
	6	286.16±11.93	277.11±17.78	298.85±7.14	281.38±8.51	294.48±14.60	**287.60 ab**
	8	282.72±21.27	286.62±13.29	282.40±1.96	283.99±3.04	267.87±10.29	**280.72 bc**
	10	292.82±6.83	291.62±6.92	296.05±8.34	292.95±8.05	284.91±13.40	**291.67 a**
	Treatment average	**283.92**	**278.22**	**281.88**	**279.57**	**276.69**	

RI: Redness index. C: Control, R: Red, B: Blue, UVA: Storage under continuous UV LED, UVAh: Storage in dark conditions after 1 h of UV LED treatment.

As shown in Table 1, the redness index (RI) values increased in all treatments on the second day of storage, and the RI values of the control group were the highest. From this result, it was thought that the strawberries in C started to senescence earlier than the other treatments, followed by UVA, B, UVAh, and R. Additionally, the RI values were also higher in C than in the other treatments on day four, but then decreased afterwards.

Furthermore, although the RI values in B and UVAh were higher on day six, the differences among the treatments were not statistically significant ($p<0.05$). The objective evaluation of fruit color was performed using Minolta colorimeter according to the color coordinate system developed by CIELAB. In this system, colors can be expressed closer to human perception, and all colors are shown with three vertical axes. According to this system, a* color values represent colors from green to red, and b* color values represent colors from blue to yellow. The hue angle value calculated from these values represents the true color of the fruit, and the redness index indicates the intensity of the red. In the CIELAB color coordinate plane, L* values vary between 0 and 100, while the L* value approaches zero indicates a decrease in brightness, while an increase toward 100 indicates an increase in brightness (Mcguire, 1992). Generally, the LED treatments did not have any significant differences in color values considered, as stated by (Chong et al., 2022). There are limited numerous studies showing LED light's effect on fresh produce, and in these studies, the authors focused on the antimicrobial effect of LED lights. In one of these studies, the authors stated that the LED lights including UVA did not affect the biochemical quality of red leaf lettuce, whereas the other said that UVA-LED reduced browning in fresh-cut apples and pears. The color of strawberries in UVAh is lighter than in the other treatments, which means the L* values of these fruits are higher, whereas the RI values are lower. An increase in RI values can indicate that the color becomes darker, and therefore the fruits mature more. The fruits treated with UVAh responded to this abiotic stress by delaying ripening. Thus, it can be concluded that UVAh treatment retards the senescence of fruit compared with other treatments.

3.3. TSS

The TSS content was measured as 7.06% at the start of the study (Table 2).

Table 2. TSS, fructose, glucose, and total sugar contents of strawberries treated with different wavelengths of LEDs

		Treatments					
	Storage duration (days)	**C**	**R**	**B**	**UVA**	**UVAh**	***Time average***
TSS (%)	0	7.06±0.26	7.06±0.26	7.06±0.26	7.06±0.26	7.06±0.26	**7.06 ab**
	2	7.12±0.43	8.54±0.63	7.47±0.98	8.22±0.70	6.54±0.45	**7.58 a**
	4	7.03±0.12	8.13±0.58	6.75±0.59	8.63±0.65	6.80±1.02	**7.40 ab**
	6	7.06±1.01	6.88±0.61	7.06±0.31	6.69±0.24	6.98±0.23	**6.93 b**
	8	6.32±0.69	6.96±0.78	7.06±0.31	5.01±0.54	5.76±0.38	**6.22 c**
	10	5.82±0.36	5.91±0.27	4.22±0.40	4.77±0.24	4.78±0.25	**5.10 d**
	Treatment average	**6.74 b**	**7.25 a**	**6.60 b**	**6.68 b**	**6.32 b**	
Fructose content (%)	0	0.69±0.30	0.69±0.30	0.69±0.30	0.69±0.30	0.69±0.30	**0.69 c**
	2	1.34±0.51	1.25±0.08	0.92±0.15	1.20±0.19	0.97±0.12	**1.13 ab**
	4	1.29±0.11	1.00±0.04	1.20±0.14	1.08±0.11	1.11±0.21	**1.14 ab**
	6	1.11±0.23	1.01±0.45	1.11±0.25	1.14±0.10	0.79±0.01	**1.03 b**
	8	1.33±0.13	1.41±0.24	1.18±0.26	1.29±0.61	1.30±0.10	**1.30 a**
	10	0.75±0.11	0.82±0.14	0.99±0.10	0.77±0.27	0.86±0.07	**0.84 c**
	Treatment average	**1.084 a**	**1.028 a**	**1.014 a**	**1.026 a**	**0.954 a**	
Glucose content (%)	0	0.74±0.16	0.74±0.16	0.74±0.16	0.74±0.16	0.74±0.16	**0.74 b**
	2	2.30±1.03	1.64±0.18	0.74±0.15	1.00±0.23	0.79±0.20	**1.30 a**
	4	0.81±0.12	0.61±0.11	0.77±0.26	0.95±0.21	0.69±0.23	**0.77 b**
	6	0.81±0.56	0.66±0.21	0.57±044	0.29±0.19	0.29±0.20	**0.52 c**
	8	0.29±0.37	0.31±0.18	0.15±0.03	0.30±0.15	0.10±0.02	**0.23 d**
	10	0.18±0.03	0.19±0.11	0.22±0.12	0.14±0.09	0.19±0.09	**0.18 d**
	Treatment average	**0.86 a**	**0.69 ab**	**0.53 bc**	**0.57 bc**	**0.47 c**	
Total sugar content (%)	0	1.43±0.36	1.43±0.36	1.43±0.36	1.43±0.36	1.43±0.36	**1.43 c**
	2	3.64±1.02	2.89±0.25	1.66±0.28	2.20±0.42	1.76±0.32	**2.43 a**
	4	2.10±0.23	1.61±0.15	1.98±0.39	2.03±0.31	1.80±0.43	**1.90 b**
	6	1.92±0.79	1.66±0.63	1.68±0.52	1.42±0.24	1.09±0.19	**1.55 c**
	8	1.62±0.44	1.72±0.40	1.33±0.29	1.58±0.69	1.40±0.11	**1.53 c**
	10	0.94±0.13	1.01±0.24	1.21±0.21	0.91±0.36	1.04±0.11	**1.02 d**
	Treatment average	**1.94 a**	**1.72 ab**	**1.55 b**	**1.60 b**	**1.42 b**	

C: Control, R: Red LED, B: Blue LED, UVA: Storage under continuous UV LED, UVAh: Storage in dark conditions after one hour of UV LED treatment.

It then increased in all treatments except UVAh on day two of storage. The highest increase in the TSS content was measured in the R treatment, followed by UVA, B, C, and UVAh. After this time, the TSS content of the strawberries decreased during storage. Moreover, the average values given in Table 2 show that the highest TSS content was found in R (7.25%), and the differences between R and the other treatments were statistically significant ($p<0.05$).

Because TSS of strawberry fruit is changed according to the maturation stage of fruits and in harvest time, and the maturation stage is not homogenous among the fruits, there is no standard value of TSS as in kiwi fruits or grapes. In fact, in a study, it was determined that the amount of TSS of strawberry fruits harvested at six different stages varied between 5.19 and -9.19 °Brix (Basak et al., 2022). In addition, in another study conducted with the Albion variety, the TSS value was found to vary between 7.00 and -8.10% (Polat et al., 2016). In the present study, the initial TSS value of the Albion strawberry cultivar was within these limits, but after the fourth day of storage, it decreased in all treatments. On the other hand, it was also found that the amount of TSS in the fruits was higher in the R application than in the other treatments. Light treatments cause abiotic stress in horticultural crops. The plant or plant part activates a defense mechanism for responding to abiotic stress, resulting in an increase in the bioactive component of fruit and vegetables. In this study, the red LED light treatments were more effective on the TSS content than the other treatments, and the TSS content of the strawberries stored under the red LED light was the highest. Therefore, this result showed that the storage of strawberries under red light was successful in retarding TSS loss. In another study, it was found that the total soluble solids of strawberries stored under white, red, and blue light decreased over time (Noor et al., 2022). On the same line, a similar trend was detected in this study. On the other hand, the highest TSS content was measured in the R treatment, and differences among LEDs were also significant, as concluded by (Noor et al., 2022). Additionally, it could be said that the senescence of the strawberries was slowed by the R and UVAh treatments in this study.

3.4. TA

The titratable acidity (TA) of the strawberries in R decreased during the first 2 days of storage, whereas the acidity increased in the other treatments. The highest TA level was found in the K treatment on day four of storage, followed by B, R, UVAh, and UVA. Additionally, when the average of the treatments was considered, the highest TA level was detected in C, and the differences between C and the other treatments were statistically significant ($p<0.05$) (Figure 4).

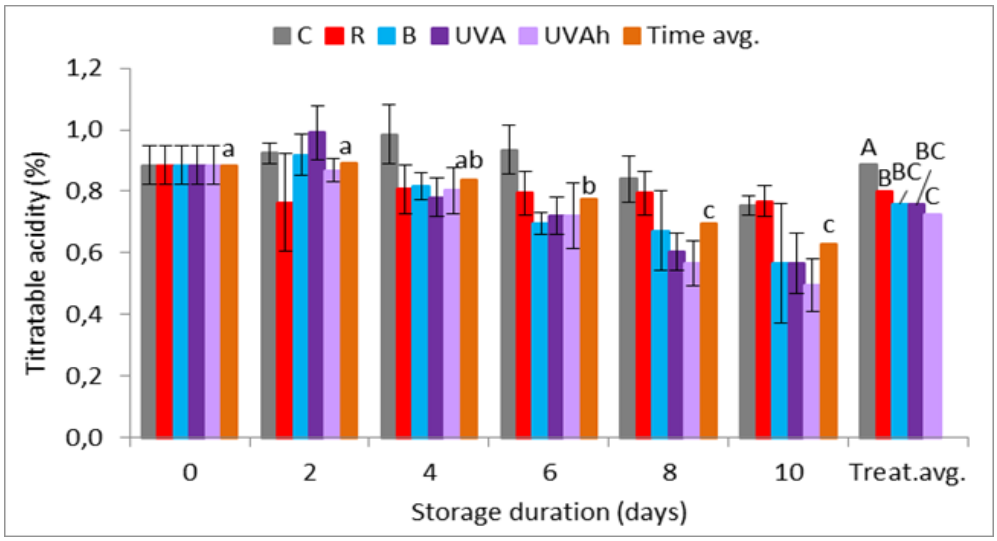


Figure 4. The changes in titratable acidity content of the strawberries during 10 days storage at a temperature of 4±1oC. C: Control, R: Red LED, B: Blue LED, UVA: Storage under continuous UV LED, UVAh: Storage in dark conditions after one hour of UV LED treatment. Lower cases letters are shown differences among the storage time. Capital letters are shown differences among the treatments.

LED treatments decreased TA levels after the second day of storage compared with the control group. This result was also supported by (Wang et al., 2022), who concluded that BL + SA treatment decreases titratable acidity contents. Among the treatments, however, the R treatment was more effective in retaining TA levels than the other LED treatments.

3.5. TSS/TA

The TSS/TA percentage of strawberries was lower in C than in all LED treatments (Figure 5). In addition, the differences between C and LED treatments were found to be crucial. According to the TSS and TA results, TA values were decreased by LED treatments, whereas TSS increased. Therefore, it could be concluded that LED treatments improve the taste of strawberries by reducing TA. Generally, studies on the TSS/TA content of strawberries treated with LED light were conducted during the growing period. Unlike our work results, only one study revealed that the TSS/TA ratio of strawberries treated with red, blue, and white light was decreased (Jiang et al., 2023). These contradictions could have originated because the PPFD of LED lights treated to strawberries is different.

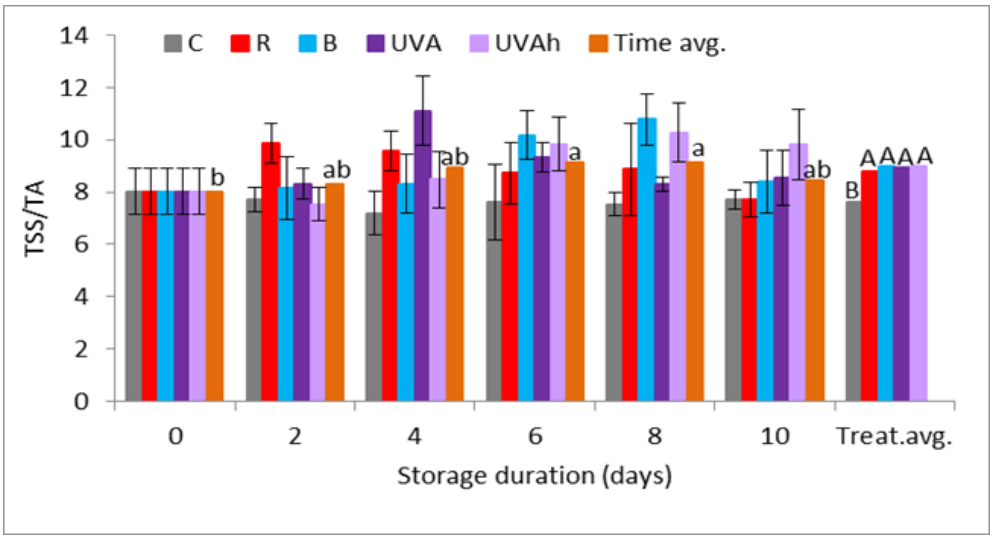


Figure 5. The changes in TSS/TA content of the strawberries during 10 days storage at a temperature of 4±1oC. C: Control, R: Red LED, B: Blue LED, UVA: Storage under continuous UV LED, UVAh: Storage in dark conditions after one hour of UV LED treatment. Lower cases letters are shown differences among the storage time. Capital letters are shown differences among the treatments.

3.6. Fructose, glucose, and total sugar content

Both glucose and fructose levels of strawberries increased sharply after two days of storage. While the glucose content decreased dramatically after this time and, continued to decline during storage, the fructose levels decreased more slowly. As seen in Table 2, when the treatment averages were examined during the storage period, the fructose content in C (1.08%) was higher than that in the other treatments, but it was not statistically significant. Nevertheles, a similar trend was obtained by glucose measurement, and the differences between C and the other treatments except for R were significant ($p<0.05$). The total amount of sugar changed as it did for glucose, and the highest total sugar content was found in C (1.94%), followed by R (1,72%), UVA (1,560%), B (1.55%), and UVAh (1,42%) treatments. Additionally, statistical differences were similar to those of the glucose content (Table 2). In the study, it was found that the fructose, glucose, and total sugar content of the strawberries in C were higher than those in the other treatments, followed by R. In this case, it could be concluded that the strawberries in C and R started to age earlier those in the other treatments. In contrast, the C, R, UVA, B, and UVAh treatments delayed senescence, and the most effective treatment was UVAh in this context. Moreover, the TSS content and color values of the strawberries in UVAh supported this result.

Jiang et al. (2023) concluded that blue and red LED light increased the soluble sugar content of strawberries compared with 0 days of storage. In this study, similar results were detected for fructose and total sugar, but glucose content first increased and then decreased during storage.

3.7. Fruit firmness

The fruit firmness was 5.38 N at the beginning of the study, which started to decrease in the C (4.70N) and UVA (5.23 N) treatments but increased in the other treatments (Figure 6) after the second day of storage. Additionally, the highest firmness was measured in B, whereas the lowest firmness was measured in C on the second and fourth days of storage. Generally, fruit firmness did not show pronounced changes in C and R and nearly retained the initial level of firmness at the end of storage. However, it changed to a slight decrease-increase after the sixth day of storage, and then sharply decreased in B, UVA, and UVAh. When Figure 6 is examined, it can be seen that the difference between R and UVA treatments is statistically significant ($p<0.05$). According to the results of firmness measurements, it can be concluded that all LED treatments were effective in terms of promoting fruit firmness after 4 days of storage. However, afterwards, the firmness of the strawberries decreased to a level below that of the control group in all LED treatments. Therefore, it seems that the LED treatments were successful in protecting fruit firmness after four days of storage. Additionally, the R LED treatment retained fruit firmness (5.37 N) longer than the other treatments. (Zhang et al., 2022) stated that 405 nm (blue) LED application did not affect the texture of strawberries. Similarly, in the present study, it was concluded that blue light had no effect on fruit firmness, and red light was more effective at the end of storage.

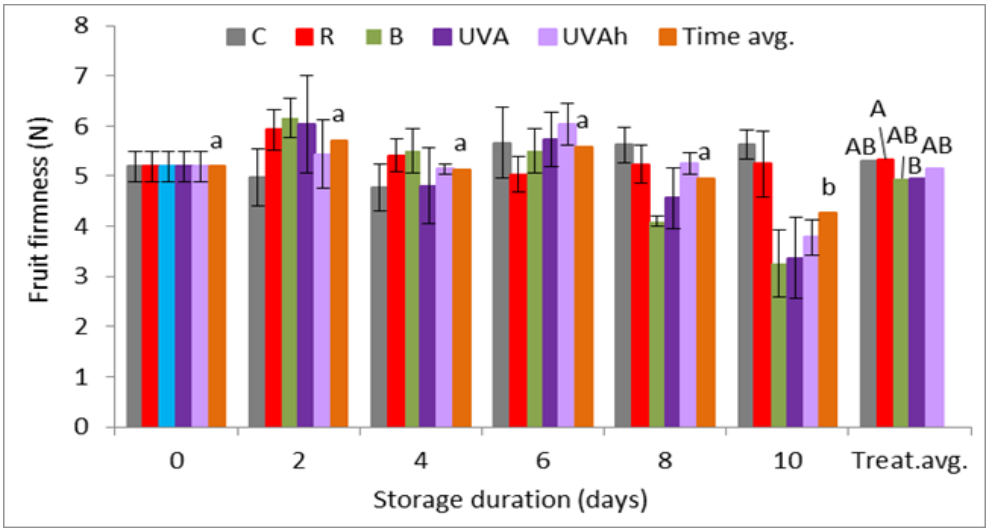


Figure 6. The changes in fruit firmness content of the strawberries during 10 days storage at a temperature of 4±1oC. C: Control, R: Red LED, B: Blue LED, UVA: Storage under continuous UV LED, UVAh: Storage in dark conditions after one hour of UV LED treatment. Lower cases letters are shown differences among the storage time. Capital letters are shown differences among the treatments.

3.8. Weight losses

The weight loss of strawberries in all treatments increased during storage (Figure 7). The highest weight loss occurred in the UVA and UVAh treatments (0.65% and 0.50%, respectively), followed by B (0.44%), C, and R (both 0.17%). Additionally, when the average of the treatments was examined, the differences between C, R, and B, UVA, and UVAh treatments were significant ($p<0.05$). In the study, the lowest weight losses were found in C; therefore, it, can be concluded that the all LED treatments lead to an increased loss of weight in strawberries. However, among the LED treatments, the UVA treatment caused the most weight loss compared with the other treatments. LED light treatment is an abiotic factor that affects fruit metabolism, thus causing weight loss during storage.

Thus, ultraviolet irradiation is also a strong abiotic factor for fruit and creates a stronger effect than the other LED treatments. Therefore, the weight loss of strawberries under UVA was higher than that of other LEDs. Meanwhile, the weight losses never exceeded the marketable limits, and the loss was not reflected in the appearance of the strawberries.

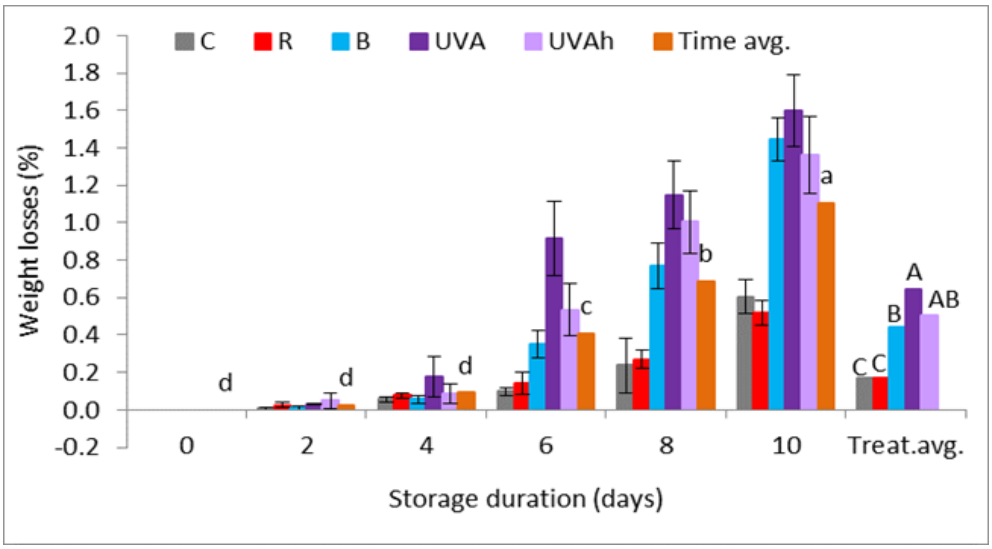


Figure 7. The changes in weight loss content of the strawberries during 10 days storage at a temperature of 4±1oC. C: Control, R: Red LED, B: Blue LED, UVA: Storage under continuous UV LED, UVAh: Storage in dark conditions after one hour of UV LED treatment. Lower cases letters are shown differences among the storage time. Capital letters are shown differences among the treatments.

4. Conclusions

This study aimed to investigate the effect of lighting during the storage of 'Albion' strawberries using LED lights with different wavelengths as well as dark storage and dark storage after 1 h ultraviolet LED lighting on the postharvest quality of the strawberries. In this study, the storage of strawberries under continuous red LEDs was effective in improving the anthocyanin content. A significant effect was detected on the TSS content and fruit firmness, and a reduction in weight loss was also observed. When evaluating the color values, it was observed that the UVA LED treatment was effective on L*, whereas UVAh was effective on the hue angle and redness index of the strawberries. Moreover, LED light irradiation did not affect the glucose, fructose, and total sugar content, whereas it reduced the TA levels of the strawberries. Consequently, red LED lighting during storage was successful in increasing the biochemical quality of the strawberries compared with the other treatments. However, more studies are needed to investigate the effect of continuous UVA and dark storage after 1 h UVA treatments on the quality of strawberries.

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Onur YAVUZ: data collection, draft manuscript preparation. **Rezzan KASIM**: study conception and design, analysis and interpretation of results. **Mehmet Ufuk KASIM**: analysis and interpretation of results. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

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Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgment

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Sustainable methods for growing turmeric: Evaluating the effects of synthetic and organic fertilizers on vegetative and reproductive attributes

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A B S T R A C T

This study aimed to assess the influence of various biofertilizer sources on turmeric (*Curcuma longa* L.) cultivation in Eastern Nepal. The research was conducted at G.P Koirala College of Agriculture and Research Centre, Sundarharaicha, Morang, Nepal, from April 2023 to January 2024. The experiment utilized a randomized complete block design (RCBD) with seven treatments including T1: Recommended dose (RD) of NPK, T2: Goat manure (GM), T3: Organic manure (OM), T4: Poultry manure (PM), T5: Vermicompost (VC), T6: Farmyard manure (FYM), T7: Control, replicated three times. Turmeric cultivation practices were implemented following standard agronomic procedures. The recommended dose of synthetic fertilizer, NPK, exhibited the highest enhancement across multiple vegetative and reproductive growth parameters of turmeric, with notable increases in plant height, leaf number, tillers per plant, primary and secondary fingers per clump, fresh rhizome yield, dry yield, and dry recovery percentage. Among the organic sources, goat manure and poultry manure also showed promising results in enhancing turmeric yield and quality. Specifically, NPK recorded the highest fresh rhizome yield at 21.30 tons ha^{-1}, while goat manure and poultry manure yielded 20.35 tons ha^{-1} and 18.69 tons ha^{-1}, respectively. In contrast, the lowest fresh rhizome yield was observed in the control group, indicating minimal enhancement in yield without fertilizer supplementation. The results highlight how organic farming methods may be a good substitute for traditional chemical fertilizers in the context of sustainable turmeric production.

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1. Introduction

Turmeric (*Curcuma longa* L.) is a perennial herbaceous plant belonging to the ginger family, Zingiberaceae (Ferdous et al., 2018), extensively cultivated in the tropical and subtropical regions of the world, including Nepal (Baral et al., 2021; Chandana et al., 2024a). It holds significant cultural, culinary, and medicinal value (Chapagain et al., 2021), with its rhizomes being prized for their vibrant color, Flavors, and therapeutic properties (Hossain et al., 2007). Turmeric has been cultivated across diverse ecological zones of Nepal, spanning from the eastern to western regions, encompassing the inner terai, foothills, and mid-hills up to an altitude of 1600 meters above sea level (Mehata et al., 2022a; Rajbanshi et al., 2023;). Its cultivation demands minimal care and management, making it a popular choice either as a standalone crop or for intercropping within fruit orchards or agro-forestry setups on less fertile land (Khan et al., 2023; Majhi et al., 2024). Serving as a vital source of income, turmeric holds immense importance in Nepal's agricultural landscape, playing a crucial role in supporting the livelihoods of numerous farmers nationwide (Bairagi, 2022). As of 2078/79 BS, the total yield of turmeric per hectare in Eastern Nepal, averaging between 18 to 22 tons ha^{-1} under favorable conditions, has been reported by (Timsina et al., 2011). In Eastern Nepal, turmeric cultivation serves as a vital component of agricultural practices and plays a crucial role in the socio-economic fabric of the region (Chapagain et al., 2021; Datta et al., 2017). The cultivation of turmeric in Eastern Nepal is typically characterized by smallholder farmers employing traditional farming techniques (Chapagain et al., 2021). However, despite its importance, turmeric production faces various challenges, including declining soil fertility, pest and disease pressures, erratic weather patterns, and fluctuating market prices (Baka et al., 2021). Among these challenges, maintaining soil health and fertility stand out as critical factors influencing turmeric growth and yield (Chávez-Mejía et al., 2021).

In recent years, Nepal's turmeric production has seen fluctuations due to various factors like climate, soil health, and farming practices (Yadav et al., 2024b). Despite being a major global producer, with yields averaging 18 to 22 tons per hectare annually, the sector faces challenges hindering its full potential (Timsina et al., 2011; Mehata et al., 2023c). Reliance on chemical fertilizers has led to environmental degradation, soil depletion, and food safety concerns (Mehata et al., 2023b). Transitioning to sustainable agriculture is imperative to mitigate these issues and enhance soil fertility while bolstering community resilience (Mekonnen and Garedew, 2019). Synthetic fertilizers, prevalent in conventional farming, pose risks such as soil degradation, water pollution, and health hazards for farmers and consumers alike (Ojikpong, 2018; Ishwar et al., 2024). Thus, urgent action is needed to shift towards environmentally friendly practices for the long-term viability of Nepal's turmeric industry (Chandana et al., 2022b). Considering these challenges, there exists a compelling imperative to explore alternative approaches (Chhetri et al., 2020), particularly focusing on organic farming methods, to foster a more ecologically harmonious and economically viable agricultural system in Nepal. Organic manures, derived from plant or animal sources, offer a holistic solution for enhancing soil fertility, improving soil structure, and promoting balanced nutrient uptake by crops (Kumar et al., 2023). Moreover, organic farming practices contribute to the conservation of biodiversity (Yadav et al., 2024a), reduction of greenhouse gas emissions, and overall resilience of agroecosystems (Baral et al., 2021).

Despite the recognized benefits of organic manures, their efficacy in enhancing turmeric growth and yield in the specific agroclimatic conditions of Eastern Nepal remains underexplored. This study systematically assesses various organic manure sources, including compost, farmyard manure, green manure, and biofertilizers, to elucidate their comparative effectiveness in enhancing soil fertility, promoting healthy plant growth, and maximizing turmeric yield, with the specific aim of evaluating their impact on the growth and yield of turmeric in Eastern Nepal.

2. Materials and methods

2.1. Experimental site

The field experiment was carried out from April 2023 to January 2024, in the research field of G.P Koirala College of Agriculture and Research Centre located at Sundarharaicha, Morang, Nepal, to evaluate the efficacy of several biofertilizer sources on vegetative and reproductive traits of Turmeric. The climate of this area is tropical type.

The average annual temperature of this area ranges between 21.81 to 34.46 °C, and the average yearly precipitation is 131.88 mm. Geographically it is located at 26⁰ 40′ 49.9″ North latitude and 87⁰ 21′ 16.7″ East longitudes with an elevation of 151 m. The soil characteristics of the experimental site were analyzed in qualitative measures with the help of a soil test kit box (Table 1). A soil test kit box is a portable tool used to assess soil health by measuring pH, nutrient levels, and sometimes moisture content. It measures soil properties through colorimetric tests, where chemical reactions produce color changes that are compared to a reference chart. While these kits offer quick and convenient assessments, they provide less accurate and detailed results compared to laboratory analyses, making them less reliable for critical agricultural decisions. The nutrient content of the soil was analyzed only once before the research was conducted just to check the available nutrient level. The average highest temperature and lowest temperature throughout the study period were 36.74 °C and 11.08 °C, respectively, with an average amount of precipitation of 209.03 mm. The meteorological data of the research area throughout the study period was presented in Figure 1.

2.2. Plant materials

The 'Kapor Kot Haledo-1' variety was used for a study which was obtained from the National Agricultural Research Council (NARC). The main characteristics of this variety is that the rhizomes have a tough brown skin and bright orange flesh, known for their pungent and bitter taste. This variety, known for its adaptability, has a growth cycle of approximately 180-200 d. It exhibits promising yields and is typically harvested when the leaves start to turn yellow and dry, signaling optimal rhizome development.

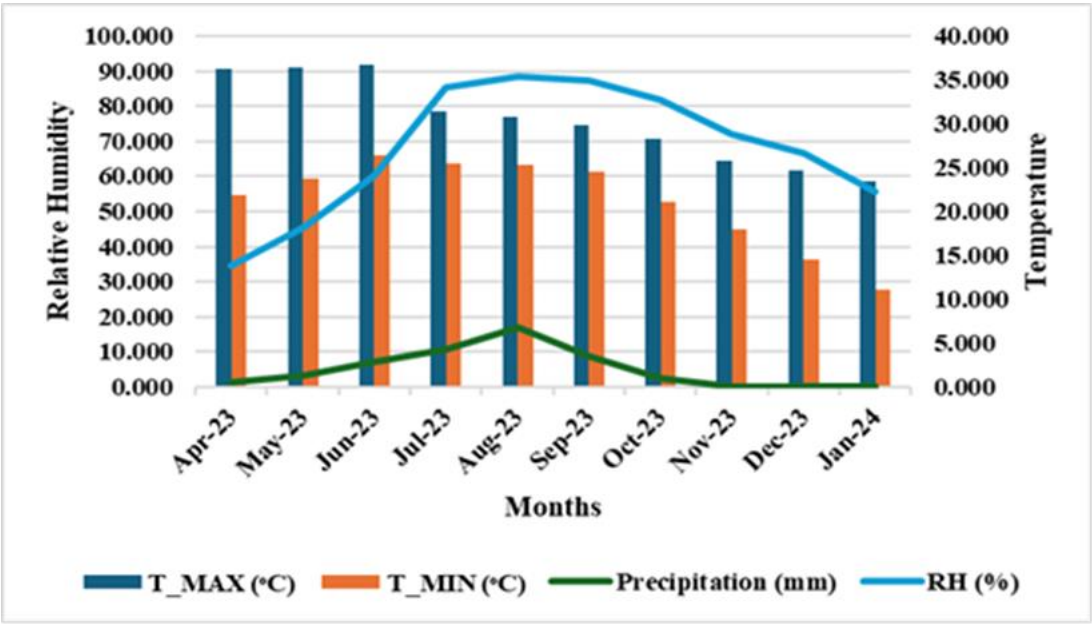

Figure 1. Meteorological data of the study site during the study period

Table 1. Soil characteristics of experimental site

Serial Number	Soil Characteristics	Nutrient Content	Properties
1	Nitrogen (N)	0.15%	Slightly High
2	Phosphorous (P)	58.83 mg kg^{-1}	Medium
3	Potassium (K)	221.33 mg kg^{-1}	Low
4	Organic matter	3.68%	Medium
5	Soil texture	-	Clay loam soil
6	Soil pH	-	6.8

2.3. Research design and cultural practices

The research was designated in RCBD with seven treatments replicated three times. There were altogether 21 plots. Each plot was designated 6 m² (3 m x 2 m) with a total area of 126 m². The gap between the two replications was maintained at 1m, and between the two treatments was 0.5 m. 80 plants per plot were kept with spacing between rows to row 0.3m and plant to plant 0.25 m. Turmeric cultivation began with land preparation, which involved ploughing and levelling to ensure proper drainage. Rhizome selection was crucial, with growers opting for disease-free, healthy ones. Planting depth was around 5-7 cm, with a spacing of 30 cm between rows and 25 cm between plants. Full dose of potassium and phosphorous and half dose of nitrogen was applied prior to planting in the specific plot whereas full dose of all biofertilizer sources were applied in their respective research plot one week prior to planting. Half dose of nitrogen was applied during rhizome development phase after weeding. Regular weeding was conducted to remove competing vegetation that could hinder the growth and yield of turmeric. Manual weeding techniques were utilized, with special focus given during the early growth stages when turmeric plants are most vulnerable. Effective irrigation ensured consistent soil moisture for turmeric growth. We used a mix of furrow and ridge systems, supplemented by manual watering as needed, to maintain ideal conditions for rhizome development while preventing waterlogging. The irrigation was applied at 5-7 days interval during germination, vegetative growth phase and rhizome development phase, however, during maturity phase the frequency of irrigation was reduced and light irrigation was done. The details of all treatments and their respective doses employed in our research are outlined in Table 2.

Table 2. List of treatments and their doses

Serial Number	Treatments	Symbol	Doses
1	RD of NPK	T1	60: 50: 120 kg ha^{-1}
2	Goat manure	T2	10 tons ha^{-1}
3	Organic manure	T3	6 tons ha^{-1}
4	Poultry Manure	T4	7 tons ha^{-1}
5	Vermicompost	T5	4 tons ha^{-1}
6	Farmyard Manure	T6	30 tons ha^{-1}
7	Control	T7	untreated

2.4. Data collection

From each experimental plot, twelve plants were randomly selected to gather the required data. Data collection occurred every fifteen days. The raw data encompassed vegetative parameters such as plant height, leaf number per plant, tillers per plant, leaf length, leaf area, and days to 50% sprouting. Additionally, reproductive traits including the number of primary fingers per clump, number of secondary fingers per clump, fresh rhizome yield, dry yield, and dry recovery were recorded.

2.5. Statistical analysis

The data was initially input in chronological order for both the replication and treatment blocks utilizing MS Excel 2021 (Microsoft Corporation, Washington, USA). Following this, ANOVA analysis was carried out using statistical software (R Studio, Version 4.2.2, Boston, Massachusetts, USA). To assess mean differences among different treatments at a significant level of 5%, Duncan's Multiple Range Test (DMRT) was employed.

3. Results and discussion

3.1. Effects of different sources fertilizers on vegetative growth parameters

Table 3 presents the data on turmeric plant height across various fertilizer treatments investigated during our study period. The results reveal significant differences in plant height among these treatments at a level of significance 0.1%, 1% & 5%. Initially, the average plant height was 32.43 cm, progressively increasing to a peak of 102.66 cm at 180 days post-planting.

The treatment receiving the recommended NPK dose exhibited the tallest plants at 90 days post-planting (39.33 cm), reaching a height of 109.63 cm at harvest. This finding aligns with Kadam and Kamble (2020), who also observed similar trends in plant height with organic manures. The consistent and optimized nutrient release from NPK fertilizers likely contributed to sustained growth throughout the plant's life cycle. Biofertilizers such as organic manure, goat manure, vermicompost, and poultry manure showed the second-highest plant heights, ranging from 103-105 cm at harvest. This pattern is consistent with Datta et al. (2017), who reported comparable results, particularly noting vermicompost's effectiveness. The gradual nutrient release from these organic sources supports steady nutrient availability, fostering robust and sustained plant growth across developmental stages. Unlike the rapid nutrient release of NPK fertilizers, organic sources offer balanced and prolonged nutrient availability, promoting healthier plant growth. The control group, receiving no fertilizer, recorded the lowest height (92.88 cm) at harvest, echoing findings by Kadam and Kamble (2020) with a similar control group height of 93.33 cm.

The application of different fertilizers reveals noteworthy variances in the leaf count of turmeric plants throughout our research period, as depicted in Table 3. These results exhibit significant differences in leaf numbers across various fertilizer treatments at a significant level at 0.1% and 1%. This finding is consistent with the study conducted by Kadam and Kamble (2020), aligning closely with our own observations. The notable disparities in leaf count among fertilizer treatments, observed in both studies, likely arise from variations in nutrient compositions and release rates. These factors profoundly influence leaf development and foliage growth, demonstrating statistical significance at a level of less than 0.01%. The mean leaf count recorded at harvest stands at 8.93. In contrast, Chapagain et al. (2021) reported leaf count of just 7.69 slightly lower results compared to our findings. The highest leaf count observed in our study could be attributed to various factors, including differences in soil fertility, climatic conditions, cultivation techniques, and varietal disparities, all of which influence leaf development and overall plant vigor. Notably, the recommended NPK dose yielded the maximum number of leaves, reaching 10.86, a result consistent with findings from Kadam and Kamble (2020). This dominance of leaf count with NPK fertilizers in both studies, compared to various biofertilizer sources, likely stems from the balanced nutrient composition tailored to specific variety needs, optimal environmental conditions supporting robust foliage growth, and genetic factors enhancing leaf proliferation. Additionally, the precise nutrient delivery of NPK fertilizers likely played a role in maximizing leaf production compared to biofertilizer alternatives, a conclusion also supported by Khan et al. (2023). Among the biofertilizer sources, organic manure and vermicompost recorded the highest leaf numbers at 9.20 and 9.40, respectively, followed by goat manure at 9.00. Similar results were noted by Kadam and Kamble (2020) with different organic manure sources. Poultry manure, on the other hand, exhibited the second-lowest leaf count at 8.53. Although Kadam and Kamble (2020) reported slightly higher leaf numbers with poultry manure, this discrepancy may arise from variations in nutrient release rates, composition, and soil interactions. The lowest leaf count was observed in treatments without any fertilizer input, i.e., the control group, at 7.26. This finding aligns with those reported by Hossain et al. (2007) and Datta et al. (2017), indicating that inadequate nutrient supply hampers optimal plant growth. The absence of fertilizer input deprives plants of essential nutrients, hindering leaf development and overall foliage abundance compared to fertilized counterparts.

Likewise, the tillers per plant exhibit significant differences across the various treatments employed in the study, as illustrated in Table 4. These results demonstrate very high significance levels at less than 0.001%. The overall average of tillers per plant stands at 2.49. The highest number of tillers was observed in the group receiving the recommended NPK dose, reaching 3.76, followed by poultry manure at 3.21 and goat manure at 2.80. In contrast, Chandana et al. (2022b) reported significantly higher tiller counts with poultry manure and vermicompost, nearly double our findings. This discrepancy could be attributed to variations in nutrient availability influenced by soil composition, microbial activity, environmental conditions like temperature and moisture levels, genetic factors of the cultivars used, and cultivation practices, all contributing to these differences. Similarly, a study by Hossain et al. (2007) recorded tiller counts with the recommended NPK dose at 5.2, almost twice our findings. This variance may stem from differences in soil fertility, climatic conditions, varietal characteristics, and cultivation practices, all potentially accounting for the twofold difference in tiller numbers. The tiller count per plant varied significantly across treatments, with the control group recording the lowest count at 1.36, findings supported by Ferdous et al. (2018) and Datta et al. (2017).

Conversely, the recommended NPK dose resulted in the highest leaf length (35.58 cm), leaf width (14.20 cm), leaf area (326.33 cm^2), and days to 50% sprouting. Chandana et al. (2022b) reported similar leaf area results, while Datta et al. (2017) and Ferdous et al. (2018) documented comparable findings for leaf length, width, and area with the recommended NPK dose. These parameters were notably higher with NPK due to its balanced nutrient composition, vital for robust leaf development and timely sprouting. Among biofertilizer sources, goat manure and poultry manure demonstrated noteworthy results, with goat manure treatments exhibiting a leaf length of 32.93 cm, leaf width of 13.44 cm, leaf area of 285.56 cm^2, and requiring 27.00 days for 50% sprouting, while poultry manure treatments showed a leaf length of 31.07 cm, leaf width of 13.20 cm, leaf area of 264.47 cm^2, and 26.66 days for 50% sprouting. However, Ferdous et al. (2018) noted longer leaf lengths ranging from 25 cm to 50 cm with green manure treatments, potentially influenced by environmental factors like temperature and light intensity. The control group exhibited the lowest values for leaf length (25.45 cm), leaf width (10.84 cm), leaf area (178.23 cm^2), and days to 50% sprouting (24.33 days), findings corroborated by Chávez-Mejía et al. (2021) and Datta et al. (2017). These minimal results can be attributed to inadequate nutrient supply for optimal vegetative growth and development in the absence of fertilizers.

Table 3. Effects of various sources of fertilizers on plant heights and leaf number of turmeric

Treatments	Plant height (cm)							Leaf number						
	90 DAP	105 DAP	120 DAP	135 DAP	150 DAP	165 DAP	180 DAP	90 DAP	105 DAP	120 DAP	135 DAP	150 DAP	165 DAP	180 DAP
NPK	39.33^{a}	65.39^{a}	73.06^{a}	93.12^{a}	101.83^{a}	105.80^{a}	109.63^{a}	3.06^{a}	3.73^{a}	4.80^{a}	6.60^{a}	9.40^{a}	10.80^{a}	10.86^{a}
Goat manure	32.52^{b}	58.70^{b}	65.11^{bc}	85.16^{b}	94.24^{b}	99.84^{b}	104.55^{b}	2.80^{ab}	3.20^{bc}	3.60^{bc}	5.53^{c}	8.26^{c}	8.93^{bc}	9.00b^{c}
Organic manure	32.59^{b}	59.08^{b}	67.52^{bc}	87.05^{ab}	96.29^{ab}	99.60^{b}	103.02^{b}	2.73^{ab}	3.13^{bcd}	3.53^{bc}	5.60^{c}	8.40^{c}	8.93^{bc}	9.20^{b}
Poultry manure	32.94^{b}	58.34^{b}	66.52^{bc}	88.72^{ab}	97.84^{ab}	100.13^{b}	103.00^{b}	2.80^{ab}	3.20^{bc}	3.60^{bc}	5.60^{c}	8.13^{cd}	8.40^{cd}	8.53^{cd}
Vermicompost	31.48^{bc}	58.38^{b}	68.74^{ab}	91.12^{ab}	99.28^{ab}	101.59^{b}	103.81^{b}	2.86^{ab}	3.46^{ab}	4.06^{b}	6.13^{b}	8.86^{b}	9.20^{b}	9.40^{b}
FYM	30.47^{bc}	55.91^{b}	62.89^{cd}	84.59^{b}	94.82^{b}	98.06^{b}	101.70^{b}	2.33^{b}	2.800^{cd}	3.13^{c}	5.20^{cd}	7.86^{d}	8.20^{d}	8.26^{d}
Control	27.68^{c}	53.25^{b}	59.51^{d}	77.64^{c}	86.82^{c}	89.67^{c}	92.88^{c}	2.26^{b}	2.66^{d}	3.12^{c}	4.86^{d}	7.00^{e}	7.20^{e}	7.26^{e}
Grand mean	32.433	58.439	66.196	86.775	95.878	99.244	102.66	2.695	3.171	3.695	5.647	8.276	8.809	8.933
SEM	1.688	2.770	2.178	2.794	2.427	1.491	1.489	2.9295	0.213	0.235	0.198	0.173	0.237	0.275
CV (%)	6.375	5.806	4.029	3.943	3.100	1.839	1.776	11.862	8.218	7.789	4.291	2.556	3.291	3.767
F-value	***	*	**	**	**	***	***	NS	**	***	***	***	***	***

*: Significant at 5% level of significance. **: Significant at 1% level of significance. ***: Significant at 0.1% level of significance. NS: Non-significant. SEM: Standard error of the mean. CV: Coefficient of difference. DAP: Days after planting

3.2. Effect of different sources of fertilizers on reproductive growth parameters

Table 4 showcases the response of various reproductive traits to different fertilizer sources utilized in the study, including primary fingers per clump, secondary fingers per clump, fresh rhizome yield, dry yield, and dry recovery percentage. Significant variations among these metrics were recorded at a significant level ($p< 0.001$). The 'Kapor Kot Haledo-1' variety of turmeric treated with the recommended dose of synthetic fertilizer, NPK, exhibited the highest reproductive growth across all mentioned parameters. The NPK treatments yielded the highest number of primary and secondary fingers, at 4.57 and 5.23, respectively. This outcome can be attributed to the balanced nutrient composition of NPK, providing essential elements crucial for robust rhizome and finger development, thereby promoting optimal growth and productivity compared to other fertilizer sources. Following closely were the numbers of fingers from goat manure at 4.28 and 4.87, respectively. Similarly, poultry manure (4.22) and vermicompost (4.15) recorded the highest number of secondary fingers among other treatments. Possible reasons behind this may include variations in nutrient availability and release rates influencing finger proliferation. Similarly, the control groups exhibited the lowest number of primary and secondary fingers, with counts of 3.12 and 3.53, respectively. This could be attributed to the lack of essential nutrients like nitrogen, phosphorus, and potassium in the control group, leading to growth retardation and ultimately resulting in a lower number of fingers.

In comparison, farmyard manures achieved the lowest number of primary and secondary fingers per clump compared to other biofertilizers used in the study. However, contrary to our findings, Ferdous et al. (2018) reported the highest reproductive traits such as primary and secondary fingers per clump, fresh rhizome yields, and dry yields with the combined application of chemical (NPK) and organic fertilizer sources. Discrepancies between their results and ours could be attributed to variations in soil composition, climate conditions, and microbial activity, which influence nutrient availability. Additionally, differences in fertilizer application rates, timing, and sources may also contribute to variations in plant growth, highlighting the impact of environmental and fertilizer management practices. Furthermore, the study by Kadam and Kamble (2020) also corroborated our results, as their findings closely matched ours.

Similarly, the application of various fertilizers on turmeric demonstrates significant results for fresh rhizome yields at a 5% significance level. The overall mean rhizome yield recorded among these treatments was 18.62 tons ha^{-1}. The maximum yield was observed in the recommended dose of NPK, totaling 21.30 tons ha^{-1}. According to Rajbanshi et al. (2023), their findings on the effects of organic sources of nutrients and biofertilizers on turmeric growth, yield, and quality indicated the highest rhizome yield with chemical fertilizer in combination with organic sources, closely mirroring our results. The superior yield achieved with the recommended dose of NPK can be attributed to several factors. NPK fertilizers offer a balanced and readily available supply of essential nutrients crucial for optimal turmeric growth and rhizome development. Unlike organic sources, NPK fertilizers ensure precise nutrient delivery, minimizing deficiencies and maximizing yield potential. Additionally, efficient nutrient uptake from NPK fertilizers may enhance photosynthesis, increase biomass accumulation, and ultimately lead to higher rhizome yield compared to other treatments. Among the biofertilizer sources used in the study, goat manure (20.35 tons ha^{-1}) and organic manure (19.59 tons ha^{-1}) recorded superior results, followed by vermicompost (18.91 tons ha^{-1}) and poultry manure (18.69 tons ha^{-1}) respectively. These findings are strongly supported by Kadam and Kamble (2020) and Chhetri et al. (2020). Additionally, a previous study by Hossain et al. (2007) also reported similar results compared to ours. Moreover, farmyard manure (16.01 tons per hectare) and the control group (15.53 tons per hectare) exhibited similar and the lowest rhizome yields. This could be attributed to inadequate nutrient supply. Both farmyard manure and control groups likely lacked sufficient essential nutrients crucial for robust rhizome development, resulting in diminished yields compared to treatments receiving synthetic or balanced nutrient supplementation. The findings reveal that the dry yield was significantly influenced by the application of various biofertilizers and chemical fertilizers at a significant level of $p<0.01$, as indicated in Table 4. The overall grand mean of dry weight across treatments was recorded at 3.98 tons ha^{-1}. The highest dry yield was observed with the recommended dose of NPK at 4.79 tons ha^{-1}, followed closely by goat manure (4.58 tons ha^{-1}) and poultry manure (4.03 tons ha^{-1}), respectively. These results are consistent with a previous study by Hossain et al. (2007), which also reported similar dry yield ranges from 4 to 5 tons ha^{-1}. The superior performance of these treatments in both studies can likely be attributed to the balanced nutrient supply provided by these fertilizers. NPK, goat manure, and poultry manure likely supplied essential nutrients in optimal ratios, facilitating vigorous growth and maximizing dry yield compared to other treatments. Similarly, the results determined 3.83 tons ha^{-1} for organic manure, 4.03 tons ha^{-1} for poultry manure, and 3.76 tons ha^{-1} for vermicompost. Chandana et al. (2022b) also reported similar findings in their study on the effect of organic manures and biofertilizers on turmeric varieties. In contrast, the control group exhibited the lowest dry yield at 3.20 tons ha^{-1}, a result supported by Kumar et al. (2023). This minimal yield in the control group can be attributed to the absence of nutrient supplementation. Without fertilizer application, plants in the control group likely experienced nutrient deficiencies, impeding their growth and resulting in diminished dry yield compared to treated groups. Furthermore, the results indicate that dry recovery was significantly affected by the application of various fertilizers at a significant level of $p<0.001$. The highest dry recovery percentage was observed with goat manure (23.57%) and the recommended dose of NPK (23.50%), followed by poultry manure (21.54%) and organic manure (21.49%), respectively. In contrast, a study by Ferdous et al. (2018) reported the highest dry recovery percentage with synthetic fertilizer along with a biofertilizer source, a finding echoed by Baka et al. (2021). The lowest dry recovery percentage was observed in the control group (19.64%) and farmyard manure (19.57%), results closely aligned with previous findings documented by Hossain et al. (2007) and Datta et al. (2017). This minimal recovery percentage in these treatments may be attributed to inadequate nutrient availability and imbalanced soil conditions.

Table 4. Effect of various sources of fertilizer on several attributing characters of turmeric

Treatments	TP	LL	LW	LA	DS	PF	SF	FR	DY	DR
NPK	3.76^{a}	35.58^{a}	14.20^{a}	326.33^{a}	27.66^{a}	4.57^{a}	5.23^{a}	21.30^{a}	4.79^{a}	23.50^{a}
Goat manure	2.80^{bc}	32.93^{b}	13.44^{ab}	285.56^{b}	27.00^{ab}	4.28^{ab}	4.87^{a}	20.35^{a}	4.58^{ab}	23.57^{a}
Organic manure	2.12^{de}	27.04^{e}	12.28^{c}	226.48^{d}	26.00^{bc}	3.76^{bc}	3.78^{bc}	19.59^{a}	3.83^{cd}	21.49^{b}
Poultry manure	3.21^{ab}	31.07^{c}	13.20^{b}	264.47^{c}	26.66^{ab}	3.59^{cd}	4.22^{b}	18.69^{abc}	4.03^{bc}	21.54^{b}
Vermicompost	2.45^{cd}	28.58^{e}	11.23^{d}	197.07^{e}	25.33^{cd}	3.42^{cd}	4.15^{b}	18.91^{ab}	3.76^{cd}	20.61^{c}
FYM	1.74^{ef}	27.15^{e}	11.23^{d}	196.25^{e}	25.33^{cd}	3.20^{cd}	3.69^{bc}	16.01^{bc}	3.71^{cd}	19.64^{d}
Control	1.36^{f}	25.45^{f}	10.84^{d}	178.23^{e}	24.33^{d}	3.12^{d}	3.53^{c}	15.53^{c}	3.20^{d}	19.57^{d}
Grand mean	2.494	29.688	12.351	239.203	26.047	3.712	4.215	18.629	3.989	21.422
SEM	0.296	0.627	0.401	8.600	0.477	0.260	0.259	1.390	0.299	0.192
CV (%)	14.528	2.585	3.978	4.402	2.242	8.574	7.522	9.136	9.166	6.096
F-value	***	***	***	***	***	***	***	*	**	***

*: Significant at 5% level of significance. **: Significant at 1% level of significance. ***: Significant at 0.1% level of significance. NS: Non-significant. TP: Tiller per plant. LL: Leaf length (cm). LW: Leaf width (cm). LA: Leaf area (cm^2). DS: Days to 50% sprouting. PF: Number of primary fingers per clump. SF: Number of secondary fingers per clump. FR: Fresh rhizome yield (t/ha). DY: Dry yield (tons ha^{-1}). DR: Dry recovery (%)

Without proper fertilization or supplementation, turmeric plants in these treatments likely struggled to absorb and utilize nutrients efficiently, resulting in reduced dry matter accumulation and lower recovery percentages compared to treatments receiving nutrient inputs.

4. Conclusion

This study emphasizes the pivotal role of organic fertilizers in promoting sustainable turmeric cultivation in Eastern Nepal. Among various fertilizer treatments, the recommended dose of synthetic fertilizer, NPK, significantly improved both vegetative and reproductive growth parameters of turmeric, including plant height, leaf number, tillers per plant, primary and secondary fingers per clump, fresh rhizome yield, dry yield, and dry recovery percentage. Additionally, organic sources like goat manure and poultry manure showed promising results in enhancing turmeric yield and quality. These organic fertilizers deliver essential nutrients sustainably, promoting vigorous plant growth and contributing to soil health and biodiversity conservation. The findings underscore the potential of organic farming practices to mitigate soil degradation and ensure the resilience of Nepal's turmeric industry, offering viable alternatives to chemical fertilizers. Further research is needed to optimize nutrient management strategies tailored to diverse agro-climatic conditions, ultimately enhancing turmeric cultivation practices and securing farmers' livelihoods nationwide.

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest. All authors have read and agreed to the published version of the manuscript

Authors' Contributions

Chandani SUNUWAR: Data curation, Funding acquisition, Methodology, Writing – original draft, Writing – review & editing. **Soniya KOIRALA:** Conceptualization, Data curation, Methodology, Software, Visualization, Writing – original draft. **Ravi ACHARYA:** Investigation, Supervision, Validation, Writing – review & editing. **Nisha CHAUDHARY:** Data curation, Writing – original draft. **Uma Devi BHANDARI:** Data curation, Methodology, Writing – original draft. **Melina RAI:** Data curation, Writing – original draft. **Supriya NIRAULA:** Data curation, Resources, Writing – original draft. **Rupesh Kumar MEHTA:** Conceptualization, Software, Visualization.

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