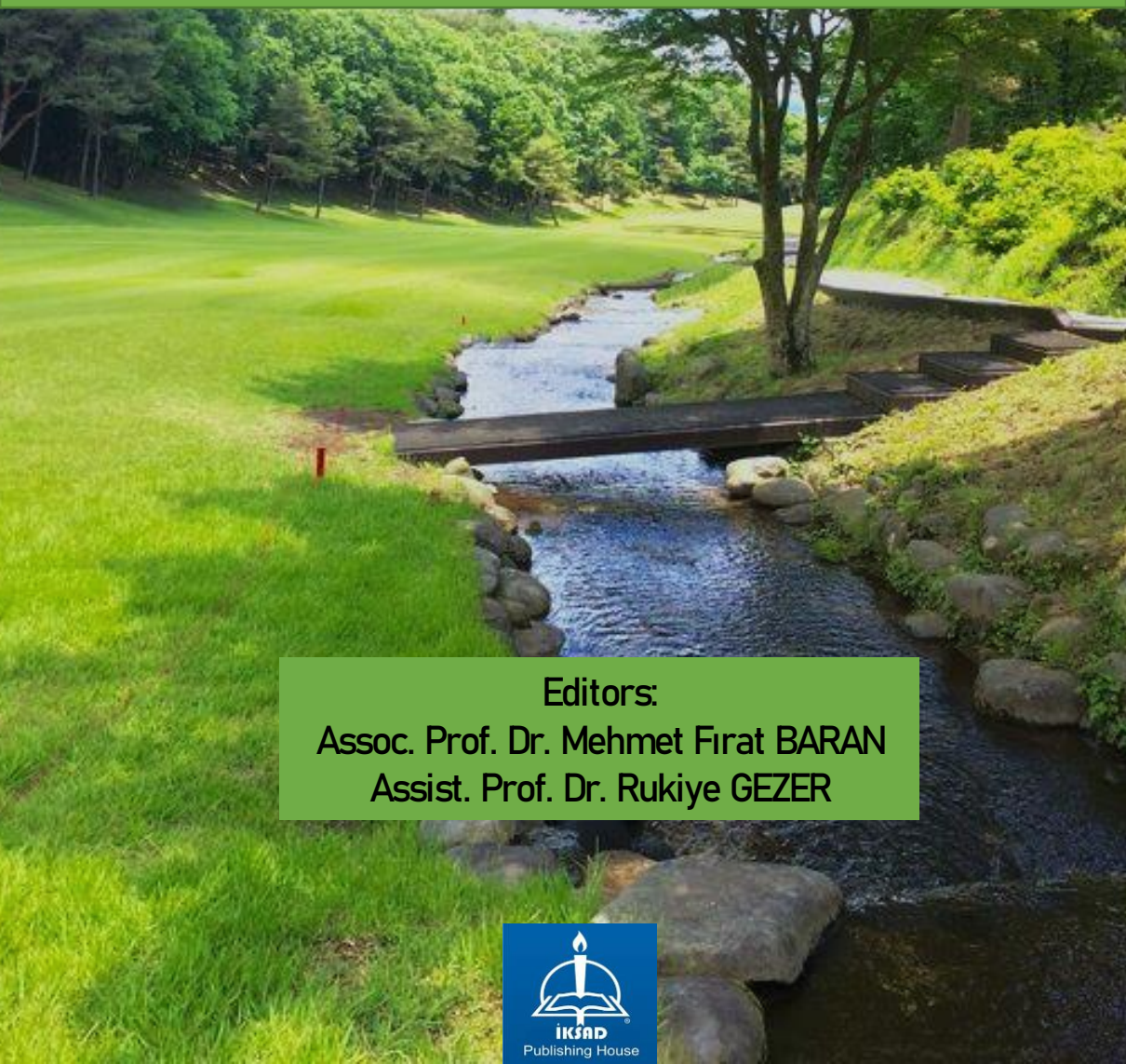
SCOPE AND IMPORTANCE OF AGRICULTURAL STUDIES



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PREFACE

The scope of agricultural research must include a description of the systems being investigated, their functions, and their boundaries. It is essential to figure out the type of study model required and the intended users. When a farmer is growing a crop in an area with rules and forecasts of how much economic yield the crop will produce, it can be useful to know the optimal management techniques that satisfy productivity, profitability, and environmental protection goals. Similar to this, studies that include livestock production need to be planned to forecast the performance of herds or individual animals under various combinations of breeds and management. They may forecast the number of animals of various ages by gender, body mass, or the amount of milk each lactating cow produces each day, all of which are influenced by herd management and the commercialization of meat, calves, and/or milk. The goal and scope definition of bioenergy scenarios and studies of organic agriculture systems frequently fail to adequately differentiate the unique characteristics of the respective farming system, and frequently only a small number of impact categories are assessed within the impact assessment, making it impossible to conduct a thorough analysis. In general, the goal, methods used, attributional or consequential perspective, chosen system boundaries, functional units, used data basis (experimental data vs. modelled data), assumptions made regarding farming practises (including yields), applied characterization factors, analyses of results, assessed impact categories, life cycle, impact assessment, and conclusions can all be included in the research goal and scope.

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CHAPTER 1

ROLE OF CYTOKININS ON FLOWERING OF ORNAMENTALS

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Cytokinins, which include benzyladenine (BA), isopentenyl adenine (2iP), kinetin (KIN), thidiazuron (TDZ), BAP, CPPU, ZEA, and others, play a variety of roles in plant growth and development, and physiological data also suggests that they play a part in the transition from one floral stage to another. As an important phytohormone, cytokinins were initially discovered to be catalysts for cell division and shoot development in culture. Observations indicating cytokinins activate cellcycle genes and interact with genetic controls of stem cell quantity within the shoot apical meristem now at least partially explain these effects (Yanai et al., 2005; Leibfried et al., 2005; Gordon et al., 2009). According to numerous studies (D'Aloia et al., 2011; Werner and Schmülling, 2009; Kieber and Schaller, 2014; Zürcher and Müller, 2016), cytokinins are also important for many other aspects of plant growth and development, including cell proliferation and differentiation, vascular cambium activity, chloroplast development, response to nutrients, leaf senescence, shoot and root branching, and seed germination. A phosphorelay system that mimics bacterial two-component signaling systems mediates cytokinin signaling (Müller and Sheen, 2007).

The ability of cytokinin signaling to induce shoot production in callus demonstrated its importance in the setting of shoot apical meristem development (Patton & Meinke, 1988). Leaf epidermis cell divisions are regulated by cytokinin (Vaten et al., 2018). Additionally, cytokinins have been linked to numerous aspects of root growth, including maintaining bilateral symmetry, vascular development, determining the size of the root meristem, and the production of root nodules (Wybouw et al., 2019).

1. Cytokinin signalling

Cytokinins are synthesized by the ISOPENTENYL TRANSFERASE (IPT) and LONELY GUY (LOG) enzymes, whereas cytokinin conjugation act mainly through CYTOKININ OXIDASE (CKX) enzymes. Cytokinins are next perceived by the ARABIDOPSIS HISTIDINE KINASE2-4 (AHK2-4) receptors which initiates a phosphorylation signaling cascade. This phospho-relay starts with auto-phosphorylation of the receptors and will ultimately lead to phosphorylation and activation of B-type ARABIDOPSIS REPSONSE REGULATORS (ARRs) through the ARABIDOPSIS HISTIDINE PROTEINS (AHPs). The active ARRs will then induce cytokinin responsive

genes, such as those encoding the cytokinin signaling repressors A-type ARRs or CYTOKININ RESPONSE FACTORS (CRFs) (Wybouw et al., 2019).

2. Cytokinin action in flowering

Cut flowers and potted plants are examples of ornamental products with a finite display life. Senescence and abscission of leaves and flowers, poor color development during flower opening or rapid color fading, desiccation, and gravitropic bending are the key factors that cause these ornamentals to lose their ornamental life. Controlling the quantities and actions of the many plant growth regulators involved in these processes is one way to deal with these issues (Chernov et al., 2007).

Senescence in flowers is the final stage of the processes that bring about their loss. Since many emerging nations are focused to the international trade in fresh flowers for commercial purposes, this occurrence is a significant barrier for all floricultural enterprises. The current state of postharvest technology is used to extend the vase life of cut flowers by using preservatives in the form of energy sources like sucrose and other sugars, biocides, mineral ions, growth regulators, or various metabolic inhibitors, and it offers a practical solution to the global cut flower market. There are numerous physiological and biochemical investigations that provide information on the lipid peroxidation, loss of membrane integrity, and protein degradation necessary for petal senescence (Rani & Singh, 2014). Cytokinins also plays a key role during several stages of flower development. In the whole plant kingdom, they are crucial to the formation of the gynoecium (Akagi et al., 2018). During the development of female gametophytes, cytokinins are crucial for determining cell fate (Yuan et al., 2016).

A coordinated genetic program that is partially mediated by variations in the concentration of ethylene, abscisic acid (ABA), and cytokinin controls the senescence of plants. Senescence, or wilting or abscission, of whole flowers or portions of flowers, is the final stage of floral development. Cytokinins prolong senescence in vegetative and floral tissues, in contrast to ethylene and ABA's effects. In some flowers,

the amount of cytokinin and senescence are inversely correlated. The rose and carnation's cytokinin content is highest in immature blooms and declines as the corolla opens and develops. There have been claims that rose cultivars with longer vase lives contain more cytokinins than rose variants with shorter vase lives. Carnations, roses, Gerberas, and petunias' senescence was postponed by cytokinin treatment (Chang et al., 2003).

The vital developmental stage in the life cycle of vascular plants that bear seeds is flowering. It symbolizes their passage from the vegetative to the reproductive stage, specifically the conversion of the vegetative meristems into inflorescence meristems once the plant has transitioned from the juvenile to the adult stage (Poethig, 2003). For plants to successfully reproduce throughout their life cycle, flowering at the right moment is crucial. Environmental and endogenous cues are used to fine-tune the shift from vegetative growth to reproductive development (Wu et al., 2019). Environmental signals and the primary elements, such as photoperiod, light intensity, light quality, temperature, water availability, etc., cause flowering in the majority of plants. When subjected to favorable and unfavorable conditions, respectively, plants produce flowering promoters and inhibitors, and these signals can be transmitted from leaves or fruits to the bud meristem (Winterhagen et al., 2020). Among these, cytokinins and gibberellins are recognized to play a role in certain plant species' reproductive development processes (Gordon et al., 2009).

More frequently, growth regulators like cytokinins and gibberellins are used to speed up flowering and boost flower quality (Pogroszewska & Sadkowska, 2008; Janowska, 2013). A crucial characteristic that affects the economic potential of plants used in floriculture is large blossom size. Many plants used in floriculture have flowers that are noticeably bigger than the comparable wild species. Increased petal number and larger individual petals are two morphological alterations that contribute to large flowers. A larger floral

meristem or the homeotic transformation of stamens and carpels into petals both cause an increase in the number of petals. However, due to the rarity of mutations in the genes responsible for blossom size, a large flower characteristic takes a long time to breed (Nishijima, 2012). Application of cytokinin and gibberellin together has a synergistic effect on petunia corolla expansion (Nishijima et al., 2006) (Fig. 1.).

Benzyladenine is largely used in ornamental plants as a growth regulator that prevents the branching of in vitro produced plants. As an example, *Zantedeschia aethiopica*'s ability to produce blooms is improved by benzoladenine (Luria et al. 2005). Benzyladenine increases the quantity of 1st-order lateral shoots in *Campanula persicifolia* cv. Alba (Pogroszewska & Sadkowska 2008). In turn, *Astilbe arendsii* Amethyst plants produced more inflorescence branches when benzyladenine was sprayed to the foliage (Pogroszewska et al., 2007).

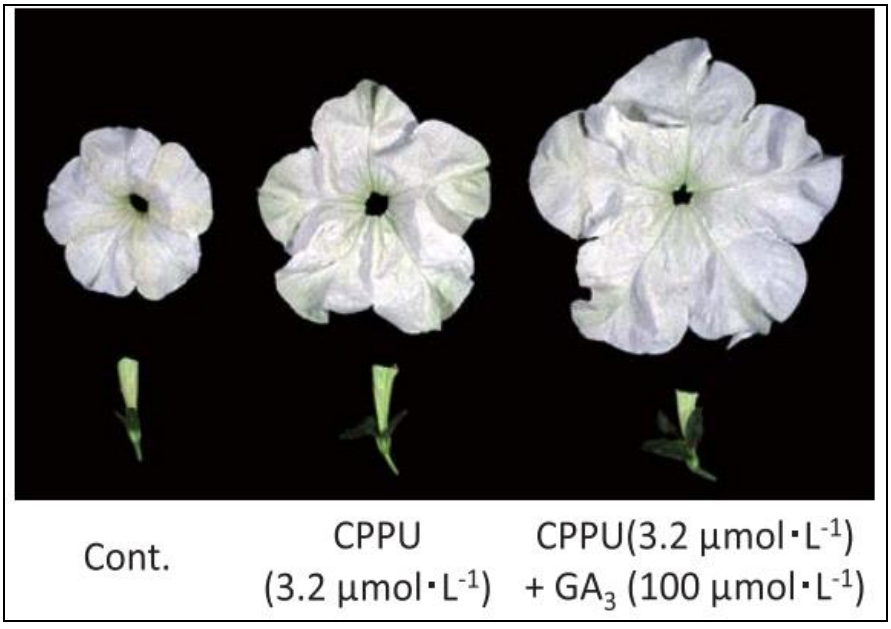

Fig. 1. Application of cytokinin and gibberellin together has a synergistic effect on petunia corolla expansion. The "Pearl White" cultivar was examined. Young flower buds were repeatedly treated with cytokinin and gibberellin (Nishijima et al., 2006).

When cytokinins are applied to a plant's apex during noninductive photoperiods, they can cause a number of apical alterations, such as cell divisions that typically take place before floral initiation (Bernier and Kinet, 1986). In the short-long-day plant *Leucospermum cordifolium* x *L. lineare* R. Br. and the short-day plant Schlumbergera truncata, BA improved the floret count and capitulum size when given during floral initiation (Napier et al., 1986). (Ho et al., 1985). The number of phylloclades (branchs) increased when cytokinins were administered to Schlumbergera under noninductive photoperiods (Halevy, 1985).

In *Helianthus annuus* L., cytokinins and GA have been successful in boosting the amount of pistillate ray florets (Sladky, 1986). However, this mixture reduced the amount of flowers generated by BA alone while causing phylloclade elongation, flower bud initiation, and lateral phylloclade development in Schlumbergera under inductive SD (Ho et al., 1985).

3. Effect of cytokinins on ornamental flowers

An enhanced methodology for the micropropagation of the crucial *Scoparia dulcis* L. species for ethnomedicine was designed by Premkumar et al. (2011). They determined that cytokinins are the key factor to induce the direct shoot regeneration and flowering of S. dulcis. For the purpose of inducing shoot bud formation, explants were inoculated on MS basal medium supplemented with kinetin and 6-benzylaminopurine. Different auxins, including 3-indoleacetic acid, 3-indolebutyric acid, and -naphthylacetic acid, were evaluated alongside 2.32 M KI and 4.44 M BAP to see if they could improve shoot induction. The regenerated shoots were planted in MS medium that had been diluted and treated with varying amounts of IAA, IBA, or NAA. The plantlets were transferred to pots with vermiculate and sand after developing roots, and they were then kept in a growth room with 70% to 80% humidity and a 16-hour photoperiod. The plantlets were moved to the garden after acclimation, and the survival rate was measured. The MS media supplemented with a mixture of two cytokinins (2.32 M KI and 4.44 M BAP), 2.85 M IAA, 10% CM, and 1 483.79 M adenine sulfate was the most effective for inducing shoots on young leaf explants. After 13 days of culture, a single young leaf explant was able to produce 59 shoots. In a medium supplied with a mixture of KI and BAP, flowering was triggered.

The influence of BA, GA3 and IAA applied successively onflower bud formation in shoot apices of *Pharbitis nil* has been investigated by Galoch et al., (2002). The shoot apices of seedlings grown in non-inductive continuous light and those exposed to a subinductive (12 h) dark period were separated. The inductive night was substituted by the simultaneous introduction of BA and GA3, which induced plantlets to flower in entirely non-inductive continuous light (optimal concentration of BA: 107-106mol dm3, GA3: 107-106 moldm3) and stimulated this process under sub threshold induction. The flowering of explants was unaffected by these hormones when they were administered in reverse order (GA3 first, then BA). The stimulating effects of BA and GA3 were reduced by IAA. The influence of phytohormones on flowering may result from the change of growth correlations within the shoot apical meristem.

In three stages during the ontogeny of growth that correspond with the progressive transition of buds from apical dominance to dormancy, Subbaraj et al., (2010) evaluated the effects of cytokinin and gibberellin, applied alone and in sequential combinations, on bud fate. With the shift to dormancy, cytokinin's ability to induce branching alone became less effective. A successive administration of gibberellin was required to maintain branching during this transition. Branching was not stimulated by gibberellin alone. With the onset of dormancy, gibberellin's ability to induce blooming alone became less effective. Any flowering during this transition occurred only after the sequential application of cytokinin. Flowering was not induced by cytokinin on its own. By working together, cytokinin and gibberellin are able to reduce bud dormancy and promote flowering in Zantedeschia.

In the study conducted by Abad Farooqi et al., (1994), Kinetin, at a concentration of 10 mg/l, allowed *Rosa damascena* plants to produce more blooms, whereas GA3 prevented flowering. GA-like activity was high in the leaves of non-flowering plants, but blooming plants had much greater levels of specific cytokinin activity.

The cytokinin 6-benzylaminopurine (BAP) was applied to the leaves of "*Boronia heterophylla* F. Muell" to induce the growth of several lateral shoots by Richards, (1985). The short BAP-induced laterals later blossomed into flowers in the spring if plants were allowed to overwinter in a polyhouse. The resulting plants were dense, heavily branched, and provided a lovely floral display. The BAP concentration that worked best was 50 mg l1 spread across 4

applications. Application made more frequently significantly decreased flowering. Manually pinching the leading shoots was an ineffective method for promoting branching and resulted in fewer flowers. Transferring plants between a polyhouse and a glasshouse with environmental control revealed that B. heterophylla requires a protracted period of cold for adequate bloom development.

The effects of several chemically pure gibberellins and cytokinins on the yellowing of Alstroemeria leaves were discussed by Jordi et al., (1995). Both the leaves of cut flowering stems and a model system made up of detached leaf tips were used to measure the loss of chlorophyll. It was established that plant growth factors had an equal impact on chlorophyll loss in both systems. Different gibberellins and cytokinins postponed leaf senescence. The outcomes showed that some gibberellins (GA4 and GA7) are significantly more efficient than GA3, which is typically applied as a postharvest therapy for Alstroemeria cut flowering stems, in postponing chlorophyll loss.

The gaseous hormone ethylene stimulates flower senescence in many plant species, including the rose (Rosa hybrida), while the cytokinin (CTK) class of hormones inhibits it. But it is unclear what molecular processes underlie these opposing effects (Wu et al., 2017).

Fig. 2. Boronia heterophylla (Lullfitz, 2004)

Because cytokinin is critical for pistil development (Kieber & Schaller, 2018), it is considered a major phytohormone responsible for expression of the female sex (Khryanin, 2002). In order to encourage floral feminization in several plant species, such as Mercurialis annua, Plukenetia volubilis, and Sapium sebiferum, exogenous application of cytokinin is typically used (Ming et al., 2020). Unisexual flower growth has been widely divided into two categories based on the pattern and method (Luo et al., 2020). In type I, which is the more common variation, flowers begin as bisexual and change to unisexuality after the development of the androecium or gynoecium is completed. These mature flowers have reproductive organs that have already aborted. In type II, flowers only begin androecium or gynoecium and appear to be unisexual from the beginning. These flowers do not have aborted reproductive organs when they are mature. In terms of the developmental mechanisms that determine the sex of unisexual flowers, type I flowers must abort their reproductive organs at a specific point, whereas type II blooms must initiate their reproductive organs (Diggle et al. 2011). A feminization role for cytokinin in sex determination is clearly suggested by its effect on female primordia during flower development in Sacha inchi (Plukenetia volubilis, Euphorbiaceae). Sex determination occurs at early developmental stage, and exogenous cytokinin treatment can take effect on the meristem of male flowers to lead it to feminizing (Luo et al., 2020).

In Gladiolus cv. Candyman, Kumar et al. (2010) investigated the impact of bio-regulators on growth, flowering, and corm formation. According to the findings, BA at 50 ppm increased the number of shoots per plant while BAP at 25 ppm increased the weight of cormels per plant. When Baskaran and Misra (2007) investigated how plant growth regulators affected the growth and flowering of gladiolus, they discovered that BA @ 100 ppm produced the most shoots per corm and BA At 25 ppm produced the fewest shoots and leaves per plant.Sajjad et al. (2015) conducted research on how Gladiolus grandiflorus L.'s numerous sprouting, floral, and corm-associated properties are affected by pre-plant soaking of corms in growth regulators. According to the findings, BA @ 150 ppm displayed the greatest number of sprouts per corm and decreased plant height. In an experiment, Manasa et al. (2017) examined how gladiolus cv. Summer Sunshine's vegetative characteristics were affected by growth regulators. The findings showed that the largest number of suckers per plant

and the maximum number of leaves per plant were recorded at 100 ppm of BA. Baskaran et al. (2009) studied the performance of gladiolus by spraying and dipping the corms with different growth hormones at different concentration and they reported that propagation co-efficient was maximum in BA @ 100 ppm as spray method of treatment.

In an experiment by Mohammadi et al. (2013) to determine the effects of benzyl adenine (BA), gibberellic acid (GA), and salicylic acid (SA) on tulip cut flowers, they discovered a decreased rate of anthocyanin contents during the experimentation period, with the best results coming from BA or GA treated plants. According to Nelofar et al. (2005), BA @ 200 ppm recorded the longest vase life when tulip cut stems were pulsed for 4 hours.

Zantedeschia aethiopica was the subject of a study by Luria et al. (2005) to determine how planting depth and density, leaf removal, cytokinin, and gibberellic acid treatments affected flowering and rhizome formation. By five times compared to the control, the dipping rhizomes in BA @ 350 ppm improved blossom yield.

Gibberellic acid and benzyl adenine's effects on the yield of Allium karataviense Regel 'Ivory Queen' were examined by Pogroszewska et al. in 2007. The total yield of bulb number per m2 and total yield of bulb weight per m2 were found to increase with the use of BA. Bharathi and kumar (2009) noticed that foliar spray of kinetin @ 200 ppm increased the flower diameter in tuberose cv. Suvasini.

According to Janowska et al. (2009), using benzyl adenine reduced the flowering time of the poppy anemone "Sylphide" by 3–7 days. Its tubers were submerged in benzyl adenine, which resulted in the development of blooms with shorter pedicels. By using benzyl adenine, the tubers produced just one-third as many leaves and developed leaves with shorter stalks and blades.

Gibberellic acid and benzyl adenine's effects on tuberose (Polianthes tuberosa L.) cv. Asil et al. (2011) conducted research on Goldorosht Mahallat and found that BA at 100 ppm and 200 ppm recorded decreased spike length, rachis length, and leaf length, resulting in maximum floret diameter and vase life of cut flower.

Emami et al. (2011) studied the impact of gibberellic acid and benzyl adenine on the development and flowering of the lily (Lilium longiflorum) and found that the combination of gibberellic acid at 75 ppm and benzyl adenine at

75 ppm produced the highest number of buds per plant (4.6) and total chlorophyll content, while benzyl adenine at 150 ppm and 75 pp.

The impact of plant growth regulators on the vegetative characteristics of lily was the subject of an experiment by Naji et al. (2015). The findings demonstrated that BA @ 50 ppm recorded maximum plant height, number of leaves, leaf area, fresh leaf and stem weight, and dried stem weight.

According to Faraji and Basaki (2014), plants sprayed with BA @ 200 mg per L had a maximum vase life of 12 days and considerably higher protein content in the petals. The highest effective dose of BA to slow down chlorophyll breakdown was 100 mg/L.

4. Conclusions

Major cytokinins used in the international studies include benzyladenine (BA), isopentenyl adenine (2iP), kinetin (KIN), thidiazuron (TDZ), BAP, CPPU and ZEA. They play a variety of roles in plant growth and development, and physiological data also suggests that they play a part in the transition from one floral stage to another. Among these, Benzyl adenine and kinetin is effective in improving the growth of plants and yield of flowers.

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CHAPTER 2

A BRIEF OVERVIEW OF THE HISTORY OF CORN EVOLUTION

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1. Introdution

Corn (*Zea mays* L.) is a crucial industrial product that can be utilized to people and animal nutrition and also a raw material for the food, oil, biofuels, and sugar industries. A variety of agroclimatic zones can be used to cultivate corn, a crop known for its great adaptability. Due to its benefits, corn is one of the most important cereal crops in the world (Washburn et al., 2020).

Corn is one of the few plants that has been cultivated for approximately 8,000 years. The origin and history of corn are still unknown, however, there are several possibilities that are being discussed right now despite the lack of obvious knowledge. Teosinte, which is regarded as a wild relative of corn in taxonomy, is one of the plants that are found in the native flora of Honduras, Guatemala, and Mexico (Shultz, 2008). There are seven genera in the tribe *Maydeae* that have been recognized, containing groups from the Old and New Worlds. *Coix*, *Chionachne*, *Sclerachne*, *Trilobachne*, and *Polytoca* are plants from the Old World, whereas *Zea* and *Tripsacum* are plants from the New World. The indigenous Mesoamericans developed the precursors of corn into a number of pre-Columbian corn strains within a few centuries of hybrids (García-Lara and Serna-Saldivar, 2019).

2. The History of Corn Breeding

Corn has experienced development for more than 10,000 years. There are seven types of corn dent, flint, pop, flour, sweet, pod,and waxy. These subspecies have different usage areas due to the structure of different endosperm and the difference in vegetative characteristics. One of the most important areas of human endeavor today is plant breeding. Plant breeding has continuously advanced, paralleling the development of human civilization. For thousands of years, the American Indians used archaic breeding techniques (Haullauer et al., 1988). The choice of this grain's forebears by early farmers aided in the modernization of corn. The modern period of plant breeding began more recently as a result of Mendel's studies. Inbreeding depression in corn was recognized by Darwin as a significant aspect of corn breeding. Its significance for scientific inquiry has been maintained since Darwin (Washburn et al., 2020). Self-pollinated corn had a smaller size than cross-pollinated corn, according to Darwin. George Harrison Shull expanded on his findings in 1908 after using self-pollinating corn plants to produce inbred lines for multiple

generations before combining them to produce hybrids (Hake and Ross-Ibarra, 2015). In the first place, farmers in particular exploited corn breeding and seed manufacturing. Many open-pollinated varieties of corn have been obtained by the intentional breeding of introduced kinds and the sale of corn seeds, both of which date back thousands of years. However, as superior corn hybrids became widely available throughout the majority of the USA in the early 1900s, on-farm corn applications made by breeders and farmers to increase grain yield began to decline (Kutka, 2011).

Corn landraces have been kept alive by traditional farming practices because they were chosen by farmers for the valuable agronomic qualities they were thought to possess. For this reason, corn landrace is a variety that exhibits remarkable yield stability and average yield production with inadequate agricultural implementation. It also has a great capability to tolerate abiotic stress and resist biotic stress. A specific genetic variety found in landraces is vital for corn breeding (OECD, 2003). In the majority of the world's corn crop, corn hybrids are taking the place of open-pollinated corn varieties. Existing landraces served as the foundation for the creation of open-pollinated corn cultivars. Traditional breeding involves the creation of open-pollinated corns utilizing cautious techniques to change the genome of plants within the genetic range of the corns naturally (Acquaah, 2016).

Corn breeding for hybrid production began in the 1930s, and by the end of the 20th century, hybrids were responsible for over fifty percent of all corn production, which increased annually. In order to create hybrids, genetic improvement in corn is a constant process. In this regard, other cereals, due to their nature, are not as suitable to hybridization to the same extent as corn because corn differs from other cereals in that the male and female flowers are in separate organs and are wind-pollinated. Before the use of synthetic nitrogen fertilizers or the chemical control of weeds and insects greatly increased beginning in the 1950s with the Green Revolution, production increased (Duvick, 2005). Heterosis is a procedure by which diverse genotypes are crossed to produce a corn hybrid that is superior in growth, weight, yields, and vitality. By utilizing cross-breeding to choose the best hybrid offspring with a specific desirable characteristic, that trait can be developed. By backcrossing the chosen line with the recipient line over a number of generations, it can also be added to the best recipient line that was chosen. The required features can

be achieved by reducing undesirable phenotypic combinations (Ahmar et al., 2020).

A fundamental process that can be utilized to obtain inbred lines is corn hybrid. Inbreed lines, a word used in hybrid breeding, are primarily intended to prevent inbreeding depression. Determining the genetic content of the resources used as parents to improve corn hybrids is also crucial. Candidate lines are evaluated in field trials as both inbred and hybrid lines. Phenotypic evaluations of a large number of candidate lines under various environmental climate circumstances over a long period of time are necessary for selection techniques in corn breeding (Guerrero et al., 2014). The most popular technique for creating inbred lines as parents of hybrids is pedigree breeding. The backcross breeding technique is frequently employed in corn breeding to pass on one or more desirable traits from the parent to the repeatedly bred and most suitable parent. The backcross breeding process has steps that are comparable to those in the pedigree system. Stadler discovered gamete selection in 1944, which is another method used in traditional breeding and is used to choose the elite gametes that produced better inbred lines. The primary difference between these strategies is how well the parent's recurrent genotype is recovered, which affects how long it takes to produce good inbred lines (Haullauer et al., 1988).

The majority of agronomic traits taken into account by plant genetic improvement projects are quantitative in nature, controlled by several genes with quantifiable modest impacts, and influenced by environmental factors as well. According to the breeding objective, the selection criteria alter. In the past, the choice of agronomic traits has been used in phenotypic description. Because of how strongly influenced by environmental factors phenotypic descriptions are, they do not provide a precise picture of the genetic structure of the plant (Benkherbache et al., 2016).

3. Innovative Approaches to Corn Breeding

Corn breeders will logically look for novel strategies to speed up or improve this process. Established approaches to corn breeding are currently ineffective at increasing corn production. But, traditional breeding may take more than ten years for a new variety to emerge. The primary goal of current biotechnology is to use genetic distribution variations to create crops and plant-

based genetic constructs that have the desired features (Eckerstorfer et al. 2014).

The emergence of molecular markers in the 1980s substantially aided in understanding breeding-targeted features and offered the opportunity to increase selection effectiveness (Ali et al., 2020). Molecular markers are commonly utilized to develop answers to issues that arise in corn breeding. When purely using traditional methods, the selection and breeding processes are time-consuming. In hybrid breeding, phenological selection has been effectively replaced by molecular technologies such as marker-assisted selection (MAS) and the quantitative trait locus (QTL) in the early selection process. By consistently obtaining pure lines with high relationships for combination, it is feasible to generate high-quality, high-yielding hybrid corn types with great flexibility. Rather than using self-fertilization, haploid plant production looks to be a promising alternative way for hybrid breeding (Konstantino et al., 2012). In order to increase corn yield and quality, increase the expression of desirable characteristics, and shorten the breeding cycle, biotechnology must be employed in corn. Quantitative trait loci (QTLs) can be employed in large numbers of recombinant inbred lines to map the genetic basis of yield-related traits and heterosis and collect information about corn hybrids. Due to their significance in the field, quantitative traits like yield have been studied for a long time in genetic research. QTL (Quantitative Trait Loci) are genes that regulate the genetic change of quantitative traits. Quantitative or complex traits are traits for which genetic variation in phenotypic variety is continuously distributed, with population variance frequently matching, on a reasonable scale, a statistically normal range. Since the 1990s, methods of analyzing quantitative trait loci (QTL), which may include tiny target genes, have become available (Yonemaru et al., 2010).

The basis of the double haploid technique is the doubling of the chromosome sets of haploid plants and the rapid development of 100% homozygous pure lines. The development of homozygous pure lines is done in a shorter time by chromosome doubling with the haploid technique. Elite parental inbred lines play a significant role in hybrid corn breeding performance. In order to obtain roughly pure inbred lines, the development of parental inbred lines in corn relied almost exclusively on more than 6–8 generations of recurrent self-fertilization and selection. In order to further crop

breeding, double haploids technique appears to be widely used, particularly in hybrid corn (Chaikam et al., 2019). Haploid plants are those that have the same number of chromosomes in their somatic cells as the number of chromosomes in the gametes of the plant species to which they belong. The finding in this case was that each locus in haploid plants only has one series of alleles. Pure inbred lines can be produced in just one generation by doubling the number of chromosomes in haploid plants, such as through the use of mitotic inhibitors. The maximal genetic variation, parental pure inbreed-lines, faster breeding period, simplified logistics, reduced costs and labor, and the occurrence of ideal marker method circumstances are the main benefits of double haploid in corn hybrid breeding (Geiger and Schönleben, 2011).

Modern biotechnology has recently made it achievable to alter plants genetic structure in ways that are not conceivable to duplicate naturally through breeding or other traditional means. Gene transfer is the process of transferring genes from one organism to another using contemporary biotechnology techniques; the new organism created as a result is known as a genetically modified organism (GMO). *Bacillus thuringiensis* corn, the first transgenic variety of corn resistant to corn rootworm larvae, was made available in 1996. Recent years have seen a rise in the importance of genetic alteration in plant breeding, particularly for a commodity with significant economic importance like corn. Biotic and abiotic stress tolerance, such as drought tolerance, and herbicide tolerance are all traits that have been improved in transgenic corns (ISAAA, 2020).

4. Concluison

One of the most commercially significant crops worldwide is corn. In the 20th century, improvements in corn genotype through corn breeding were responsible for more than 50% of the production gains. The science of breeding started with people living in the prehistoric period searching for and finding plants with superior characteristics. Because of improved characteristics, improved cultural applications, and improvements in corn breeding, the production of corn today has increased significantly. Today, modern techniques have brought breeding studies to a very different point.

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CHAPTER 3

PRECISION CELL AGRICULTURE AND PRECISION FERMENTATION

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INTRODUCTION

By 2050, the population of the world is estimated to be 9.5 billion. Meeting the food demand for this growing population will be limited due to the effects of limited resources, soil, water and climate change. To improve the nutrition of the world's population, food production will need to be doubled, and if traditional agricultural models continue to be followed, this will result in a reduction in overall environmental sustainability and an increase in greenhouse gas emissions of 80%. For most of the world's population, animal protein is a critical source of nutrients for a balanced diet, and global demand for this protein is estimated to double by 2050. It has been reported that approximately 78% of consumers in the USA rely on meat as a source of protein. With increased meat consumption, the increase in food-borne diseases due to *Escherichia coli*, *Salmonella* and *Campylobacter* and the rapid proliferation of resistant bacteria with overuse of antibiotics used in farm animals is worrying. In parallel with the developing biotechnology, microorganisms have been begun to be synthesized proteins that they could not synthesize before. The amount of protein synthesized has been gradually increased. The idea that soil and water resources will remain insufficient to meet the growing population's food demand is widely accepted. The solution to this problem is to produce ready-to-eat products through a method called precision fermentation in bioreactors using microorganisms designed by genetic engineering. While traditional farming is largely dependent on external stochastic factors such as weather, in precision fermentation, risks in production have been reduced because bioreactors help to fully control cellular farming conditions such as temperature, acidity and access to critical nutrients for cell growth. It has been managed to create highly valuable components that improve the sensory properties and functional qualities of cultivated or plant-based products meat through precision fermentation a method that proteins, vitamins, enzymes, natural pigments and oils can be produced with. Precision Fermentation is a much more advanced method than traditional fermentation in order to increase safety, efficiency, flavour, nutrition, and general quality. It is crucial to take advantage of the opportunities provided by Industry 4.0 technologies for producing food that is healthier and more sustainable.

1.CELLULAR AGRICULTURE

Consumer interests in food have moved recently in favour of a "healthier" diet. The shift away from consumption of animal products in favour of plant-based ones of trend (especially in Western nations) is particularly notable and so pertinent to the subject of this study. Also, Due to ethical and/or animal welfare concerns, consumers are limiting their usage of animal products and switching to plant-based alternatives in an effort to minimize their carbon footprint. (Godfray et al., 2018).

Livestock farming produces greenhouse gases such as methane, carbon dioxide and nitrogen oxide. These gases are responsible for 14.5 percent of total anthropogenic greenhouse gases, and these ratio shows how much animal agriculture contributes to global warming. (FAO, 2017). A recent reserch by Xu and colleagues (2021) showed that the production of animal food products (including feed) accounts for 57% of the greenhouse gases from food production. In addition, the expansion of animal husbandry production also consumes water resources. It is needed 20.7 tons, 5.9 tons and 4.5 tons of water, respectively for each kilogram of beef, pork, poultry grown. (Oki et al., 2003). Although the high cost of the animal husbandry industry to environmental stability, some nutrients and their role in industrial food production cannot be easily substituted. For instance, animal-based proteins, such as egg and milk proteins, are crucial to structure nutritional quality and formation of various food products. Instead of relying on animal husbandry for the production of the most crucial animal protein components, biotechnology offers attractive alternatives using food industry microorganisms. In this case, cellular agriculture or precision fermentation means the use of fermentation technologies and recombinant food-grade microorganisms for the production of certain organic molecules such as food proteins. (Rischer et al., 2020; Ercili-Cura and Barth, 2020; Chai et al., 2022). At the same time, this technology also has the potential to produce animal-free cosmetics, fashion products and leather alternatives.

Interest and investment in cellular agriculture, which was introduced by New Harvest (a not-for-profit, donor-funded research institute) around 2015, and is generally described as the production of ingredients of animal origin without animal husbandry (e.g., using bioreactors), has increased (Mattick, 2018). Cellular farming is an developing field of research and application where

traditional products can be produced through a biotechnological cell culture process as an alternative to products traditionally obtained from animals (meat, milk, leather, etc.). In cell-cultured meat production, in particular, requires culturing and propagating animal cells *in vitro*, mounting them on a scaffold and feeding them with a serum to stimulate growth in a bioreactor. Although still a developing technology, cellular farming methods can produce a significant amount of meat from just a few animals by extracting the necessary cells instead of breeding animals for their meat. The implementation of cell-based meat production is expected to reduce water use, land use, and greenhouse gas emissions (especially methane) by twice. (Hocquette, 2016). However, it is difficult to optimise the production conditions because of lots of media components with non-linear and interactive effects between media, matrix material, cells, and reactor environment (Brunner and al., 2010). Typically, the culture medium used for processes in cellular agriculture consists of a basal medium (such as the common Dulbecco's Modified Eagle Medium [DMEM]) including, vitamins, amino acids, glucose, and salts supplemented with fetal bovine serum (FBS) for better cell survival. FBS is an unidentified, animal-based serum composed of hormones, proteins, and other substances of big molecular weight.

To date, research decisions such as the selection of cell types and cell species in cell-based meat production have been substantially driven by environmental impact and market size, rather than the suitability of types and species of cells suitable for large-scale bioreactor cultivation (Rodriguez-Fernandez, 2019).

1.1. PRECISION CELLULAR AGRICULTURE

Unlike to cultured meat in which differentiated stem cells cultivated under *in vitro* conditions are used, some egg and milk proteins can be expressed in a controlled combination or singly for usage in the products such as animal-free egg whites or milk, cheese, ice cream, and yoghurt. This field of study, in which only targeted proteins are expressed and derived, is called precision cellular agriculture (PCAg), a subset of cellular agriculture. The term PCAg is inspired by the term “precision fermentation” defined in The Good Food Institute’s State of the Industry report and used to refer to the cellular production of particular compounds ranging from lipids to organic acids (Teng

et al, 2021; Terefe, 2022). Most of the PCAg research is carried out by private companies, so there are very few peer-reviewed publications in this area. Precision fermentation will hold an important place in the food and beverage components market. Investors are pouring capital into the market, suppliers are forming partnerships with biotechnology companies to explore growth opportunities, and new component concepts are being rapidly introduced.

While the power of fermentation has been used for thousands of years to modify, enrich and preserve foods, and in recent years to produce vitamins, supplements, and medicines, the use of fermentation to mimic or recreate animal proteins is relatively new. About 57 percent of the 136 companies that focus on fermentation for alternative proteins have been established in the last three years, and the first animal-free dairy products have only taken their place on the shelves by 2020. Although it is still in its early stages, the fermentation industry has made significant progress in a short time. At the moment, at least 30 animal-free dairy products are available for retail sales in the United States and more products are under development. Fermentation companies are spread across 31 countries and every major region of the world. And manufacturers are trying to find new ways to collaborate, including an industry alliance and a number of strategic partnerships. (GFI, 2022 reports).

2. FERMENTATION

Fermentation is the synthesis of new molecules from carbohydrates or other organic compounds in foods by means of microorganisms and/or their enzymes to preserve the food, increase its organoleptic properties, increase its commercial and nutritional properties, and the resulting product is called fermented food. Microorganisms in GRAS status (Generally accepted as safe) are responsible for the bioprocess of most of the known foods and beverages (For example, dairy products, cheeses; *Lactococcus*, *Lactobacillus*, *Streptococcus thermophilus*, *Propioniobacterium*, *Penicicillium roqueforti*, Alcoholic beverages; *Zymomonas*, *Saccharomyces*, Yeast bread; *Saccharomyces*, meat products; *Pediococcus*, *Lactobacillus*, *Microoccus*, *Staphylococcus*, fermented vegetables; *Leuconostoc*, *Lactobacillus*, Soy sauce; *Aspergillus*, *Tetragenococcus halophilus* etc.). In fermented food, microorganisms and the compounds they synthesise such as bacteriocins,

organic acids or alcohols coexist. Since the bacteria involved in these processes cannot grow at pH below 4, fermentation is a self-limiting process.

Fermentation has been used to improve the nutritional value, digestibility, stability, aroma and flavour of bread, chocolate, coffee, olives, wine, and the plant-based sources such as many vegetables (cabbage, cucumber, onion, tomato, pepper) and legumes (peas, wheat, soya sauce, soya beans, beans, lentils) as well as meat/dairy products. It is common perception that plant-based products are less nutritious than animal products. Also, extensively used additives and ultra-processed foods have not been accepted by conscious consumers in recent years. The relationship between processed foods and health problems such as obesity, cancer and cardiovascular diseases is a controversial issue. Foods produced by fermentation have become frequently consumed foods today because they contain minimal additives, has been increased nutritional value, and have significant positive effects on health. (Owusu-Apenten and Vieira, 2023).

It was reported that the microorganisms used in food fermentations consisted of 264 species as of 2012, of which 195 were bacteria and 69 were yeasts and moulds. The most common bacteria for food fermentation appear to be 32 *Actinobacteria* species and 94 *Lactobacillus* species. (Bourdichon et al., 2012). There is also a wide variety of fermented products. It is estimated that there are about 3,500 fermented foods worldwide. (Tamang and Kailasapathy, 2010). The tendency to use traditionally prepared fermented foods is higher in communities that are financially stressed and decreases with increased prosperity. Fermented foods have been stated to be suitable for famine relief and support survival (Owusu-Apenten and Vieira, 2023).

It is possible to examine fermentation, which has recently been used in fermented food production, under three main headings.

2.1 TRADITIONAL FERMENTATION

Traditional fermentation has been used for centuries as a means of preserving food and increasing the nutritional value of food. Fermentation has been used to preserve foods such as fish, meat, milk, vegetables, legumes, cereals and fruits. Fermented foods are produced through the controlled growth of microorganisms and the transformation of components in food by the action of microbial enzymes. Among the well-known fermented foods and beverages

are yogurt, kimchi, kefir, cabbage, cheese, tempeh, wine, beer and natto (Bourdichon ve ark, 2012). However, in traditional fermentation, the microorganisms used are not generally well characterised and the underlying processes are not well optimised. (Sanni, 1993).

Fermented foods and drinks have been produced since the Neolithic Period in 10.000 BC. This process started as a positive result of repeated microbial contamination of animal and plant materials and resulted in fermented product that lasted for a long time. The repetition of the environmental qualification of these fortuitous events using handmade or pottery containers has enabled people to provide a minimum safety-standard in the fermentation process based on (i) spontaneous growth of the desirable microorganisms present in the raw material or (ii) the addition of parts obtained from previous well-resulted fermented products to a fresh bottom layer. (Owusu-Kwarteng et al., 2022).

Food preservation was the primary reason that hunter-gatherer societies adopted fermentation. However, other remarkable differences such as increased nutrition, changing texture and biological functionality in food by fermentation have encouraged the use of it in various societies. Remarkably, the use of the primitive fermentation process has taken place simultaneously but independently on each continent, depending on accessibility of raw materials and cultural aspects. (Owusu-Kwarteng et al., 2022). For example, in Europe and the Middle East, natural dairy products (e.g., kefir, and airag) are frequently consumed while in Oceania and East Asia, grains, vegetables and fermented products such as natto, kimchi, and sikhae based on eating habits continue to be a part of the daily diet. Other fermented foods produced without the addition of external bacteria include pickled cabbage and pickles of other vegetables. Such fermented foods are usually produced on a small scale and in developing countries.

However, certain starter cultures are now being selected and implemented to control these processes. Selected microorganisms are frequently used in the food sector, particularly in the production of wine, beer, bread, cheese, and yogurt. The primary purpose of the use of starter cultures in the application is to provide standardization of the fermentation process, increase safety parameters and optimize processing time. (Yamaner, 2010; 2020). Examples of starter cultures are *Rhizopus oligoporus* for tempeh,

Lactococcus, *Lactobacillus*, *Streptococcus thermophilus*, *Propioniobacterium*, *Penicicillium roqueforti* for dairy products and cheeses, *Bacillus subtilis natto* for natto, *Aspergillus oryzae* for miso, kefir grains containing yeasts such as *Kluyveromyces marxianus*, *Saccharomyces unisporus,* and *Saccharomyces cerevisiae* in addition to acetic and lactic acid producing bacteria for kefir (Dimidi et al, 2019).

2.2 BIOMASS FERMENTATION

In this fermentation system, the goal is to obtain single cell proteins (SCP). Edible microorganisms (e.g., bacteria, algae, or filament fungi) are used as an alternative to traditional food sources produced by conventional agriculture as a source of both edible bio-mass and functional components (proteins, polysaccharides, vitamins, lipids/omega-3 fatty acids, dietary fibre, and minerals) (Barros de Medeiros e al., 2022). In the World Economic Forum's 2019 white paper list, it was stated that the non-plant-based protein alternative in widespread use is mycoprotein-based Quorn (WEF, 2019). Algae, and especially microalgae (such as *Spirulina platensi*, *Arthrospira platensis*, *Dunaliella salina* and *Chlorella vulgaris*) have been developed and cultivated as a source of protein and other food components due to their nutritional composition, metabolic flexibility, and functional diversity (Garofalo et al., 2022). For example, microalgae are promising components for the production of nutritious meat analogues, but there are still technical difficulties in texturing microalgae, removing undesirable odours and colours, and expanding the scale of growing and processing conditions. (Fu et al., 2021).

Fermentation has also been used for the production of small food components (e.g., antioxidants, colorants, aromas, enzymes and vitamins). Recent research of extracts obtained from freshwater algae (*Spirogyra sp*., *Cosmarium* sp., and *Cosmarium blytii*) has shown that they have high carbohydrate content, contain free amino acids (glutamic acid, aspartic acid and proline) and exhibit high antioxidant activity, which has made them potential sources of functional components for food and feed (Santiago-Díaz et al, 2022).

Food waste is increasingly used as a fermentation medium for the production of antioxidants, natural preservatives, colourant, enzymes and other food components. Thus, the use of an unused or underutilised resource as a medium has the potential to both reduce food waste and create

high-value products. Recently, single-cell protein has been produced by fermentation of food waste by *Yarrowia lipolytica* (Yang et al., 2022).

Compared to traditional protein sources, biomass fermentation can quickly produce very high protein content in addition to other nutrients. Furthermore, wastes and food by-products can be utilised as substrates for conversion into feed products and high-value food. This is an environmentally friendly strategy that can promote an energy-efficient and sustainable economy.

2.3 PRECISION FERMENTATION

The term “precision fermentation” has emerged in recent years to refer to fermenting processes that are optimized by using specially designed microbial hosts as 'cell factories' to produce highly valuable functional food components such as enzymes, lipids, vitamins, carbohydrates, sweeteners, antioxidants, colorants, and preservatives in high purity and efficiency [GFI, 2020 reports]. Precision fermentation is defined as a food trend that emerged in the fourth industrial revolution of the food industry (Hassoun et al., 2022). The fact that the components obtained by precision fermentation is produced by traditional industrial methods such as agriculture, livestock farming, food search, bulk extraction or organic synthesis is often not environmentally sustainable.

Fermentation on an industrial scale of food-related products (such as glutamate and enzymes) using wild-type microorganisms is not very new. However, according to its widespread definition “precision fermentation' is practically synonymous with metabolic engineering, which often includes genetic manipulation of microorganisms for non-local products. Metabolic engineering encompasses in many methods and approaches, including, high-throughput and combinatorial library screening, bioinformatics, next-generation sequencing, synthetic biology, molecular cloning, enzyme engineering, comparative multi-omics analysis, machine learning and kinetic modelling.

Fungi play a leading role in traditional food fermentation, from bread, wine and beer production to cheese, sauce, meat and vegetables production. Unlike precision fermentation, a lot of commercial conventional food fermentations remain mostly by itself and undefined. In recent years, however, precision methods are increasingly being investigated and applied to traditional

fermentations, both to speed up the process, increase product yield and reduce process costs, and to improve food safety, quality, nutritional and flavour profiles. There are especially highly efficient scanning strategies, the multi-omics, and CRISPR-Cas9 genome editing tool among these methods.

If we compare all three fermentation methods, in traditional fermentation, microorganisms naturally present in the food or added as starter cultures synthesise compounds that can change the texture and taste properties of the food by using the sugar in the food under anaerobic conditions. Microbial culture, which exists naturally or is subsequently added as a starter culture, continues to exist in the final product. Usually high-value raw materials are used as raw materials. In the process of making yogurt, the use of milk as a raw material can be given as an example. It has seen very little or no processing in the final product that can take place in the group of unprocessed foods. Yogurt, bread, cheese and alcoholic beverages are produced using traditional fermentation technology.

In biomass fermentation, fast growing microorganisms are used to produce biomass with high protein content. In the production of microorganisms, unlike traditional fermentation, agricultural wastes are used instead of rich raw materials. In addition, the entire reproduced microorganism is consumed as a rich source of protein. As in traditional fermentation, the final product is among minimal or no-processed products. Using bacteria, fungi, algae, cosmetic products, many products related to bovine and ovine livestock that can be consumed as human food are produced on a commercial scale.

In the synthesis of specific functional food components in precision fermentation, microorganisms designed using recombinant DNA technologies and synthetic biology techniques are used. Unlike traditional fermentation, the microorganism used in the fermenting process is not present in the final product. As in biomass fermentation, high-value compounds are synthesized using cheap and waste raw materials. These compounds are not just proteins as in bio-mass fermentation. In addition to proteins, it also contains aroma components, vitamins, pigments, oils and antioxidants.

2.3.1. PROCESS STAGES IN PRECISION FERMENTATION

The first stage of precision fermentation is the selection of the target molecule. The molecule targeted for production by fermentation can be protein (animal-free milk protein, egg white protein), lipid (to provide a meat-like flavour profile to plant-based foods), pigment, aroma or vitamin. Microorganisms that are usually isolated from nature cannot be used to this goal. It is therefore necessary to design microorganisms capable of synthesizing the target molecule using techniques such as genomic modification and genomic regulation (CRISPR-Cas9). The selection of raw materials is very important for the large-scale production of designed microorganisms in fermenters and for the synthesis of products. Agricultural waste is usually preferred to be sustainable and cost low. Food waste is increasingly used as a fermentation medium for the production of antioxidants, natural preservatives, pigments, enzymes and other food components. Also, using an unused or less-used resource reduces food waste and also has the potential to create high-value products. Some examples of food waste fermentation include (1) carotenoids and flavonoids from vegetable or fruit waste, (2) bioactive peptides from whey from cheese producing and grain processing waste, (3) antioxidative compounds from livestock and seafood processing by-products (Martí-Quijal et al., 2021, (4) enzymes (glucoamylase) from various substrates such as tea waste, wheat bran, rice bran, and corn stalks; and (5) microbial red, orange and yellow pigments derived from various food industry wastes as media (e.g. whey, tomato waste, fruit pits, cottonseed meal, etc.) (Panesar et al., Kaur and Panesar 2015; Mehri et al., 2021).

The second stage of precision fermentation is the fermentation stage. Recent developments in Industry 4.0 technologies have made great progress in precision fermentation thanks to artificial intelligence, bioinformatics, systems, and computational biology (Teng et al, 2021; Hassoun et al, 2022). In precision fermentation, large-scale fermenters that can easily be controlled various parameters such as pH, temperature, oxygen and salt concentration to monitor and optimise the process, as well as more cost-effective food production methods that can provide quality standardisation by detecting potential anomalies during fermentation (e.g., mutation) and stable productivity have been developed (Tyndall et al., 2022).

The third phase of precision fermentation is called the “downstream phase”, this is the stage that requires different processes to obtain a pure product. After target molecule synthesis in the fermenter, the downstream process starts. This phase requires different processes depending on whether the target molecule is inside the cell or released outside the cell. For this purpose, it is subjected to a series of purification processes so that the final product is free of microorganisms or production media components used for production purposes. A drying process is usually carried out to turn the final product from liquid to solid powder. In the final phase, the final product is tested to determine whether the product contains the intended characteristics and to make sure that it does not contain unwanted particles.

2.3.1.1. PREPARATION OF MICROBIAL CELL FACTORIES

Microbial cells are important model cells in the production of recombinant proteins for reasons such as that they can easily be produced in fermenters, have a simple physiology, and many of them have all the genome sequences identified. Microorganisms that are generally recognised as safe (GRAS) or non-harmful are used for food applications. Two basic strategies are used to create a microbial production plant: the bottom-up approach (genome synthesis), and the top-down approach (genome reduction)

Identification of genes that are not necessary for the recombination of microbial cells to be used in industrial applications is vital. Calculated analyses are used to identify the basic genes that microorganisms need to sustain their lives. Basic genes are often involved in basic metabolism, cell wall metabolism and DNA metabolism. In many applications, phage residues, unknown genes and abundant non-essential genes involved in different metabolic pathways have been identified. The removal of these non-essential genes resulted in microbial cell factories that had smaller genomes but were able to reproduce faster and produce higher amounts of industrial products. Recently, due to advances in genomic fusion technologies and the presence of low-cost DNA synthesis methods, chemically synthesized genomes have also been built using a bottom-up approach and used as microbial cell factories. (Chi et al., 2019).

The first goal in the development of a recombinant protein is to obtain a nature-identical amino acid sequence of the target protein. Post-translation

changes (e.g., phosphorylation, glycosylation) should be considered as affecting both the structure and functional properties of a protein. Innovative synthetic biology approaches to the production of recombinant proteins for food components generally aim to: (1) increase protein expression per unit of biomass (i.e., increase quantity and yield), (2) increase protein secretion, (3) improve specific physicochemical properties such as pH or temperature resistance of the recombinant protein product (Cramer et al., 2018), 4) eliminate allergens in a non-catalytic way. (Bhatt et al., 2021).

Several nations have financed research projects on this topic. The Minimum Genome Factory (MGF) project, which aims to produce microbes with reduced genomes for industrial use, was started in Japan in 2001. *E. coli* MGF-01, which has a reduced genome size and displays superior growth and higher threonine synthesis than the parental strain, is one of the best instances of the project's outcomes (Mizoguchi et al, 2007). The gram-negative soil bacterium *P. putida*, which is widespread, satisfies many biological requirements for creating a synthetic cellular plant, such as metabolic variety, robustness, and ease of manipulation (Dos Santos et al., 2004). The whole chromosomal sequence of *P. putida* KT2440, the most well-studied saprophytic laboratory *Pseudomonas*, has been made available since 2002 (Nelson et al., 2002). Studies with this strain yielded mutant strains whose genome was deleted up to 7.4%.

Gram-positive microorganisms are a group of phylogenetically diverse cells, including lactic acid bacteria, and are now generally considered safe (GRAS) with their uses as probiotics or food fermentation strains. The genetics of these strains tend to be less variable, which makes changes at the molecular level more difficult. *Bacillus subtilis*, a non-pathogenic gram-positive bacterium, is known for its superior capacity in protein secretion. (Zweers vet al., 2008). In addition, the wide variety of natural two-component systems found in *Bacillus* species and interbacterial communication systems called "quorum-sensing" have made them the preferred cellular factories for the industrial production of various products including chemicals, proteins, and biopolymers. (Gu et al., 2018). *B. subtilis* strain MGB874 was obtained by extracting the sequence of 874 kb (20%) from the original *B. subtilis* 168 by stepwise genome deletions. MGB874 cells are used in industry due to remarkable improvements in extra-cellular protease (2,5 times), and cellulase

(1,7 times) productivity (Morimoto et al., 2008). Over the past twenty years, extensive research on *Lactococcus lactis*, recognized as GRAS, has revealed a collection of genetic tools and recombinant technologies. (Song et al., 2017). Today, *L. lactis* is an important bacteria of economic value used in food fermentation. (Cavanagh et al., 2015). Nisin, extensively used in the biotechnological production of important molecules such as aroma additives, and sweetening compounds is produced by *L. lactis* (Özel et al., 2018),

Fungi are eukaryotic, and saprophytic microorganisms with strong environmental adaptation, which makes them favourable microbial hosts for precise fermentation. The natural tendency of many species of fungi to synthesize high levels of commercially valuable food compounds (carotenoids, organic acids, fungal pigments, and polyketides) makes them suitable and efficient hosts for the production of these products on an industrial scale. From the point of view of metabolic engineering, one of the most important advantages of using fungi over bacteria is that they are tolerant to heterologous eukaryotic proteins and enzymes due to their eukaryotic structure and are able to synthesize them functionally, carry out correct protein folding and post-translation modifications. (Lyu et al., 2019).

Different recombinant strains of *Saccharomyces cerevisiae,* often used in traditional fermentation and in GRAS status, have been developed by many commercial companies. These recombinant strains are used in the production of a wide range of commercial products. Biotechnology company Evolva uses recombinant *S. cerevisiae* strains for the commercial production of vanillin (aroma), nootkatone and valence (aromatic compounds), L-arabinose (sugar substitutes) and resveratrol (functional components) while Cargill (USA) uses it for the production of steviol glycosides, a natural sugar substitute marketed as EverSweet™ (Nxumalo et al., 2020; Shi et al., 2022). Recombinant strains obtained from of *Yarrowia lipolytica*, *Komagateella phaffi*, *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei*, *Mucor circinelloides*, *Eremothecium gossypii*, *Penicillium oxalicum*, in addition to *S. cerevisia*, are used in precision fermentation processes to manufacture a product on a commercial scale (Chai et al., 2022).

Studies are carried out to speed up the process, increase product yield and reduce process costs while improving the nutritional, safety, quality and flavour profiles of food by making genetic modifications to the microorganisms

used in traditional fermentation. For example, two engineered *S. cerevisiae* strains have recently been granted FDA GRAS status for the production of two commercial craft beers with different flavour profiles, one of which was derived by gene transfer from *Rhizopus oryzae* for sour beer, while the other (yBBS002) synthesises recombinant basil and mint enzymes to impart hop aromas. There have also been some efforts to adapt methods of precision fermentation to solid-state fermented foods. The RGT2 and SNF3 genes of *S. cerevisiae*, isolated from Nuruk (a conventional Korean microbial fermentation initiator), have been degraded through CRISPR-Cas9 to ease glucose suppression during bread dough fermenting and to eventually obtain bread with improved leavening. (Lee et al., 2022). Another study showed that excessive expression of ASP3 in Nuruk strains or the removal of the URE2 or GZF3 gene decreased the content of acrylamide in potato chips, and baked bread, thereby significantly improving their safety profiles (Lee et l., 2022).

2.3.2 PRODUCTS OBTAINED BY PRECISION FERMENTATION

2.3.2.1 PROTEIN PRODUCTS PRODUCED BY PRECISION FERMENTATION

The use of precise fermentation in food is not new; it has been used in the manufacture of rennet since the 1980s. Rennet is a series of protease enzymes found endogenously in the stomachs of young ruminants and used as an enzyme for coagulating milk casein proteins in cheese production. In the 1970s, this time-consuming and costly process was used to meet the cheese demand. Instead of this difficult method, it is produced by fermentation using bacteria, yeast and mould. The rennet from fermentation has been praised for providing a pure and stable supply, free from contaminants including other proteins and enzymes found in calves' stomachs. In 1990, fermentation-derived rennet was the first genetically modified food product granted by the United States Food and Drug Administration (USFDA) (Gladwell, 1990).

The global dairy industry is undergoing a shift. With global milk production estimated at 843 million tons in 2018 alone (FAO 2019), dairy products continue to be a significant agricultural product. However, when the environmental impacts of dairy production are examined, it is stated that global livestock production produces more greenhouse gases than the transport sector.

(Rojas-Downing et al., 2017). Although ethical concerns about dairy production tend to be less than other sectors of livestock farming, Croney and Anthony (2011) state that there has been a general rise in ethics concerns that have led to increased interest in plant-based diets.

Focus has been placed on eukaryotic expression hosts that can produce animal proteins that are nature-equivalent through the proper post-translational modifications as a result of recent discoveries that favour the manufacture of animal proteins utilizing animal-free systems. Single-celled Crabtree-negative yeast such as *Komagataella phaffii* (*Pichia pastoris*) provide an ideal expression system and are widely used (Juturu and Wu, 2018).

Milk contains two main classes of proteins, and caseins (α- and β-caseins) whey (primarily β-lactoglobulin and α-lactalbumin). Due to their widespread use in the food industry, the expression of recombinant milk proteins is of great interest and can replace milk proteins with natural origin (especially whey-derived proteins) in many applications. Whey constituents (β-lactoglobulin [Invernizzi et al., 2004], α-lactalbumin (Vanhooren et al., 2005), lactoferrin (Iglesias-Figueroa et al., 2016]) and lysozyme (Brierley et al., 1990) using *K. phaffii* and ovine β-lactoglobulin using both *S. cerevisiae* and *K. lactis* (Rocha et al., 1996) have been expressed. Using a batch-fed fermenter and the methanol-inducible alcohol oxidase 1 promoter (PAOX1), bovine lactoferrin up to 3.5 g/L was generated (Iglesia, 2016).

Beef β-casein has been expressed using PAOX1 in *K. phaffii* (Choi and Jiménez-Flores, 2001). *Aspergillus oryzae* and *Aspergillus awamori* have both been expressed additional human milk proteins having protective properties (e.g., lactoferrin) (Ward et al., 1992; Ward et al., 1995). Both *K. phaffii* and *S. cerevisiae* have been used to express the human caseinomacropeptide, a κ-casein fragment (residues 106-169) (Kim et al., 2005). Methods of production of non-animal milk oils and proteins that can be used in the production of foods such as cheese, butter, cream have been identified and patented (Pandya et al., 2016).

Private companies have also allocated funds to obtain animal-free milk proteins. Perfect Day (Berkeley, CA; formerly known as Mufri) began its work in 2014. The company, whose mission is to make cows, people and the planet happier, has developed the first products to be used in ice-cream with regulatory approval for a new non-animal milk protein in early 2019

(https://perfectday.com/our-story/, 2023). According to Perfect Day, the final product will have a longer shelf life than normal milk and will be safer along with the advantage of being free of hormones, antibiotics and lactose (Perfect Day, 2019). Expression products manufactured by Perfect Day include β-lactoglobulin, α-lactalbumin, κ-casein, β-casein and possibly others. In particular, β-lactoglobulin is already used by Perfect Day in product analogues such as ice cream, cream cheese and protein powder supplements.

The market for food protein components is dominated by milk and egg proteins. Both whey and egg proteins are difficult to replace with plant proteins due to their unique structural properties. Biotechnology offers attractive alternatives using food industry microorganisms instead of relying on livestock farming in the production of the most critical animal protein components. The ingredients of animal-free eggs are also produced by precision fermentation (Mahadevan et al., 2021).

Ovalbumin (OVA) is a storage protein, which is the predominant protein in the egg white, and constitutes 54% of the total protein of the egg white. (Huntington and Stein, 2001). Therefore, in order to provide a non-animal source of these important proteins, fungal host *Trichoderma reesei* has been used for the biotechnological production of recombinant chicken ovalbumin (TrOVA) (Aro et al., 2023). Whether these food proteins have been efficiently expressed in a fungal host was investigated using two different promoter systems. TrOVA protein has been successfully produced in bioreactor scale at a quantity level of 2 g/L. Recombinant TrOVA protein demonstrated excellent foaming properties and heat-induced gelation in experiments (Aro et al., 2023).

Among the egg white proteins, as ovalbumin ranks first with 54%, ovotransferrin ranks second with 11%. Ovotransferrin was also expressed using *K. phaffii* and a yield of ∼100 mg/L was obtained using methanol induction through the AOX1 promoter. (Mizutani vet al., 2004). Recombinant ovotransferrin was able to bind iron functionally and had circular dichroism and fluorescence spectra structurally similar to native ovotransferrin (Mizutani et al, 2004).

Chicken egg lysozyme (HEL) is one of the sweet proteins and makes up 3.5 percent of the total protein. Using *K. phaffii*, approximately 400 mg/L lysozyme was obtained through AOX1 promoter. (Masuda et al., 2005). In particular, GRAS approval has been obtained for the use of *K. Phaffii* in egg

white protein synthesis to be used in food products (FDA, 2021). The recombinant ovomucoid can be found in an alcoholic beverage marketed by the company "Pulp Culture" and has also been successfully used in a macaron application (Watson, 2022). In addition, The company Onego Bio (Espoo, Finland) continues its work on the expression of recombinant ovalbumin using *Trichoderma reesei*, a fungus with filament.

It has been reported that AllG and Mayamilk companies produce milk proteins, Better Dairy company produces dairy proteins and The Every Company produces animal-free egg proteins by means of precision fermentation (Augustin et al, 2023). Algama company has developed an egg-free mayonnaise using microalgae (GFI, 2020).

Western diets including meat have a greater negative impact on the environment compared to more sustainable plant-based diets. However, many consumers are reluctant to reduce the amount of meat they eat. Therefore, those who prefer plant-based diets remain limited to small populations. A growing number of consumers, known as flexitarian, avoid livestock meat as part of their diets. These meat alternatives for flexitarians are welcome and play a role in consumers' choice of protein alternatives.

A potential way to accelerate the widespread transition to plant-based diets is to produce meat that satisfies the tastes of meat consumers directly from plants. In the studies conducted, it has been determined that the heme molecule in the meat is a catalyst in the creation of the taste and aroma of the meat. In plant-based meats, leghemoglobin protein (LegH) (a close structural orthodontist of myoglobin) derived from soybeans plays a very important and parallel role: After cooking, it unfolds and releases the heme cofactor to catalyse reactions that can turn the same common biomolecules obtained from plant sources into the array of compounds that give meat's unique flavour and aroma. Although the amino acid sequence of LegH is quite different from that of animal hemoglobin and myoglobin, its 3-dimensional structure is quite similar (Gupta et al., 2011).

Soy (Glycine max) leghemoglobin protein (LegH) expressed in *Pichia pastoris* (*K. phaffi*) (LegH Prep) gives plant-based food products a meat-like flavour profile. The safety of LegH Prep has been assessed by a series of *in vivo* and *in vitro* tests. Using the *in vitro* chromosome aberration test and the bacterial reverse mutation assay (Ames test), the genotoxic potential of LegH

Prep was evaluated. LegH Prep was determined not to be mutagenic and clastogenic in each test, respectively. Systemic toxicity was evaluated in a 28-day diet study in male and female Sprague Dawley rats. No deaths associated with LegH Prep have been. There were no clinical findings, body weight changes, ophthalmological, clinical pathological, or histological modifications related to the use of LegH Prep (Fraser et al., 2018).

In 2016, Impossible Foods company launched its first meat-like product, Impossible Burger, using leghemoglobin protein derived from plants (Reyes et al., 2021). The company states that 95 per cent less land and 74 per cent less water is used in the production of this burger, and approximately 87 per cent less greenhouse gases are emitted compared to the ground beef burger patty from cows. A plant-based burger contains more protein, less total fat, less cholesterol, and less food energy than a same size hamburger patty made from beef. In comparison to a plain beef patty, it has higher sodium and saturated fat. In May 2018 and December 2018, respectively, Impossible Burger acquired their certificates for being Kosher and Halal.

Meat is not only consumed by humans and is also consumed as pet food. Pet food is a huge industry with great opportunities for impact. Pet food is not only a $30 billion market, but it is also responsible for about 25-30% of the environmental impact of meat eating in the United States. If cats and dogs in the United States were a country, they would be the fifth largest country in the world in meat consumption. Founded in 2017 by IndieBio co-founder Ryan Bethencourt, Wild Earth introduce into the market its high-protein dog feed in 2019 after launching the Aspergillus oryzae-based dog feed prototype in 2018. Bond Pet Food has adopted a different approach by using microorganisms to produce specific animal muscle proteins. (GFI, 2020). Combining precision fermentation with genetic recombination, the company has managed to produce chicken, beef, fish and other meat proteins that are the same as those derived from animals. After the genetic code obtained from animals transferred into a food-class yeast, these yeasts were produced the same meat proteins as those obtained from animals by fermentation in large tanks (https://www.newprotein.net/news/bond-pet-foods-fermented-protein-cats-dogs).

Among other precise fermentation derivatives that can be used as alternative proteins in non-animal meat analogues are recombinant collagen

and muscle protein. (Lu et al., 2021). Traditionally, gelatin is made from animal connective tissue as a by-product of the grating of meat. Gelatin is a partially broken collagen protein. Because of its gelating properties, gelatin is employed in numerous food applications as well as cosmetics. Precision fermentation is used by Geltor, a pioneer in the production of animal-free gelatin, to create its collagen product series. Gelatin is a partially damaged collagen protein and is traditionally obtained from animal connective tissue as a by-product of the meat grating process. Gelatin is used in many food applications and in cosmetics due to its gelating properties. Geltor, a leader in the field of animal-free gelatin, uses precision fermentation to produce its collagen product series.

Plant-based seafood is one of the least developed fields of alternative proteins. The solution to unlocking this area may lie in fermentation, particularly with microalgae. "Quorn's fishless fingers" is the only fermented seafood commonly found (GFI, 2020). A fermentation company, Aqua Cultured Foods, situated in Chicago has unveiled the first prototype of mycoprotein calamari (GFI, 2022).

It is present in proteins that leave a sweet feeling in the tongue. Low-calorie sweeteners hold an important place in the food industry. Brazzein isotopes have been detected to be 400 to 1500 times sweeter than sucrose by weight. It has been reported that *K. Phaffii, K. Lactis* and *A. Oryzae* were used to produce such proteins by precise fermentation and successful results were obtained (Neiers et al, 2021).

2.3.2.2 OILS PRODUCED BY PRECISION FERMENTATION

Although the main objective is to replace animal proteins, microbial fermentation technologies are also utilised to produce solid and liquid oils. These can be included in plant-based products to help imitate the sensory experiences of traditional animal foods' taste, tissue, and oral sensation. A wide range of synthetic biology approaches have been applied to enhance fatty acid and lipid production in fermentation, including increasing substrate supply for acyl-CoA-dependent systems, decoupling fatty acid production from growth, increasing carbon flux to the pentose phosphate pathway, and manipulating the expression of natural and recombinant lipid-processing acyl transferases and thiol esterase enzymes to match the desired lipid product.

Nourish Ingredients (Australia) has developed a prototype of a fermentation process that produces lipids with structures that mimic animal fats, using microorganisms with modified lipid synthesis pathways, without using palm or coconut oil. Motif FoodWorks and Perfect Day have also announced new attempts to create oil solutions for plant-based meat applications and dairy products.

Among patented processes are the production of and docosahexaenoic acid (DHA) eicosapentaenoic acid (EPA) from seaweeds (*Crypthecodinium cohnii*), thraustochytrids and yeast. The interest in long-chain polyunsaturated fatty acids (DHA and EPA) has grown due to their health advantages (Augustin et al., 2023).

Many different types of fish oils contain omega-3 fatty acids, which have traditionally been utilized as dietary supplements. These important fatty acids were traditionally obtained by grinding up fish, but they are also abundant in some microalgae species that may be generated by fermentation. Now, researchers and companies are using microalgae as highly scalable bio-factories for omega-3 fatty acids and other beneficial ingredients for the alternative protein industry. For instance, the microalgae Odontella is utilized to make a variety of healthful seafood products made from algae, including tuna, salmon, caviar, scallops, and shrimp.

2.3.2.3 SECONDARY METABOLITE PRODUCTION BY PRECISION FERMENTATION

Plant flavonoids are in more demand than ever, and conventional labour-intensive and too expensive extraction techniques are no longer able to meet this demand. The biotechnological generation of several plant secondary metabolites through microorganisms has so begun to gain important in recent years. Biotechnology's microbiological advancements have accelerated the manufacture of high-quality goods. So far, the most common microorganisms used as cellular factories of flavonoids are *E. coli, S. cerevisiae* and in some cases other species (e.g., *S. venezuelae*). Detailed analyses have been published in this field. The development of bacterial flavonoid engineering began with the engineering of *E. coli* in 2003. Recently, different types of flavonoids such as flavanones isoflavones flavones, and flavonols, have been successfully produced by recombinant *S. cerevisiae* or *E. coli* (Mohammad et al, 2020).

Significant amounts of vitamin B12 have also been produced from wheat bran using co-fermentation of *Propionibacterium freudenreichii* and *Lactobacillus brevis* . This vitamin can be used to enrich plant-based meat products to compensate for deficiencies that may occur in vegans who eat plant-based sources.

2.3.2.4 CARBONHYDRATE PRODUCTION BY PRECISION FERMENTATION

Human milk oligosaccharides have been produced using genetically modified *E. coli* strains. Produced by microbial fermentation and found in human milk, two oligosaccharides, lacto-N-neotetraose (LNnT and 2'-fucosylactose (2-FL)), have been approved by FSANZ (Food Standards Australia New Zealand) for adding baby formula (Bych et al, 2019).

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CHAPTER 4

USE OF ORGANIC ACIDS AS ALTERNATIVE FEED ADDITIVE TO ANTIBIOTICS IN LAYING HENS AND BROILER CHICKENS

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INTRODUCTION

Protein needs have increased as parallel to population growth. In order to close the protein deficit, animal production must be increased accordingly. This situation has made the poultry sector important. Poultry breeding has many advantages such as easier production than livestocks, shorter production time, relatively cheaper protein source.

Feed additives are used to improve performance and product quality, and protect the health of animals (Pandey et al., 2019). One of these additives is organic acids (Ebeid and Homidan, 2022). With the prohibition of the use of antibiotics as growth promoters, organic acids have also investigated as an alternative feed additive to antibiotics (Haq et al., 2017; Dittoe et al., 2018).

The effect of organic acids and their salts in animal nutrition is given in Figure 1 (Lim et al., 2015).

	Effective form	Effects
Feed	H^+	- pH reduction
	H+ and Anion	- Reduction of acid-binding capacity
		- Reduction of microbial growth
		- Antibacterial effects
Intestinal tract	H^+	- pH reduction in stomach and duodenum
	Anion	- Improved pepsin activity
	H^+ and Anion	- Complexing agents for cations (Ca^{++}, Mg^{++}, Fe^{++}, Cu^{++}, Zn^{++})
		- Antibacterial effects
		- Modulation of microbial population
Metabolism		- Energy supply

Figure 1. Effect of organic acids and their salts in animal nutrition (Lim et al., 2015).

The organic acids commonly used in poultry nutrition are summarized in Figure 2 (Hajati, 2018).

Acid	Chemical name	Formula
Formic	Formic acid	HCOOH
Acetic	Acetic acid	CH_3COOH
Propionic	2-Propanoic acid	CH_3CH_2COOH
Butyric	Butanoic acid	$CH_3CH_2CH_2COOH$
Lactic	2-Hydroxypropanoic acid	$CH_3CH(OH)COOH$
Sorbic	2,4-Hexandienoic acid	$CH_3CH:CHCH:CHCOOH$
Fumaric	2-Butenedioic acid	$COOHCH:CHCOOH$
Malic	Hydroxybutanedioic acid	$COOHCH_2CH(OH)COOH$
Tartaric	2,3-Dihydroxy- Butanedioic acid	$COOHCH(OH)CH(OH)COOH$
Citric	2-Hydroxy-1,2,3- Propanetricarboxylic acid	$COOHCH_2C(OH)(COOH)CH_2COOH$
Benzoic	Benzenecarboxylic acid	C_6H_5COOH

Figure 2. The organic acids commonly used in poultry nutrition (Hajati, 2018)

There are studies on the use of organic acids in laying hens (Rahman et al., 2008; Dahiya et al., 2016; Rezaeipour et al., 2022; Miranda et al., 2023), broiler chickens (Kamal and Ragaa, 2014; Ghazvinian et al., 2018; Manvatkar et al., 2022; Sadeghian et al., 2023) and quails (Khan et al., 2016; Üstündağ and Özdoğan, 2019; Aliverdi-Nasab et al., 2023).

This chapter aims to provide information about organic acids, their importance and potential uses in laying hen and broiler chicken.

USE OF ORGANIC ACIDS IN LAYING HENS AND BROILERS

There are many studies investigating the effects of various organic acids on performance, product (egg and meat) quality, immune and serum biochemical parameters, intestinal histomorphologic properties and microbial population in layers and broilers. Some of these studies are summarized in Table 1.

Table 1. Some studies on the use of organic acids in laying hens and broiler chickens

Organic Acids	Poultry	Dose	Treatment Effects	References
Organic acid mixture (propionic and formic acids, and their ammonium salts)	Laying hen	0, 0.5, 1 and 1.5%	- No effects on some performance parameters (body weight, feed cossumption) and some egg quality traits (egg weight, Haugh unit, yolk index, albumen index, shell thickness and shell breaking strength) - No effects on some blood serum parameters [alanine aminotransferase (ALT) enzyme activity, cholesterol, triglyceride, VLDL (very-low density lipoprotein) and HDL (high-density lipoprotein) concentreations] - Increased some blood serum parametres [aspartate aminotransferase (AST) activity, albumin and total protein concentrations]	Yeşilbağ and Çolpan (2006)
Organic acid mixture			- No effects on feed intake and body weight change	

(fumaric acid, salt of propionic, butyric and lactic acids	Laying hen	0, 250, 520 and 780 ppm	- Improved some performance parameters (egg production and feed conversion) - No effects on total fat and some organ weight (liver, heart, gizzard, spleen, oviduct) - Increased egg shell thickness (520 and 780 ppm organic acid-supplementated groups)	Rahman et al. (2008)
Organic acid mixture (propionic, formic and soft acids)	Laying hen	0, 1.5, 3 and 4.5 g/kg	- No effects on body weight, egg weight, egg production, feed consumption and FCR (feed conversion ratio) - Increased yolk index - No effects on other egg internal and external quality parameters - Differences on small intestine in some parameters [Increased villus height (quadratic effect), villus weight (quadratic effect),	Kaya et al. (2015)

			and tunica mucosal width (linear effect)] No effects on blood serum parameters such as albumin, cholesterol, HDL concentrations and AST, ALT activity	
Organic acid salts (sodium-butyrate, calcium propionate)	Laying hen	0, 0.5, 1 and 1.5%	- No effects on body weight gain and feed intake - Improved production performance (egg mass production and percent hen-day egg production) - Improved FCR - Increased egg weight (0.5% level of salts of organic acids)	Dahiya et al. (2016)
Organic acids (propionic, formic and malic acids)	Laying hen	0 and 200 mg/kg	- No effects on performance - No effects on egg quality traits (except egg shell thickness) - No effects on some blood serum parameters [cholesterol, glucose levels and ALP (alkaline phosphatase), ALT and AST activity]	Sarı and Kaya (2017)

			- Increased serum Ca and P levels (propionic acid supplementation)	
Benzoic acid	Laying hen	0, 1000 and 2000 mg/kg	- No effects on FCR, feed intake, laying rate and breaking rate - Decreased egg weight (2000 mg/kg benzoic acid-supplemented group) - Increased Haugh unit and albumen height - Increased duodenum crypt depth and villus height (2000 mg/kg benzoic acid-supplemented group) - Increased jejunum crypt depth (1000 mg/kg benzoic acid- supplemented group) - Increased ileum crypt depth and villus height	Gong et al. (2021)
Organic acids	Laying hen		- Increased feed intake - No effects on egg weight, egg production, FCR and egg mass (47 to 58 weeks of age)	Rezaeipour et al. (2022)

[acetic (AA), butyric (BA), and propionic acids (PA)]		0, 0.1 (PA), 0.2 (BA) and 0.3% (AA)	- No effects on albumen index, egg albumen percentage, and Haugh unit - No effects on fecal microbiota (lactic acid bacteria and coliform populations)	
Organic acids (benzoic, citric, formic and phosphoric acids) blend + Tributyrin + Organic minerals	Laying hen	-	- No effects on productive performance and internal and external quality of the egg - No effects on kidney and liver weights	Miranda et al. (2023)
Organic acid salts (ammonium formate and calcium propionate)	Broiler chicken	0 and 3 g/kg	- Increased live weight and live weight gain (0-21 days) - No effects on live weight and live weight gain in day 42. - Reduced coliform count - No effects on clostridium count (gut) - Decreased *Escherichia coli* count in gut (ammonium	Paul et al. (2007)

			formate-supplemented group) - Increased villus height - No effects on pH levels of different gastrointestinal segments	
Organic acids (citric, benzoic and tartaric acids)	Broiler chicken	0, 0.5 and 1%	- No effects on performance parameters (feed intake, body weight gain and FCR) in 0-7 weeks - No effects carcass characteristics	Talebi et al. (2010)
Organic acids (butyric, fumaric and lactic acids)	Broiler chicken	0, 2 and 3%	- Improved FCR and body weight gain - No effect on feed consumption - Increased serum calcium and phosphorus concentrations - No effects on serum, serum glutamic oxaloacetate transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), glucose and cholesterol levels	Adil et al. (2011)

			- No effects on some carcass characteristics (gizzard, liver and heart weight) - Increased jejunal villus height	
Organic acid blend (commercial product) (Note: Experiment on elayed feed access after hatch of broiler)	Broiler chicken	0, 0.1, 0.2 and 0.3% (0.3% in starter diet, 0.2% in grower diet and 0.1% fnisher diet)	- Increased villus length in jejunum - Decreased Enterobacteriaceae count in ileum (Day 25) - Decreased pectoral muscle malondialdehyde level	Cengiz et al. (2012)
Organic acids (acetic acid and citric acid)	Broiler chicken	0 and 0.25% (drinking water)	- No effects on body weight - Decreased feed consumption (citric acid) - Positive effect on total mortality of broiler chickens	Kopecký et al. (2012)
			- Improved body weight gains and FCR - No effects on cumulative feed consumption	

Organic acids (butyric, fumaric and lactic acids)	Broiler chicken	0 and 3%	- Higher serum globulin levels - Decreased serum cholesterol, total lipid and low density lipoprotein (LDL) concentrations - Increased serum calcium, phosphorus and magnesium concentrations - Decreased ALP concentration	Kamal and Ragaa (2014)
Organic acid blend (commercial product) (citric, lactic acid, phosphoric acids)	Broiler chicken	0, 0.5, 1, 1.5 and 2 ml/L drinking water	- Increased body weight, FCR, carcass yield and liver weight - Decreased *E. coli* and *Salmonella* in ileum	Sultan et al. (2015)
Organic acid blend (lactic, formic and propionic acids)	Broiler chicken	0, 0.05, 0.10 and 0.15%	- No effects on feed intake (during the starter period) - Increased feed intake (end of the grower period and throughout the rearing period)	Ghazvinian et al. (2018)

			- Improved microbial population of gut (Reduced *Escherichia coli* count and increased *Lactobacillus* count)	
Organic acid blend (coated) (fumaric, dl-malic and citric acids)	Broiler chicken	0, 0.3, 0.6 and 1 g/kg	- Increased body weight gain, improved FCR and lower mortality (1 g/kg coated organic acids blend-supplemented group) - Better some carcass traits (breast yield and relative weight of giblets (1 g/kg coated organic acids blend) - Reduced reduction in abdominal fat (1 g/kg coated organic acids blend) - Increased villi height (0.6 and 1 g/kg coated blend) - Higher humoral immune responses - Higher lymphoid organ (bursa and thymus) weight	Manvatkar et al. (2022)
Organic acids (formic acid, acetic acid and mix)	Broiler chicken	0, 0.25 and 0.5%	- Improved FCR in total experimental period - Increased total Lactobacilli count in ileum digesta	Pourreza et al. (2023)

		(mix: 0.75%)	- Reduced *Escherichia coli* count in ileum digesta	
Organic acid blend (commercial product)	Broiler chicken	Encapculated and Non-encapsulated organic acids	- Decreased total number of coliforms in the ileum and duodenum Increased lactobacilli count in the ileum (encapsulated organic acids-supplemented group) - Increased bursa and spleen weights	Sadeghian et al. (2023)

CONCLISION

There are many studies on the use of organic acids in laying hens and broiler chickens. It is seen that organic acids have positive effects on many important parameters parameters in the some studies summarized in this chapter. It is thought that there is a need for more comprehensive dose studies on these.

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CHAPTER 5

SOME PHYSICOCHEMICAL PROPERTIES AND FATTY ACID COMPOSITION OF THE MUSCLE, SKIN AND DEPOSIT TISSUES OF GEESE RAISED IN LOW WINTER TEMPERATURES

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Practical Application

Goose is a bird that can be raised in almost every region of Türkiye, but some provinces such as Kars, Ardahan and Erzurum are famous for breeding goose. However, goose raising in Altıntaş (Kütahya, Türkiye) is important due to climatic and geographic conditions. Goose meat is particularly consumed in winter season. This study revealed fatty acid composition of different tissues of geese raised in winter conditions.

1 Introduction

Meat and meat products are among the most important foods of animal origin that need to be consumed by people of all ages to preserve their physical and psychological health (Schlumpberger, 2004). With its nutritional value and its peculiar taste and smell, meat is one of the food products indispensable to humans. Beside amino acids and essential fatty acids, meat with its vitamin and mineral content is a food that can meet one's need for almost all food products even in a small amount. Also, people prefer poultry products due to the negative health effects of red meat and meat products. The winged animals in the white meat group is increasingly consumed on the grounds that it has a low cholesterol level and higher levels of unsaturated fatty acid (Hui, 2001; Richardson &Mead 1999). Chicken meat is also known as a food product rich in such vitamins as B_2, B_6 and B_{12} that feed or support the nervous system in addition to its having higher unsaturated fatty acid and lower cholesterol levels than red meat. As its muscle fibers are relatively shorter, chicken meat is a source of protein recommended to those who have problems with their digestion (Richardson&Mead 1999; Sams, 2001). Especially in Asia and Europe, consumption of goose meat is increasing rapidly (Muğlalı et al., 2002). It is a particular advantage that these animals can be raised in cold climates. The increasing prices of feed stuff and drugs have made chicken meat more expensive. In addition, factors such as chickens' low resistance to diseases and vulnerability to stress factors as well as their inability to consume roughage have contributed to the raising and consumption of goose meat. Geese do not need a special shelter like a coop, and they are not affected by cold weather conditions. They can increase performance and consume roughage in the best way. Geese are also valuable due to the high prices attached to their livers and feathers abroad (Maraşlı et al., 2000).

Goose is a bird that can be raised in almost every region of Türkiye, especially in cold provinces like Kars and Erzurum. It is raised without using any special technology or shelter and makes an important contribution to the needs of families (Anonymous, 2009a). It is extensively raised in regions where the climate is colder and there are many fresh river sources and wide grass fields (Kırmızıbayrak, 2002). Goose breeding in Altıntaş is positively affected by the following: The region has favorable climatic and geographic conditions; it costs little to raise geese; there are local storing techniques for goose meat such as salting and drying, which are also accompanied by a long winter season that makes it easier to store it; and finally the local people are largely accustomed to consuming goose meat. In addition, goose meat is a traditional food source that meets the protein intake needs of local people. It should, however, be added that local people engage in goose-raising not only for goose meat but also for the grease and feathers of the goose (Anonymous, 2009b).

This study has aimed at determining the fatty acid composition and some physicochemical properties such as fat content, pH, color, ash and dry matter in various tissues of domestic male and female geese raised in Altıntaş in the Kütahya region of Türkiye.

2 Materials and Methods

2.1 Materials

The geese included in the present study were obtained from Altıntaş district in Kütahya province in January. The male and female geese weighed about 3 kg and were slaughtered by hand in accordance with hygienic conditions and under the supervision of a vet. After the feathers of the geese were removed using the wet plucking technique and the entrails were separated, each goose was given a number to avoid any contamination and immediately put in a fridge in proper pouches and transferred to the lab under refrigerator conditions. The geese were stored at 4 ºC overnight, and then underwent a standard process of chopping with the help of an expert butcher. Thus, the pectoral muscle meat, skin and deposit lipids (abdominal fatty parts) were set apart to be used in the analysis. Three geese were used for each group. The geese had been raised with natural feed in their natural setting in Altıntaş. The pectoral muscle meat, skin and deposit lipids were removed from the geese and preserved in a pouch. The air in the pouches was vacuumed as much as possible.

2.2 Dry Matter Analysis

The % dry matter content of the samples was determined by using the drying cupboard method and the analysis was carried out at 125ºC (Gökalp et al., 1995).

2.3 Ash Analysis

After putting the samples into crucibles, they were dried in the oven at 105 ºC for 12 hours and the dried samples were then put in an ash furnace (Protherm PLP 120/5, Türkiye) and were burned at 525ºC for 18 hours. When the samples reached constant weight, their ash amounts were determined (Gökalp et al., 1995).

2.4 Total Fat Analysis

The total fat contents of the previously-dried samples were determined by using the Soxhlet extraction method (Gökalp et al., 1995).

2.5 pH

For determination of the pH values of the samples, 10g of homogenized sample was weighed and then 100 ml distilled water was incorporated into it. The pH value of the sample, which was homogenized for 1 minute by an Ultraturrax (IKA T18 Basic, Staufen, Germany) was recorded with a pH meter calibrated with suitable buffers (Gökalp et al., 1995).

2.6 Color

An automatic colorimeter (Lovibond RT Series Reflectance Tintometer, U.K.) was used to determine the color properties of the samples and the device was calibrated before each measurement. The samples for the color measurement were firstly homogenized in a blender and then placed in the capsule of the device, and thus the values of L^*, a^* and b^* were determined. L^* value indicates the brightness-darkness from black (0) to white (100); a^* shows the color dimension of green-red, and b^* shows the color dimension of yellow-blue.

2.7 Determination of Fatty Acid Compositions

The fatty acid composition of the goose meat was determined with gas chromatography. The fat samples obtained after the extraction, which was conducted with ether in the Soxhlet extraction system were taken as 100 mg and then 3 mL of hexane and 100 μL of 2N KOH in methanol were added. The mixture was homogenized with vortex for one minute. Then the sample was centrifuged at 5000 rpm for 5 minutes. After that, 1 ml of supernatant of mixture prepared was added to the vials and injected into the gas chromatography (Yalcin et al., 2011). In this study, Agilent 6890 model GC was used and detection in the analyses was performed with FID detector. An HP-88 column 100 m x 0.25 mm with an ID, of 0.2 μm, inlet temperature of 250°C, split rate of 1/50 and 2 mL/min fixed flow rate were used. The carrying gas was helium.

2.8 Statistical Analysis

Physicochemical analysis of the goose meat within the scope of the project was conducted in three replications. In the study, the muscle meat, skins and deposit lipids of the male and female geese were taken as the basis. SAS statistics software (version 6.03) was used for the evaluation of the results (SAS, 1988).

3 RESULTS

3.1 Physicochemical Properties

In this study, the pH, ash, dry matter and total fat analyses of the muscular tissue, skin and deposit lipids of the female and male goose meat were determined and the results were evaluated statistically. The findings for these parameters are shown in Table 1. The pH values of the muscular tissues, skin and deposit lipids in the samples studied were determined as 5.64, 5.94 and 5.87 in female geese, respectively, and 5.68, 5.96 and 5.85 in male geese, respectively. When the pH values of the samples were examined, no significant difference between the tissues was observed ($p > 0.05$). According to the statistical analyses, the effect of sex on the pH values of goose meat was found to be insignificant ($p > 0.05$), while the effect of tissue difference on the pH values of the goose samples for the same species was found as significant ($p < 0.05$).

The ash content of the female goose meat varied between 0.13% and 1.24%, while that of the male goose meat ranged from 0.12% to 1.18%. As seen in Table 1, the ash amount of the muscular tissue in both samples was higher than that of the skin and deposit lipids. The statistical analysis showed that the effect of sex and different tissues on the ash content of the goose meat was significant ($p<0.05$).

The dry matter contents of the different tissues of female and male goose meat were found to be between 27.4 and 92.61% and 27.61 and 95.46%, respectively. The dry matter contents of the skin and deposit lipids were found to be higher because these tissues had more fat content than that of muscular tissue. The statistical analysis showed that sex and different tissues have a significant effect ($p<0.05$) on the dry matter contents of goose meat.

According to the findings of the present study, the average fat content of female goose meat was 2.55% in the muscles, 73.3% in the skin and 94.3% in the deposit lipids. In male goose meat, however, the average fat content was found as 1.98%, 72.79% and 97.27%, in the muscles, skin and deposit lipids, respectively. When the study results were examined, it was seen that the skin and deposit lipids contained more fat compared to the muscular tissue, as expected. When the fat contents of the muscular tissues of the female and male goose meat were compared, the female samples contained more fat than the male ones. As a result of the statistical analysis, it was found that the effect of sex on the fat contents of the goose samples was insignificant ($p>0.05$), while the effect of tissue difference on the amount of fat contents in the same goose samples was significant ($p<0.05$).

3.2 Color

The color values of the male and female goose meat analyzed in the study are given in Table 1. When the results of the color parameters of the tissue samples of the female and male geese were considered, it was determined that the L^*, a^* and b^* color values of the muscular tissue, skin and deposit lipids were similar to each other.

However, when the color features of muscular tissue, skin and deposit lipids were examined for each species, it was seen that the L^* values that represent darkness-brightness quality yielded the highest value on the skin part of the goose samples, followed by the deposit lipids and muscle meat,

respectively. The *a** color values that indicate the density of color red were found to be higher in the muscular tissue than in skin and deposit lipids in both samples. The *b** values that indicate the density of color yellow were found to be the highest in deposit lipids. When the results were examined statistically, the effect of sex on the *L**, *a** and *b** color features was found to be insignificant ($p>0.05$) and the tissue difference of the color features of the same species of goose samples was found to be significant ($p<0.05$).

3.3 Fatty acid composition of the goose tissue samples

The fatty acid composition of the goose meat was determined with gas chromatography in the study, and the amounts of total saturated and unsaturated fatty acid levels of muscle tissue, skin and deposit lipids of female and male goose meat are given in Figure 1. The amount of total saturated fatty acids was found to be between 31.42% and 33.76% in female goose meat and between 29.17% and 33.07% in male goose meat. The saturated fatty acid content of female and male goose meat was found to be higher in muscular tissues than in skin and deposit lipids. The amount of total unsaturated fatty acids was found to be between 66.24% and 68.58% in female goose meat and between 66.93% and 70.83% in male goose meat. The saturated fatty acid content of female and male goose meat was found to be higher in the skin tissues than in the muscle and deposit tissues. A total of 15 different fatty acids were determined in the goose tissue samples in the study. The total saturated and unsaturated fatty acid compositions of the muscle tissues, skin and deposit lipids of female and male goose meat are given in Table 2.

It was determined that the most common fatty acid in the samples was palmitic acid (C16:0), one of the saturated fatty acids, and oleic acid (C18.1), one of the unsaturated fatty acids. The amount of palmitic acid was found to be 24.08, 23.90 and 23.90% in the muscle tissue, skin and deposit lipids of the female geese, respectively; the highest level was found in the deposit lipids of the female geese. On the other hand, the oleic acid contents of the same geese were found to be 41.89, 49.75 and 49.75% in the muscles, skin and depot lipids, respectively. The palmitic acid amount of the male goose samples in the muscle tissue, skin and deposit lipids varied at 24.80, 22.76 and 23.72%, while oleic acid content was found to be 44.34, 54.30 and 53.52%, respectively. Oleic acid (C18:1), a monounsaturated fatty acid, was found in the skin fat of the male

geese at the highest level. The amounts of linoleic acid (C18:2) and linolenic acid (C18:3), which are essential fatty acids, were found in the muscle tissue of the female goose meat at the highest level. The findings of the study revealed that the effect of sex and different tissues on the fatty acid compositions of the male and female goose meat was statistically significant ($p<0.05$).

4 DISCUSSION

In general, the body features of poultry are affected by some factors such as their age, sex and species, the amount and quality of food consumed, and the environments in which they live. In recent years, consumers' demand for the winged animals fed with organic food has increased rapidly (Fanatico et al., 2006; Kırmızıbayrak, 2002, Ponte et al., 2008).

In a study in which white and mottled geese were compared, it was reported that the pH value in the breast meat of the geese was around 5.7% and the amounts of dry matter, ash and fat were 28-30%, 2.3-2.7% and 3.07-4.55%, respectively (Yakan et al., 2012). In another study in which the effect of sex and age on the meat of the geese raised in free range farming conditions was examined, it was reported that the sex and age of the geese affected some physicochemical parameters and the color qualities of the breast and thigh meat of the geese (Kirmizibayrak et al., 2011).

The effects of season, years and sex on the fatty acid composition of the abdominal fat tissue of female and male winter Canadian geese were investigated and it was determined that the myristic acid (C14:0) content of the abdominal fatty tissues of the female geese was higher than that of the male ones. Moreover, C14:0, C16:0, C16:1, C18:1 and C18:3 fatty acids were found to vary for different years (Austin, 1993).

Also, the fatty acid composition of the back muscles and livers of geese fed with different grains was examined and it was determined that feeding geese with different grains changed the fatty acid composition (Kalayci &Yilmaz, 2014). However, in a study in which the effect of sex, age and species on the fatty acid composition was examined, it was determined that sex and age did not significantly affect the fatty acid composition (Friend et al., 1983). Liu and Zhou (2013) examined the effect of feeding geese with grass on fatty acid composition and some meat quality characteristics and found that feeding them with grass caused changes in the L* and pH values of the breast meat of the

geese, but there were significant increases in the linolenic (C18:3n-3) and eicosapentaenoic acid (C20:5n-3) amounts.

The above mentioned studies showed that factors such as sex, age, species, feeding type and season leed to some changes in some physicochemical, color and fatty acid compositions of geese. In the present study, too, it was determined that the sex of the geese did not affect the physicochemical parameters or the color qualities of the geese and oleic acid, a major fatty acid, was more prevalent in male geese.

In recent years, due to the increased living standards in many countries people have begun to be more sensitive and conscious about the effects of different foods on their health. Currently people not only consume food, they also care about the harm or benefit of foods to their health. As conscious consumers try to avoid the consumption of red meat and meat products because of some of their negative effects, there appears to be an inclination towards white meat. The winged animals in the white meat group are increasingly, and should be, consumed for such reasons as the lower cholesterol level of their meat and the higher rates of unsaturated fatty acid. These studies are intended to make consumers aware of the fact that goose meat should take a greater place in their diets and it can be consumed much more.

5 CONCLUSION

As a result, the geese raised in the Altıntaş district increase the levels of animal food production and contribute to nutrition of the local residents. It could be said that the improvement initiatives to be undertaken will positively contribute to an increase in goose meat production and to meeting local people’s need for food.

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Table 1. Some physicochemical and color properties of female and male geese

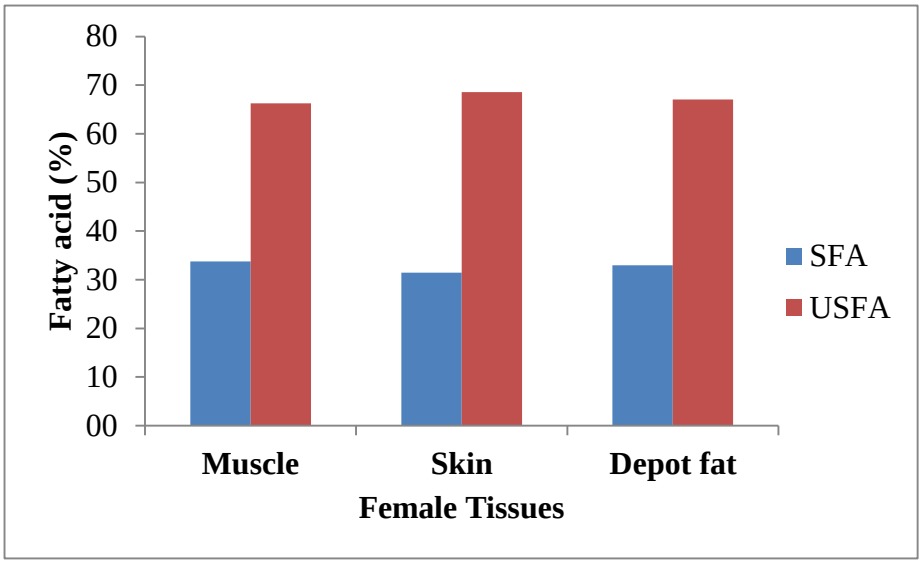
Parameters	Sex	Muscle	Skin	Deposit
pH	F	5.64^{Ac}±0.02	5.94^{Aa}±0.02	5.87^{Ab}±0.02
	M	5.68^{Ab}±0.07	5.96^{Aa}±0.02	5.85^{Aa}±0.03
Ash (%)	F	1.24^{Aa}±0.02	0.41^{Ab}±0.04	0.13^{Ac}±0.00
	M	1.18^{Aa}±0.05	0.30^{Bb}±0.02	0.12^{Ac}±0.01
Dry matter (%)	F	27.44^{Ac} ±0.63	79.07^{Ab}±1.47	92.61^{Ba} ±1.06
	M	27.61^{Ac} ±0.40	78.53^{Ab} ±0.92	95.46^{Aa}±1.59
Fat (%)	F	2.55^{Ac}± 0.21	73.30^{Ab}± 3.51	94.30^{Aa}±3.05
	M	1.98^{Ac}± 0.32	72.79^{Ab}±0.60	97.27^{Aa}±0.57
*L**	F	35.05^{Ac}±2.81	62.90^{Aa} ±2.27	56.47^{Ab} ±0.89
	M	36.30^{Ac}±2.30	61.14^{Aa}±1.57	54.26^{Ab}± 3.00
*a**	F	8.75^{Aa}± 0.49	1.62^{Ac}±0.40	3.37^{Ab}±0.15
	M	7.91^{Aa}±0.99	1.49^{Ac}±0.32	3 17^{Ab}±0.40
*b**	F	13.79^{Ab}±1.04	16.46^{Aa}±0.17	18.10^{Aa}±0.87
	M	13.79^{Ab}±1.04	15.32^{Ab}±1.60	18.76^{Aa}±0.87

F: Female, M: Male, $^{A-B:}$ The capital letters in the same column for the meat of each sex of goose are a comparison of female and male geese, and show that there are not statistical differences between the samples. $^{a-c}$ The lowercase letters in the same line are a comparison of different tissues, and the same letters show that there are not statistical differences between the samples.

Table 2. Fatty acid composition of the female and male geese (%).

	Fatty acid	Female			Male		
		Muscle	**Skin**	**Deposit**	**Muscle**	**Skin**	**Deposit**
Saturated	C14:0	0.66^{b}±0.02	0.80^{a}±0.01	0.80^{a}±0.01	0.61^{a}±0.05	0.67^{a}±0.02	0.62^{a}±0.01
	C15:0	-	0.11^{a}±0.01	0.11^{a}±0.01	-	0.11^{a}±0.01	0.09^{a}±0.00
	C16:0	24.08^{a}±0.56	23.90^{b}±1.20	23.90^{b}±1.20	24.08^{a}±0.67	22.76^{b}±0.43	23.72^{b}±0.85
	C17:0	-	0.13^{a}±0.01	0.13^{a}±0.01	-	0.13^{a}±0.01	0.14^{a}±0.03
	C18:0	9.02^{a}±0.37	6.48^{b}±0.60	6.48^{b}±0.60	8.38^{a}±0.15	5.50^{b}±0.33	7.27^{a}±0.33
Unsaturated	C14:1	-	0.08^{a}±0.01	0.08^{a}±0.01	-	0.06^{a}±0.00	0.07^{a}±0.01
	C16:1	3.71^{b}±0.03	4.79^{a}±0.07	4.79^{a}±0.07	4.01^{b}±0.12	4.69^{a}±0.04	3.52^{c}±0.02
	C17:1	-	0.11^{a}±0.01	0.11^{a}±0.01	-	0.12^{a}±0.02	0.10^{a}±0.01
	C18:1 *trans*	-	0.32^{a}±0.01	0.32^{a}±0.01	-	0.33^{a}±0.01	0.34^{a}±0.01
	C18:1 *cis*	41.89^{b}±0.98	49.75^{a}±0.10	49.75^{a}±0.10	44.34^{b}±0.10	54.3^{a}±0.25	53.52^{a}±0.50
	C18:2 *cis*	11.92^{a}±0.05	8.61^{b}±0.05	8.61^{b}±0.05	11.3^{a}±0.05	7.44^{b}±0.23	6.55^{c}±0.23
	C18:3n-6	-	0.11^{a}±0.01	0.11^{a}±0.01	-	0.10^{a}±0.00	0.10^{a}±0.01
	C18:3n-3	5.81^{b}±0.08	4.56^{a}±0.03	4.56^{a}±0.03	4.32^{a}±0.03	3.38^{b}±0.06	3.52^{b}±0.03
	C20:1	-	0.36^{a}±0.01	0.36^{a}±0.01	-	0.41^{a}±0.02	0.38^{a}±0.01
	C22:1	2.91±0.05	-	-	2.96±0.04	-	-

[a-c] The lowercase letters in the same line are a comparison of different tissues, and the same letters show that there are not statistical differences between the samples.



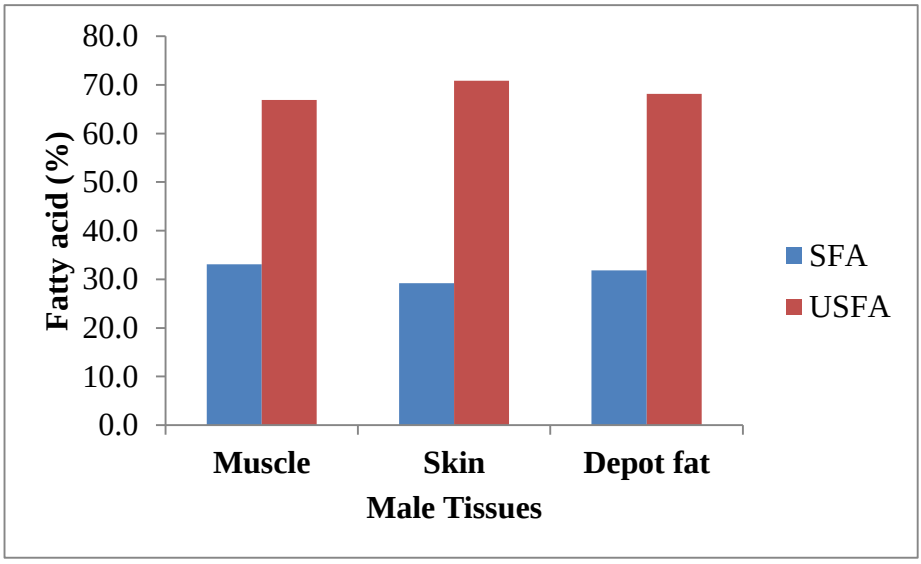


Figure 1. The amounts of SFA and USFA in various tissues of female and male geese
SFA: Saturated fatty acids, UFSA: Unsaturated fatty acids

CHAPTER 6

EVALUATION OF HERBAL AND ANIMAL BIOMASS WASTE IN FIELD AGRICULTURE AND HORTICULTURE

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INTRODUCTION

Plant production is one of the main food production methods in meeting the nutritional needs of people. Increasing population, urbanization and rapid consumption of resources once again reveal the importance of efficiency in agricultural cultivation. Agricultural activities adversely affect the structure of the soil in the long run. In order to increase yield and sustainability in plant production, the amount of organic matter in the soil should be increased. Therefore, many studies are carried out in order to obtain more products from the unit area. When the agricultural lands in Turkey are examined, they generally have a mountainous land structure. It has also been reported that 55.9% of these lands have an elevation above 1000 m and 62.5% have a slope of more than 15%. In addition, approximately 31 (24.5 million hectares) of the total 78 million ha area is considered as agricultural land (Tuğay, 2012). Today, suitable ecological conditions are required for sustainable agriculture. However, excessive fertilization for high yield and quality affects the soil structure negatively. In the face of this situation, manufacturers resort to different alternative methods in order to prevent these problems and increasing costs. Among these searches, the evaluation of biomass wastes as fertilizer is among the preferred methods. Biomass is defined as a mass of non-fossil organic matter of biological origin. In the chemical content of biomass wastes, there are atoms containing C, H, O, N together with smaller amounts of alkali, alkaline earth and heavy metals (Obernberger and Thek 2004; Kumar et al., 2009; Sözen et al., 2017). When the organic wastes in our country are examined, they constitute 65% of the general total waste. Therefore, our country has a very large organic waste capacity. Uncontrolled disposal of these wastes into the environment causes environmental pollution (Deloitte, 2014). It has been stated that combining inorganic and organic fertilizers can be a method used to both increase yield and prevent environmental problems in plants (Yusheng et al., 2005). Vegetable and animal biomass wastes are compost, biochar, bio-oil, biogas etc. is evaluated as. Solid (biochar), liquid (bio-oils) and gas (combustible synthetic gases) products are obtained by passing biomass wastes through pyrolysis stages at various temperatures. Biomass wastes have a positive effect on the soil structure and provide improvement in the parameters of yield and yield elements. In this study, studies on the working mechanisms of biomass waste, which can be considered as fertilizer, and the benefits it provides to the plant are given.

USE OF HERBAL AND ANIMAL BIOMASS WASTES AS COMPOST

It is of great importance to use organic fertilizers to increase the amount of organic matter in the soil. Barnyard manure, compost and green manure can be given as examples for these organic fertilizers (Karakurt, 2009). It has been reported that the main reason for the decrease in the amount of organic matter in the soil is the release of carbon dioxide into the atmosphere as a result of carbon oxidation. It has been determined that if the carbon lost from the soil cannot be replaced, the erosion increases even more. (Grant, 1997). Vegetable product wastes (stalk, straw, etc.) are known as biomass energy sources. The use of organic wastes by composting is among the preferred methods in terms of both economic and environmental health (Demirtaş et al., 2013; Edge and Karakuzulu, 2020). Composting is defined as an attractive disposal method that results in the most beneficial use of the end product obtained from organic wastes without causing environmental pollution (Kim et al., 2008). Thus, with the provision of appropriate temperature, humidity and ventilation in a controlled manner, organic wastes are transformed into humus-like substances through microbial organisms. Compost fertilizer increases the humus content of the soil and contributes to the regeneration of the soil ecosystem (Sagdeeva et al., 2018). It is to convert decomposable organic materials into biologically stable substances, to destroy pathogens, unwanted organisms and weed seeds that can be found in solid waste, to have maximum nutrient (nitrogen, phosphorus and potassium) content, to support plant growth and to obtain a product that can be used as a soil improver (Tosun, 2003). Ideal compost properties are given in Table 1.

Table 1. Ideal compost properties (Anaç and Okur, 1998)

Features	Requested Documents
C:N ratio	25-30
Particle Size	10 mm in ventilated systems, 50 m in long piles and natural ventilation conditions
Moisture content	50-60%
Air flow	Oxygen content should be between 10-18%
Temperature	55-60 ^{0}C
pH	5,5-9,0
Heap Height	If natural ventilation is to be done, piles of 1.5 m height, 2.5 m width and desired length are made.
Microbiological Activity	Cellulotic fungi and bio fertilizers

USE OF HERBAL AND ANIMAL BIOMASS WASTE AS BIOCAL

Biochar is the new product formed as a result of the temperature change/pyrolysis of organic materials at low temperatures (<700°C) in closed environments with little or no oxygen (Lehmann and Joseph, 2009). The term pyrolysis is the name given to the thermochemical decomposition of organic material at high temperature in an oxygen-free environment. Solid (biochar), liquid (bio-oils, etc.) and gas (combustible synthetic gases) products are formed by the pyrolysis processes of organic wastes at certain temperatures (Günal and Erdem, 2021). Pyrolysis temperatures are divided into three as slow pyrolysis, medium pyrolysis and fast pyrolysis. Slow pyrolysis is the conversion of biomass into solid, liquid and gaseous products at a temperature of 200-600°C in an oxygen-free environment, under atmospheric pressure, with a very low heating rate (Kambo and Dutta, 2015). With slow pyrolysis, 35% solid, 30% liquid and 35% gaseous products are formed (Boubacar Laouge, 2020). Medium pyrolysis is the conversion of biomass into solid, liquid and gaseous products in an oxygen-free environment, at atmospheric pressure, at 500-600°C temperature, 300-1000°C/min heating rate and 0.1-10 min retention time (Hornung et al., 2011). With medium pyrolysis, 15-25% solid, 40-60% liquid, 20-30% gaseous products are formed (Kebelmann et al., 2013). Rapid pyrolysis is the conversion of biomass into various products in an oxygen-free environment, at atmospheric pressure, at high temperature (400-600°C) and at high heating rate (200-300°C/s) (Mohan, 2006). As a result of rapid pyrolysis, 15-25% solid, 60-75% liquid and 10-20% gas products are obtained (Bridgwater 2004). It has been reported that the pH of the biochar, the amount of carbon and basic functional groups can change with the pyrolysis temperature, and the biochar yield decreases with the increase in temperature (Sun et al., 2017). Among these products, biochar undertakes many tasks such as reducing greenhouse gas emissions, soil regulation, increasing efficiency in crop production and increasing the quality of existing water in the soil (Laird, 2008).

STUDIES ON THE USE OF COMPOST AND BIOCOAL IN FIELD AGRICULTURE AND HORTICULTURE

Yağmur and Okur (2017) investigated the effect of compost and barnyard manure applications obtained from organic wastes on beans under greenhouse conditions. In their study, they applied basic nutrients N, P, K before planting in pots. In the study, the effects of compost (2, 4, 6 tons/da), barn manure + compost (1/2 + 1/2), barn manure application and N, P, K applications as control applications on beans were examined. As a result of the study, the researchers found that the effects of compost and barnyard manure applications on the dry matter, yield, total N, K, Fe, Cu and Zn amount of the

bean plant were statistically significant, and the effect on the total P, Ca, Mg, Na and Mn amount was statistically insignificant. reported that they exist. Abrishamkesh et al., (2015). The effects of biochar obtained from rice husk on lentil plant growth and alkaline soil properties were investigated. In their study, two biochars produced under different pyrolysis conditions were mixed with alkaline soils at the ratios of 0.4, 0.8, 1.6, 2.4 and 3.3 by weight. As a result of the study, they determined that biochar applications contributed to the growth of lentil plants. In addition, the researchers reported that it significantly increased soil quality, soil organic carbon, cation exchange capacity, available potassium and underground biomass of lentils. Böcek (2005) grown chickpeas in low fertile soils in different doses of compost (25, 50, 100 tons/ha), 50 tons/ha mineral fertilizer application (25 tons/ha before planting, 25 tons/ha before flowering) and control application. As a result of the study, it was determined that compost and mineral fertilizer applications gave more efficiency than the control application. However, it was determined that composts with increasing doses did not increase the amount of product. On the other hand, he reported that the biological yield was similar in all applications, and the number of pods and grains increased in chickpea as a result of compost application.

Ouedraogo (2018), in his study to determine the effect of biochar obtained from chicken manure on the prevention of Cd toxicity and mineral element concentrations in spinach plant, in greenhouse conditions, different doses of Cd (0, 25, 50, 100 mg/kg) and applied biochar (0, 5, 10 g/kg). As a result of the study, it was determined that Cd had a negative effect on the development of spinach and significantly reduced the amount of aerial parts and root dry matter in the plant. On the other hand, it was reported that the application of biochar had a positive effect on the above-ground part and root development of the spinach plant and significantly increased the amount of above-ground part and root dry matter. It was determined that Cd application decreased the P, K, Cu, Zn, Mn and B concentrations of the spinach plant above-ground parts, increased the Ca and Mg concentrations, and caused increases and decreases in the Fe concentration. It was determined that Cd application decreased the P, K, Zn and Mn concentrations of the spinach plant root, while increasing the Ca and Cu concentrations. It was observed that the biochar application decreased the Mn and B concentrations of the spinach plant above-ground parts, while increasing the P, K and Zn concentrations, while increasing and decreasing the Fe and Cu concentrations. It was reported that the application of biochar increased the P, K and Zn concentrations of the spinach plant root.

Mounirou et al., (2020) applied different doses of inorganic fertilizer (0, 50 and 100%), goat manure (5 tons / ha), biochar obtained from goat manure (10 tons / ha) separately and together, and applied to onion plants have grown. As a result of the study, it was determined that 100% inorganic fertilizer, biochar and biochar + goat manure applications and N content, P content with all organic fertilizer applications in plants without inorganic fertilizer, and K, Ca and Mg content with biochar and biochar + goat manure applications. They observed that biochar application alone significantly reduced the Fe and Cu contents of the plant, while inorganic fertilizer applications increased the Zn content. It was reported that biochar and biochar+goat manure applications significantly increased the Mn content of the plant. They found that the combined application of biochar and goat manure is more appropriate in terms of yield and yield elements in onion plants. Zhang et al., (2012) investigated the effects of biochar applications on yield, soil quality and greenhouse gas in rice-growing areas in China. In their study, they applied 10, 20 and 40 tons/ha of biochar to the soil before planting. As a result of the study, it was determined that biochar increased yield, soil pH, total organic carbon and N content. At the same time, they reported that nitrous oxide emission decreased and methane emission increased with biochar application. Clark and Cavigelli (2005) compared food waste and horse manure compost with commercial peat and tested these materials on lettuce and Brassica rapa. As a result of the study, they examined the physical and chemical properties of compost and determined that salinity was a problem in the compost obtained from horse manure, and the cost was high in compost obtained from food waste. On the other hand, they reported that it can be used in organic vegetable seedling cultivation. Togun and Akanbi (2003) compared compost obtained from different materials with synthetic fertilizers and applied them to tomato plants. As a result of the study, it was determined that there was no significant difference between compost and synthetic fertilizers in terms of plant growth and yield. However, they reported that organic-based fertilization provided an increase of 29.6% in dry matter and an increase of 36.3% in yield.

Namlı et al., (2017) examined the effects on yield and yield components by applying biochar obtained from hazelnut husk and chicken litter to wheat plants. In the study carried out in field conditions, biochars were applied at doses of 150-300 kg/da alone and with chemical fertilizers to a depth of 0-20 cm soil. Soil samples were taken after harvest and according to the results of the analysis, it was reported that biochars did not have significant effects on organic matter, N, pH, EC, lime, trace elements and heavy metals in the soil, but increased their P, K, Ca, Mg contents significantly. They also found that chicken litter biochar was more effective than hazelnut shell biochar. However,

they reported that biochars had the greatest effect on yield, plant height, and the number of grains per spike when applied with DAP fertilizer, not alone. Graber et al., (2010) mixed biochar produced from coconut fibers into the soil and grown tomato and pepper plants. As a result of the study, it was determined that biochar applications had a positive effect on plant height and leaf size, but had no effect on flower and fruit yield. In pepper, on the other hand, it was determined that leaf area, number of nodes, bud and flower-fruit yield increased compared to the control application. İnal et al., (2015) applied different doses of biochar (0, 2.5, 5, 10, 20 g/kg) and processed poultry manure (0, 5, 10 and 20 g/kg) to corn and bean plants. As a result of the study, it was determined that both poultry manure and biochar applications decreased the soil pH and Fe concentration suitable for plants, but increased the available P, Zn, Cu and Mn concentrations for plants. However, it was determined that poultry manure and biochar applications increased N, P, K, Ca, Fe, Zn, Cu and Mn concentrations in bean plants, while increasing N, P, K, Zn, Cu and Mn concentrations in corn plants, while decreasing Ca and Mg concentrations. As a result, they reported that the biochar to be obtained from poultry manure can be used for agricultural purposes. Bender Özenç and Şenlikoğlu (2017) investigated the effects of spinach plant growth in soils with compost added and nitrogen fertilizer applied under greenhouse conditions. In the study, two nitrogen fertilizer applications (with and without fertilizer), three compost materials (Hazelnut husk compost, barnyard manure and enriched compost) were applied to the spinach plant in 4 different doses (0, 2, 4 and 8 by volume). As a result of the study, it was determined that the average number of leaves, stem length, fresh yield and leaf blade width and length gave the highest values by mixing 8% dose of enriched compost. In addition, they reported that compost enriched with nitrogen fertilizer applied to the soil increased plant growth when 8% dose was applied, and it should be used regularly because it supports fertilizer applications in compost and compost products.

CONCLUSION

Increasing world population brings with it waste problems, which is one of the environmental problems. These wastes cause environmental pollution and adversely affect environmental health. Incineration of these organic wastes has become a major problem, especially in post-harvest agricultural areas. Today, thanks to the developing technology, organic wastes are put into use by passing through various stages. Plant and animal biomass wastes are considered as plant nutrition by increasing the organic matter level in the soil. Composts contribute to agricultural production by increasing the effectiveness of fertilization programs, even if they are not fully used as fertilizer. It has become possible to obtain high biochar (solid product) by pyrolysis of biomass wastes

at various temperatures. Biochars, which improve the C:N ratio in the soil, are almost in competition with chemical fertilizers. Therefore, it has soil conditioning properties. Considering the increasing input costs, the use of biomass wastes in agricultural activities provides an advantage in terms of both environmental health and fertilizer cost by eliminating waste problems. As a result, investments should be made in these areas and technical personnel should be trained in order to ensure waste management. At the same time, considering the positive effects it provides to the environment and soil, scientific studies in these areas should be widespread and their effects should be revealed.

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CHAPTER 7

PYHLOGENETIC RELATIOSHIPS OF *POTENTILLA* L. TAXA BASED ON rbcL SEQUENCES

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INTRODUCTION

Potentilla L. with over 400 species is one of the largest genus with species number and geographical distribution evaluated in the family Rosaceae (Sojak, 2008; Persson et al., 2020a; Faghir et al., 2021). The genus has wide distribution and species diversity in the northern hemisphere (Bean, 2015).

It can be stated for the genus *Potentilla* that it is very problematic and complicated in aspect of phylogenetic relationships of taxa and it's taxonomic status. Especially, the variations in morphological characters caused by different geographical and ecological conditions is one of the frequently observed situations in the species which has wide distribution like *Potentilla*. Genetic drift and gene flow are the effective mechanisms in the change of species living in areas where different ecological conditions prevail and has wide geographical distribution. Furthermore, hybridization and polyploidization are another commonly observed situations in the genus *Potentilla*. It can be stated that both of them have effective and important roles in the evolution of *Potentilla* (Potter et al. 2007; Paule et al., 2012; Persson et al., 2020a), besides it is well-known phenomenon for the genus.

Polyploid individuals can contribute for evolving of independent new lineages from the populations with time. Then hybrid individuals which have reproductive barriers and finally new species could be evolved (de Queiroz, 2005; Persson et al., 2020b).

The basic chromosome number of the genus *Potentilla* is stated with n=7 based on many researches published on the *Potentilla* taxa (Goswami & Matfield, 1975; Töpel et al., 2011). However, different ploidy levels changing from diploid to hexadecaploid are commonly observed cases in the genus (Kalkman, 2004; Persson et al., 2020a). Furthermore, single species which has multiple ploidy levels and different chromosome numbers connected with this are common observed situations in the genus *Potentilla* (Skalinska, 1950; Ilnicki & Jeremi 2008; Töpel et al., 2011).

Due to all these problematic situations, many classifications for the genus were made and still the taxonomy of the genus *Potentilla* and the phylogenetic relationships between species belonging to the genus have being tried to be understood and provide more consistent (Linnaeus, 1753; Rydberg, 1898; Wolf, 1908; Eriksson et al., 1998; Eriksson et al., 2003; Töpel et al., 2011; Persson et al., 2020b).

In other words, it can be stated that there are many problems in aspect of species of the genus *Potentilla* and these problems continue today. While many species evaluated in the genus *Potentilla* by researchers are classified in another

genus, some species evaluated in another genus are described in the genus *Potentilla* today (Eriksson et al., 2003; Lundberg et al., 2009; Paule & Sojak, 2009; Eriksson et al., 2015; Feng et al., 2017). For example, the genus *Sibbaldia* is highly polyphyletic and Dobes and Paule (2010) proposed to use the name of *P. tetrandra* instead of *Sibbaldia tetrandra*. Similarly, in the study made by Lundberg et al. (2009) it is stated that some *Potentilla* species are closely related to *Sibbaldia*. Furthermore these species were transferred to the *Sibbaldia* by Paule and Sojak (2009). Some species belonging to the genus *Sibbaldia* were evaluated in the Himalayan clade of *Potentilla* in the phylogenetic study made on the *Sibbaldia* by Eriksson et al. (2015).

All of these is the answer why the genus *Potentilla* is taxonomically problematic and why till now many taxonomists have classified the *Potentilla* taxa within different sections and genera (Sojak, 2010; Kechaykin & Shmakov, 2016). Recently, although many molecular studies are used in the solution of stated taxonomic problems, especially the studies based on the DNA sequence information are frequently preferred to understand the phylogenetic relationships in the genus (Eriksson et al., 1998; Eriksson et al., 2003; Dobes & Paule, 2010; Töpel et al., 2011; Koski & Ashman, 2016; Feng et al., 2017).

The regions containing sequence information belonging to nuclear and cpDNA and their combinations are used to provide the most comprehensive results in the various plant groups. Töpel et al. (2011) in their studies based on the nuclear and cpDNA to show phylogenetic relationships of the *Potentilla* taxa were identified six major clades named as Anserina, Alba, Argentea, Fragarioides, Reptans and Ivesioid. Similarly, these clades were identified by Dobeš and Paule (2010) and Feng et al. (2017). Furthermore, Eriksson et al. (2015) states in their molecular phylogenetic study containing *Sibbaldia* species that four *Sibbaldia* species (*Sibbaldia pentaphylla→Potentilla clandestina*, *Sibbaldia tetrandra→Potentilla tetrandra*, *Sibbaldia purpurea→Potentilla purpurea*, and *Sibbaldia sikkimensis→Potentilla sikkimensis*) were represented within the genus *Potentilla*, the Himalayan clade. Also, it is stated in the study made by Feng et al. (2017) that the Himalayan clade including former Sibbaldia species was resolved as near to *Potentilla* taxa. In other words, the Himalayan clade is mentioned within the genus *Potentilla*. However, there are still many problems in the evaluation of relations between clades and it can be stated that sometimes it is not well supported.

Although barcoding studies for plant groups are used to identify the species, to determine and solve taxonomic problems, and to evaluate and analyse phylogenetic relationships, for the purpose of performing these analysis

in the best and most accurate way the most suitable barcoding regions should be determined and then the target should be achieved by combining these regions giving best results for the related plant group.

DNA barcoding region giving effective results in a plant group may not be useful at the same level in another plant group. Yılmaz (2020) states as a result of their study on *Quercus* L. taxa that although it was recommended by the Consortium for the Barcode of Life (CBOL) for the evaluation of phylogenetic relationships, the variations containing nucleotide changes and species identification ability for the psbA-trnH IGS region are not sufficient in the studied plant group, in spite of convenient for barcoding in terms of DNA length. As a result, it can be stated that each barcoding region must analysed in aspect of their species identification and separation abilities, finally analysis should be made with the region combinations which have the most suitable and advantageous sequence information for the determined features.

For this aim, in this study, the sequence information for 81 *Potentilla* taxa based on rbcL region belonging to cpDNA were collected from National Center for Biotechnology Information (NCBI) and analysed to provide contribution to taxonomy of the genus *Potentilla*. In addition to evaluate and contribute the genus in aspect of taxonomic and phylogenetic relationships, it is aimed here to reveal the species identification and separation ability of the rbcL region selected by using as many taxa as possible. Thus it is possible to select more effective regions and will get more comprehensive results in future studies.

MATERIAL AND METHODS FOR PHYLOGENETIC ANALYSIS OF *POTENTILLA* TAXA

The sequence information of rbcL region belonging to cpDNA were preferred and examined for the evaluation of *Potentilla* taxa. All sequence information were provided from NCBI and the data containing sequence information of *Potentilla* taxa were analysed according to the compatibility of sequence information (Chen et al., 2010; Li et al., 2011; Kuzmina et al., 2012; Saarela et al., 2013; Manton, 2016; Kuzmina et al., 2017; Tan et al., 2018; Scharn et al., 2021). In other words, only compatible sequences provided from rbcL region for *Potentilla* taxa were selected and used for the analysis because of discrepancies in phylogenetic tree which could be caused by some situations like misidentifications of the taxa, missing data caused by sequencing, labelling errors in accessions. Besides compatible sequences, also the sequences belonging to the taxa provided by many researchers at different times were

extracted and examined to determine importance of related region in the taxonomic relationships the most comprehensively and remarkably within the genus. Some taxa in phylogenetic tree were represented by more than one because of their sequence information in high level in NCBI.

Totally 81 *Potentilla* taxa belonging to 105 samples based on the rbcL sequence information from cpDNA were analysed here. Afterwards, sequences belonging to the *Potentilla* taxa were aligned and performed by Molecular Evolutionary Genetics Analysis (MEGA 11) (Tamura et al., 2021). Alignment lengths, variable sites and parsim-info sites for taxa examined were computed (Table 1). Base substitution probabilities among the *Potentilla* taxa for rbcL sequences were determined and then transitional base substitutions (%), transversional base substitutions (%) and finally transition/transversion ratios for purines and pyrimidines were computed (Table 1). Nucleotide frequencies as A+T/U % and G+C % of the regions studied for *Potentilla* taxa were determined and showed in Table 1.

The dendrogram with bootstrap values on branches (with the option of hide values lower than 50 %) showing the phylogenetic relationships of *Potentilla* taxa was inferred using the Maximum Parsimony (MP) method. All positions with gaps in analysis were treated as missing data and eliminated completely for more effective analysis.

ANALYSIS RESULTS PROVIDED FROM rbcL REGION BELONGING TO THE cpDNA FOR *POTENTILLA* TAXA

The sequence information belonging to the rbcL region for 81 *Potentilla* taxa were provided from NCBI. Firstly, all taxa with rbcL sequence information were collected and after that taxa were preferred taking into account the compatibility of sequence lengths of data's loaded by different researchers in order to evaluate the taxonomic relationships more meaningfully and more comprehensively. Genbank codes for the taxa studied obtained from NCBI are given in Appendix.

Alignment length of the cpDNA region examined for *Potentilla* taxa was determined as 764 bp. The variable sites and parsimony informative sites which expose the species identification and separation ability and being important indicator for the selection of the barcoding region for further studies in plant group examined were determined in 96 and 40 nucleotides, respectively (Table 1).

Transitional substitution rate and transversional substitution rate by using the probabilities of each base substitutions were computed as 66.79 %

and 33.21 %, respectively (Table 1). In other words, the most of sequence variations of *Potentilla* taxa examined based on rbcL region were caused by transitional substitutions characterised by the substitutions between same base groups with the rate of 66.79 %. Moreover, transition/transversion ratios were computed as 5.13 for purines (k_1), as 2.88 for pyrimidines (k_2) and finally as 1.98 for overall transition/transversion ratio (R), respectively (Table 1).

Table 1: The information of *Potentilla* taxa examined based on rbcL sequences.

DNA region	Taxon (number)	Alignment lenght (bp)	Variable site	Parsim-info site	Transitional substitutions (%)	Transversional substitutions (%)	Transition/Transversion rate Purines (k_1)	Pyrimidines (k_2)	Overall (R)	Nucleotide freq. (%) A+T/U	G+C
rbcL	81	764	96	40	66.79	33.21	5.13	2.88	1.98	56.37	43.63

Nucleotide frequencies of rbcL sequences were also analysed for *Potentilla* taxa. Nucleotide frequencies for A+T/U and G+C were determined as 56.37 % and 43.63 %, respectively (Table 1). It can be stated for region related that the most of the sequence information for *Potentilla* taxa consist of A and T bases (Table 1).

Phylogenetic tree to understand better the taxonomy of the genus besides determining the species identification and separation abilities of the region examined was provided by Maximum Parsimony analysis (MP) from rbcL sequences belonging to cpDNA (Figure 1).

The using the many cpDNA regions, even using together nuclear and cpDNA regions containing gene and spacer sequences is very important to provide the most consistent and comprehensive results especially in plant groups that are taxonomically problematic. For this aim, various molecular studies based on the DNA sequence information are frequently used to contribute the understanding better as phylogenetically of the genus (Eriksson et al., 1998; Eriksson et al., 2003; Lundberg et al., 2009; Dobes & Paule, 2010; Töpel et al., 2011; Feng et al., 2015; Koski and Ashman, 2016; Feng et al., 2017).

Potentilla taxa by Töpel et al. (2011) as a result of their study based on cpDNA containing trnL/F- and trnS/G- spacers and nuclear DNA containing ribosomal ITS and ETS regions were identified within six major clades as Anserina, Alba, Argentea, Fragarioides, Reptans and Ivesioid. The clades stated by Töpel et al. (2011) were similarly determined by Feng et al. (2017). Eriksson et al. (2015) in their study on *Sibbaldia* species states the Himalayan clade

containing some *Sibbaldia* species in the genus. Furthermore, the Himalayan clade was resolved by Feng et al. (2017) as near to *Potentilla* taxa. However, there it can be stated that clades are still is not well supported and continue many problems in the evaluation of phylogenetic relationships of taxa.

Potentilla taxa in this study were evaluated based on these clades, also it is aimed here, to understand the phylogenetic relationships of taxa examined and taxonomy of the genus, besides determination the importance of rbcL region for the genus and further studies. Four groups are observed in phylogenetic tree which show the relationships of the *Potentilla* taxa based on the rbcL sequences (Figure 1).

Group IV comprising mostly of taxa belonging to the Argentea clade is the largest group in MP tree. Additionally, there are taxa evaluated within different clades in previous studies. For example, *P. biennis* was evaluated within group IV. However, it is stated in the study made by Persson et al. (2020b) that *P. biennis* is sister to the Ivesioid clade, also *P. norvegica* that was resolved within the Argentea clade based on chloroplast data and *P. biennis* are morphologically similar each other.

In this study *P. norvegica* like *P. biennis* was resolved within group IV containing the mostly of taxa evaluated within Argentea clade in previous studies. This situation seems as likely the Argentea-Ivesioid hybridization event (Persson et al., 2020b).

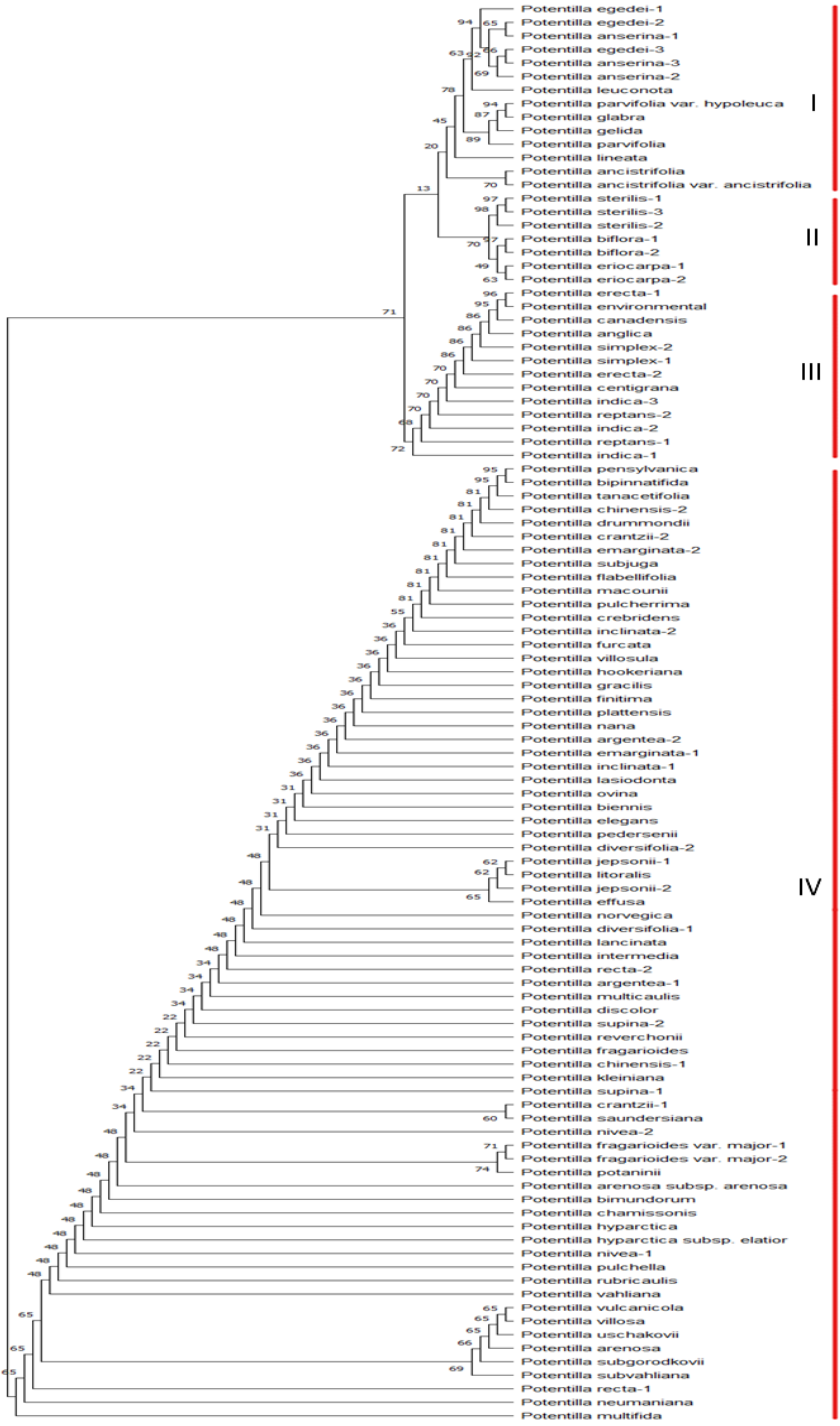

Figure 1: MP tree provided from rbcL sequences of *Potentilla* taxa.

The taxons belonging to the *P. fragarioides* that sequence information was uploaded to NCBI by different researchers was resolved in group IV in the dendrogram. However, *P. fragarioides* are evaluated with Fragarioides clade (Feng et al., 2017; Persson et al., 2020a). Group IV represented by highest number of the *Potentilla* taxa formed a distinct group with outmost species in MP tree. Also it can be stated that relationships of taxa within the group IV are very complex and confusing.

The totally 9 samples belonging to *P. erecta*, *P. simplex*, *P. indica* and *P. reptans* were clustered together within group III. These taxa are evaluated within Reptans clade in many studies by the different researchers (Töpel et al., 2011; Feng et al., 2017; Persson et al., 2020a; Persson et al., 2020b).
Group II in phylogenetic tree was represented by 3 samples from *P. sterilis*, 2 samples from *P. biflora* and 2 samples from *P. eriocarpa*. *P. sterilis* and *P. biflora* are evaluated within Alba clade in the studies based on molecular data (Töpel et al., 2011; Persson et al., 2020a).

It can be stated that phylogenetic relationships of *Potentilla* taxa for group I based on from rbcL sequence information are quite complex (Figure 1). MP tree show that the taxa belonging to *P. egedei* and *P. anserina* from Anserina clad formed a small branch within group I. Furthermore, taxa from *P. parvifolia* and *P. glabra* formed a small second branch within group I. It is stated in the study based on complete chloroplast genome sequence by Yu et al. (2021) that *P. glabra* is most related with *P. parvifolia*. Phylogenetic relationship between these two species show similarity in this study. *P ancistrifolia* evaluated within Fragarioides clade are represented in group I with outmost two taxa.

As a result, taxa from the genus *Potentilla* were clearly separated from each other and well grouped based on rbcL sequences. Furthermore, the study results show similarity with previous studies according to the evaluation of MP tree in aspect of phylogeny of *Potentilla* clades (Töpel et al., 2011; Feng et al., 2017; Eriksson et al., 2022). However, some species represented within same clade were not grouped together in dendrogram, in other words the region examined were insufficient in grouped of some taxa studied. It cannot be expected from a single barcoding region to fully reveal phylogenetic relationships. However, it is clear that the use of the rbcL region with the regions giving the best results in terms of expressed traits will be beneficial for phylogenetic relationships of *Potentilla* taxa and the taxonomy of the genus.

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Conflicts of Interest:

The authors declare that they have no conflicts of interest to report regarding the present study.

Appendix

KC483601, MN486531, MN486520, MF694761, MF572234, MF572223, MG248877, KJ204392, JN965765, JN893739, JF941383, JF941378, HQ590222, KC483682, KC483681, KC483679, KC483667, KC483656, KC483655, KC483644, KC483640, KC483639, KC483619, KC483617, KC483616, KC483615, KC483603, OP711473, LC703097, OP286942, MH092656, MK954456, MK954340, MZ359328, MN205230, MN205229, MN205227, MN205226, MN205225, MN192843, MN192842, MN192841, MN486526, MN185291, MN185290, MT921022, MH714018, MH658500, MH658488, MH658284, MH657580, MK925485, MK030046, MK030044, LC364386, MK526441, MK526440, MK526438, MK525225, MH116329, MH116327, KP643886, MG249677, MG249669, MG249666, MG249558, MG249542, MG249475, MG249349, MG249254, MG249096, MG249059, MG248968, MG248935, MG248917, MG248726, MG248591, MG248583, MG248555, MG248524, MG248465, MG248453, MG248408, MG248372, MG248288, MG248316, MG248287, MG248233, MG248196, MG247875, MG247225, MG247060, MG246835, MG246635, MG246348, MG246302, MG246248, KX678911, GQ436601, GQ436577, GU363798, KP402635, JN965768, JN892275, MT197559

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CHAPTER 8

MICROPLASTICS AND SOIL

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Introduction

The use of plastic products is becoming widespread thanks to its low cost, long-term durability and disposable feature. It is stated that approximately 8.3 billion tons of plastic has been produced in the world since the 1950s, and more than 60 percent of this is left to the marine and land ecosystems. It is stated that annual fossil fuel-based plastic production will exceed 1.2 billion tons and waste will exceed one billion tons by 2060 (Anonymous, 2023a). Plastic product production in Turkey is estimated to be 10.8 million tons in 2023 (Anonymous, 2023b). Despite the high durability capabilities of plastic products, they can deteriorate over time due to various factors. Among them, oxygen and sunlight are considered to be the most important factors of abiotic material degradation in natural environments (Chubarenko et al., 2020). The biggest reasons for the accumulation of plastic in nature are that its decomposition is very difficult and long-term (Wright and Kelly, 2017; Hale et al., 2020). The reasons why microplastics have become a global problem are that they are different in size and easy to transport (Thompson et al., 2009). There are many studies showing the negative effects of microplastic pollution in aquatic ecosystems. Although the amount of microplastic pollution in terrestrial ecosystems is higher than in aquatic ecosystems (Sea and oceans), its effects are not fully known (He et al., 2018; Horton et al., 2017; Jambeck et al., 2015). With the developing technology and population growth, the widespread use of plastic and the increase in plastic waste, dangers on ecosystems and living things are important. In addition to the benefits that the use of plastics provides in many different fields (industry, textile and food sector, etc.), it also causes important environmental problems. The physical, chemical and biological effects of microplastics, which are found in high amounts in soils, were investigated. It is aimed to reveal the effects of microplastics on the current situation by evaluating the studies on the presence of microplastics in soils. In the compilation study, it was aimed to reduce microplastic pollution and to create awareness on this issue and to create a preliminary research for future research.

Classification of Microplastics and Their Sources in Soil

The term microplastic was first used by Thompson in 2004 and defined as plastic parts smaller than 5 mm (Bouwmeester et al., 2015). Plastics according to their size are 1-nanoplastic (1 nm-100 nm), 2- microplastic (1 mm-5 mm), 3- mesoplastic (5 mm-20 mm), 4-macroplastic (20 mm-100 mm) and 5-megaplastic (>100 mm). Today, synthetic organic polymer particles smaller than 5 mm are most common in marine and terrestrial environments (Hidalgo-Ruz et al., 2012; Da Costa et al., 2016; Hartmann et al., 2019; Windsor et al., 2019). Microplastics can be transported in the soil by erosion, deteriorated under the influence of environmental conditions and stored in the soil and transferred to underground water resources. Microplastics can be taken and transported by living things (Rillig et al., 2017; Hurley et al., 2018). The top layer of the soil is an environment in which microplastics deteriorate under the influence of UV at high temperatures. This degradation lasts for many years and causes the degradation of plastics in agricultural areas (He et al., 2018). The sources of microplastics in agricultural soils and their effects on the ecological environment are given in Figure 1 (Yu et al., 2022).

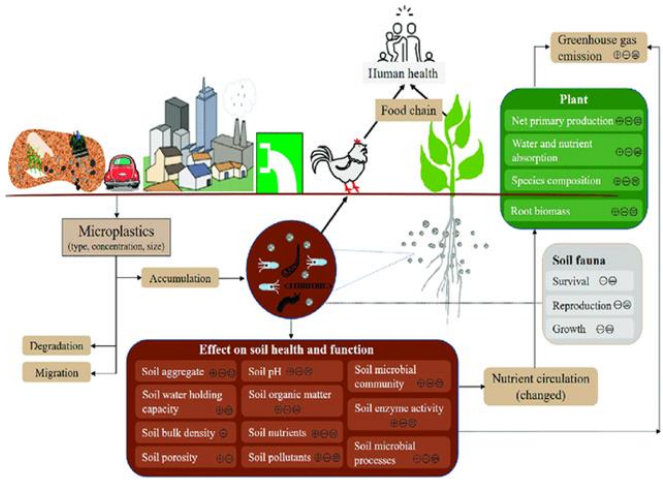

Figure 1. Sources of microplastics in agricultural soils and their impact on the ecological environment

Microplastics are grouped as primary, smaller than 5 mm and secondary (reduced in size by decomposition-degradation of plastics). Cosmetics, textiles, industrial production pellets and recycled plastic pellets are examples of primary microplastics. Road paints, industrial wastes and vehicle tire particles can be given as examples of secondary microplastics (Çelik et al., 2022; Yurtsever, 2018). Plastics collected from the fields are given in figure 2 (Örüç, 2022).

Figure 2. Plastics collected from the fields

The main sources of microplastics in the soil are industrial wastes (garbage, etc.), raw materials used in transportation (tires) and textile products (Fuller and Gautam, 2016; Yurtsever, 2015). In previous studies, the sources of microplastics found in agricultural soils consist of plastic materials such as irrigation pipes, nylon mulching, wastewater sludge, and fertilizer-pesticide crates, which have undergone environmental degradation (Helmberger et al., 2020; Ziajahromi et al., 2017). Plastic parts mixed with the soil in the fields are given in Figure 3 (Örüç, 2022).

Figure 3. Plastic pieces mixed with soil in the fields

Effect of Microplastics on Physical, Chemical and Biological Properties of Soil

As soil is a very complex structure, it is very difficult to detect and determine the effect of microplastic pollution (Campanale et al., 2022). Microplastic pollution in the soil adversely affects soil fertility and physical properties, microbial organisms living in the soil, soil quality and nutrient cycle (Bui et al., 2020). Studies show that the increase in the presence of microplastics reduces the volume weight, which is one of the physical properties of the soil, and decreases the stability of the aggregate by negatively affecting the adhesion of soil particles to each other (Gao et al., 2020; Mbachu et al., 2021). It has been determined that the presence of large amounts of macroplastics in the soil causes deterioration in the soil structure, negatively affecting the aeration and water holding capacity of the soil (Sarker et al., 2020; Zhang et al., 2018). It has been stated that microplastics have a positive effect on the mineralization of organic matter in the soil indirectly, if not directly, by accelerating microbial processes due to the carbon in their structure (Gao et al., 2020). When the effects of microplastics on the biological properties of the soil are examined, their effects on microbial diversity and the activities of living things are not fully known. It has been revealed that the presence of high amounts of microplastics in the soil, in particular, affects the Nitrogen and Carbon cycle and negatively affects

microbial activity and diversity (Liu et al., 2017; Yang et al., 2018; Ren et al., 2020). It has been shown that microplastics can increase organic carbon, organic nitrogen, phosphorus, humus and fulvic acid concentrations in soil and affect enzyme activity (Liu et al., 2017).

Conclusion and Recommendations

Plastic pollution is a global problem with serious effects, especially on soils. This pollution can pass to humans through the food chain, causing food safety problems and adverse environmental health effects. The problem of plastic pollution is quite significant, as the soil ecosystem plays a key role for other ecosystems. The incorporation of microplastics into soils poses a potential threat due to their detrimental effects on soil health. The fact that plastic wastes can stay in nature for a long time and turn into microplastics by breaking into small pieces is an important source of environmental pollution. It is known that microplastics enter soils with agricultural activities and can remain undecomposed for a long time. Microplastics have an important potential to pose a threat to the nutrition of future generations. Although measures have been taken in some countries to limit the use of microplastics, it is important to note that studies on this subject are limited in Turkey. The effects of microplastics on the environment and human health should be investigated further and awareness should be raised in the society. In addition, interdisciplinary studies are important for understanding plastic sources and their long-term effects. In the future, qualitative and quantitative evaluations of microplastics in different agricultural practices and climatic conditions may provide a better perspective on the problem. In this way, solution strategies to reduce microplastic pollution can be developed and environmental health can be better protected.

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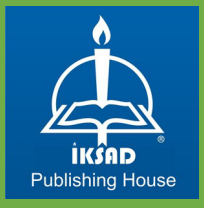

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