EFFECT OF SIRIS (Delonix regia) AND ASURO (Adhatoda vasica) LEAVES ON THE RIPENING OF BANANA

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Approval Letter

This Dissertation entitled Effect of Siris (Delonix regia) and Asuro (Adhatoda vasica) leaves on the ripening of banana presented by Elis Karki has been accepted as the partial fulfilment of the requirement for the **B. Tech. degree in Food Technology.**

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(Elis Karki)

Abstract

The aim of this study was to optimize the banana-leaf ratio for the optimum ripening of bananas and to compare the effectiveness of *asuro* and *siris* leaves on ripening. The quality attributes and storage life of bananas ripened with natural ripening agents (Leaves of *asuro* and *siris*) and those ripened with chemical agent, calcium carbide (CaC₂), at similar conditions of ripening and storage (temperature of 29-32° C and RH of 90-95%) are also compared. Bananas (*Musa cavendishii*) of same bunch were brought from the local market. Leaves of *siris* and *asuro* were collected from periphery of CCT at Dharan-14. The bananas with no abnormalities were used. The optimum banana-leaf ratio, proximate composition of banana and changes during the storage of ripened bananas. The sensory analysis of the bananas ripened with different methods was carried out to check consumer preferences. The obtained data were statistically analyzed using two-way ANOVA (no blocking) at 5% level of significance.

Banana-leaf ratio of 2:1 was found to be optimal to ripen the banana in 66 and 60 hours for *asuro* and *siris* leaves respectively under similar condition of ripening. Respiration rate of bananas at the beginning of second day of ripening was found to be 68.09 mg CO₂/Kg/hour, 71.89 mg CO₂/Kg/hour and 80.26 mg CO₂/Kg/hour for bananas ripened with *asuro* leaves, *siris* leaves and carbide respectively. From sensory evaluation, bananas ripened with natural ripening agents was preferred over carbide treated sample. Bananas treated with carbide had the lowest storage life, 4 days, while bananas ripened with *asuro* and *siris* had 6 days of storage life.

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Abbreviation	Full form
ANOVA	Analysis of Variance
SD	Standard Deviation
FAO	Food and Agricultural Organization
LSD	Least significant difference
TSS	Total Soluble Solids
USDA	United States Department of Agriculture
ССТ	Central Campus of Technology
ha	Hectar
°Bx	Degree Brix
°C	Degree Celcius
ACC	1-aminocyclopropane-1-carboxylic acid
db	Dry basis

List of Abbreviations

Part I

Introduction

1.1 General Introduction

The banana is the most popular tropical monocotyledonous fruit, grown in over 130 nations along the tropics and subtropics of Capricorn. The *Australimusa* and *Eumusa* series, which have various beginnings from the same genus, are the sources of edible bananas. *Musa accuminata* is the source of the majority of edible bananas, or they are a hybrid of *M. accuminata* and *M. balbisiana Colla*, two wild diploid species (Mohapatra *et al.*, 2010). Banana is the largest herbaceous plant, and is perennial (Karamura and Karamura, 1995). Banana is also called the "Apple of Paradise" or "fruit of heaven" or "Adam's fig" because of the multiple use of various parts from underground stem to male flower. It is botanically called *Musa Paradisiaca* (Warshini et al., 2022).

The banana (*Musa spp.*) is one of the most extensively grown and consumed fruit crops worldwide (Mohandas and Ravishankar, 2016). After rice, wheat, and maize, it is reportedly the world's fourth-largest food crop (Hailu *et al.*, 2013). Banana is a typical climacteric fruit. Commercial bananas are typically harvested when they are physiologically mature but still unripe. In order to speed up and uniformly control the ripening of bunch of bananas, ethylene or substances that release ethylene are universally applied to green bananas. This action mimics the natural maturation once ethylene production is increased exogenously (Lysenko *et al.*, 2019).

Banana ripening is the genetically designed, highly coordinate process of organ transition from immature stage to mature stage that results in an appealing edible fruit with ideal balance of color, flavor, aroma and texture (Perotti *et al.*, 2014). The changes during fruit ripening result in the production of a soft, edible, and ripe fruit with ideal quality characteristics. Fruit undergoes a number of biochemical, physiological, and structural changes during ripening that make them more alluring to consumers (Joshi *et al.*, 2017). These changes include textural change (softening of tissue), development of color, degradation of chlorophyll, development of characteristic flavor and aroma, increase in reducing sugar, decrease in acidity (Maduwanthi and Marapana, 2019). The banana, being a climacteric fruit, is typically harvested during the pre-climacteric stage and artificially ripened for commercial purposes to achieve faster and uniform ripening. Through artificial ripening, dealers can efficiently release their products at the ideal ripening stage while also minimizing losses during transportation (Maduwanthi and Marapana, 2019). Although artificially ripened fruits have a consistent and appealing exterior color, the tissue inside remains green and the fruit has a shorter shelf life. Artificial ripening speeds up the process but has an impact on the nutritional quality, sensory quality, and safety of the fruits (Hossain *et al.*, 2015).

Calcium Carbide (CaC₂) is a widely used chemical for fruit ripening because of lower cost and the widespread availability. However, use of this chemical in the fruit industry is being discouraged globally due to the risk of explosion and transmission of toxic substances like arsenic and phosphorus to consumers, making healthy fruit poisonous (Mariappan, 2004). Thus, the use of calcium carbide for fruit ripening is not mentioned in the food law of Nepal. In Nepal, in some hilly areas and in remote areas bananas are ripened using traditional method, i.e., using leaves of different plants like *siris (Delonix regia)*, raj briksha (*Casia fistula*), *asuro (Adhatoda vassica)* etc. African bush mango fruit (*Irvingia gabonesis*), *jatropha curcas* leaves, yellow papaya leaves, new *bouldia* leaves, palm nut, *dhurshilo*, apple, tomato, paddy straw are some others natural ripening agents (Ruwali *et al.*, 2022).

1.2 Statement of the problem

Banana is one of the most significant fruits traded worldwide. it has been a staple food of the human diet since the beginning of recorded history (Kaur *et al.*, 2016). The ripening of bananas is a crucial process that significantly impacts their quality, shelf-life, and consumer acceptability and during ripening bananas show marked physicochemical changes. Various methods have been employed to accelerate the ripening process artificially, including the use of chemical agents like carbide (Maduwanthi and Marapana, 2019).

CaC₂ is used commercially as a chemical ripening agent for banana ripening, although it is harmful to consumers' health and is restricted from use. It is the most commonly used artificial ripening agent because of its lower cost and availability. Concerns about the safety and environmental impact of these chemicals have motivated the exploration of alternative, natural ripening agents (Nascimento *et al.*, 2019; Nura *et al.*, 2018). Artificially ripened fruits may develop uniform and attractive surface color but the tissue inside remain green and the

fruit generally have shorter shelf-life (Hossain *et al.*, 2015). Traditional fruit ripening methods involve layering leaves and covering with hay, which is not toxic (Dhembare, 2013). The application of natural materials like the leaves of *asuro (Adhatoda vasica)* and *siris (Delonix regia)* offers a viable path toward better and ecofriendly ripening techniques. Although natural ripening agents have advantages, there is a lack of scientific knowledge on how well they work to speed up the ripening of bananas and how they affect fruit quality and storage stability.

1.3 Objectives of the study

1.3.1 General objectives

The general objective was to study the effect of *siris (Delonix regia)* and *asuro (Adhatoda vasica)* leaves on the ripening of banana.

1.3.2 Specific objectives

The specific objectives of this study are as follow:

- 1. To optimize the banana to leaf ratio for banana ripening.
- 2. To determine the time period required to ripen bananas with different ripening methods (*asuro* and *siris* leaves, and carbide).
- 3. To determine and compare the physiochemical properties of banana ripened using *siris* and, *asuro* leaves and carbide.
- 4. To evaluate the sensory properties of bananas ripened with *asuro* and *siris leaves*, and carbide.
- 5. To study the storage stability of banana ripened with *siris*, and *asuro* leaves and carbide.

1.4 Significance of the study

This study analyses and compares the different physiochemical and sensory properties and storage life of bananas ripened with calcium carbide and the leaves of *asuro* and *siris*. This study addresses the growing concern over the environmental and health impacts of chemical ripening agents. This study encourages producers and vendors to use non-toxic, eco-friendly, and effective natural ripening agents like *asuro* and *siris* leaves, which are locally available and cheap, by providing a scientific basis for the use of leaves to induce ripening of the banana and increase the storage stability of the bananas after using them. By exploring natural alternatives, it helps enhance the overall quality and safety of bananas on the market and also reduces the reliance on chemical ripening agents. Nowadays, consumer preference is increasing towards natural and organic products because of health and environmental concerns, by understanding consumer perceptions and acceptance of bananas ripened with natural leaves, consumer satisfaction can be attained.

1.5 Limitations of the study

1. Amount of ethylene produced by leaves and fruits could not be measured.

Part II

Literature review

2.1 Historical background

Banana has been a significant factor in the development of human civilization. The fruits of the genus Musa go by more than 100 common names all throughout the world. The name Musa was coined by Linnaeus and is comparable to a number of Arabic phrases for the fruit. However, the name may possibly be related to Antonius Musa, a Roman physician who lived in the first century B.C. Honoring the Greek deities, "Muses" is another explanation for the name. The term "banana" was adopted in the New World to describe the fruit's peel (Morton, 1987).

Banana that is a significant tropical fruit is one of the oldest fruits that humans are aware of. The banana was initially cultivated in south-east Asia's hot, tropical climates (Bahadur et al., 2020). Sanskrit literature such as the "Ramayana," Kautilya's "Arthashastra," and the Tamil classic "Shilappadikaram" all make explicit mention to banana (Mohandas and Ravishankar, 2016). Bananas are thought to have originated in Southeast Asia, specifically in the Philippines, Malaysia, Indonesia, and Papua New Guinea and cultivation started at around 8000 to 5000 BCE (Ploetz et al., 2007). Bananas were introduced to new locations over a period of thousands of years when people migrated and traded across the Pacific Islands. Around the 5th century, bananas found their way to Africa via trade and migration. Bananas were discovered by European explorers on their voyages to the Caribbean and Central America. Bananas were introduced to the Americas by the Portuguese and Spanish in the 15th and 16th centuries. Commercial banana growing began in the 19th century in nations such as Honduras, Costa Rica, and Panama. Transportation advancements, particularly the construction of railroads and, subsequently, the Panama Canal, allowed the shipping of bananas to North America (Gowen, 2012; Simmonds, 1962). The Cavendish subgroup of bananas, which gained popularity in the mid-20th century, dominates the modern banana industry (Seah and Nair, 2004).

2.2 Taxonomy of banana

Musaceae is the family that includes bananas (*Musa sp.*). Although the world checklist of selected plant families identified over 70 species of *Musa*, only a small number of those are edible. *Musa acuminata* and *Musa balbisiana* are the two species of wild bananas. These two species are the sources of almost all edible parthenocarpic bananas today. *Musa paradisiaca* is the name given to the resulting hybrid of *Musa acuminata* and *Musa balbisiana* (Valmayor et al., 2000).

Kingdom: Plantae

Subkingdom: Tracheobionta

Division: Magnoliophyta

Class: Liliopsida

Order: Zingiberales

Family: Musaceae

Genus: Musa

Source : Valmayor et al. (2000)

2.3 Morphology of banana

The tropical plants known as bananas (*Musa spp*.) have a distinctive shape. They differ from standard trees in that they contain a dense structure made of overlapping leaf sheaths called pseudostem, rather than a genuine trunk. Despite lacking woody structure, this pseudostem gives the plant solidity and support as it grows. The banana plant has big, elongated leaves with parallel veins and a central midrib that can reach several meters in length. Because of their size and structure, these leaves are prone to tearing in severe winds. The banana plant's shape is tailored to its tropical habitat, allowing for growth, reproduction, and resilience to environmental influences such as wind and weather (Karamura and Karamura, 1995).



Source: (Acevedo et al., 2021)

Fig. 2.1 Parts of banana plant

2.3.1 Root system

Banana has adventitious root system. The root axes arising from subterranean rhizome are called cord roots which are relatively straight and cylindrical having length of 3-5 m and diameter of 4-10 mm. These cords produce primary laterals and secondary laterals are derived from primary laterals (Draye *et al.*, 2005; Price, 1995). Just behind the root apex (cord), there are root hairs that can grow up to 2 mm long (Laville, 1964).

2.3.2 Rhizome

The subterranean (underground) stem of the banana is rhizome also known as corm. It is compact actual stem at the pseudostem's base. It grows horizontally up to 0.3 m length from which arial shoot is raised. Corm internodes are short. Lateral buds on the rhizome produce suckers, commonly referred to as "followers," which enable subsequent growth that perpetuates the corm's life. Each shoot is determinate (Carr, 2009; Norman *et al.*, 1984).

2.3.3 Sucker

Sucker development results from adventitious buds produced on corm. The suckers are main source of vegetative planting material and subsequent vegetative generation. The maiden sucker (a huge, non-fruiting ratoon with foliage leaves) carries on the growth cycle after the initial plant produces fruit and dies (Simmonds, 1962). Although wild species can potentially be reproduced by seed, bananas are vegetatively propagated through suckers. Three unique stages make up the development of suckers: peeper (young suckers having just scale leaves), sword (suckers bearing narrow sword leaves), and maiden (big but not producing ratoon with foliage leaves). A "mat" is the collective noun for the collection of suckers and the mother plant that it surrounds. Since bananas are a crop that is vegetatively propagated, the quantity of suckers a plant produces is particularly significant to growers (Pillay and Tripathi, 2007; Swennen *et al.*, 1984).

2.3.4 Pseudostem

The arial shoot of the banana plant is known as a banana pseudostem, that can grow up to 10-15 m in certain wild species and 2-8 m in cultivated forms. The curled and wrapped leaf sheaths that emerge from the compact true stem at the pseudostem's base make up the pseudostem. Leaf bases that are tightly curled in an opposite-clockwise spiral to create a cylindrical structure. The pseudostem's leaf sheaths provide the sole physical support for the shoot's vertical axis (Stover and Simmonds, 1987). A pseudostem yields one bunch of bananas before death and being replaced by a new pseudostem (Anhwange *et al.*, 2009; Karamura and Karamura, 1995).

2.3.5 Leaf

Banana leaves are made up of a "blade" (lamina) and a "stalk" (petiole) with the dimension of 3-3.5 m length and 65 cm width (dimension differs according to cultivar) (Kole, 2007). A solitary mature leaf's and a plant's overall leaf area range from 2 to 3 m² and 17 to 25 m², respectively (Stover and Simmonds, 1987). Adaxial refers to the leaf's upper surface, while abaxial refers to its lower surface. Each leaf emerges as a rolled cylinder from the pseudostem's core. Older leaves are forced outward, where they dry out, perish, and fall off as fresh leaves grow at the meristem. In their growth cycle, most banana plants produce between 30 and 40 leaves. A bunch of fruit must have a minimum of 8–10 functioning leaves

to mature properly. In the summer, banana leaves can unfold at a rate of one every week, but in the subtropics, they can only be generated once every month (Kole, 2007; Morton, 1987). Leaf material is widely utilized in basket and mat weaving, food wrapping for cooking and sale, food coverings, and eating utensils (Kambuou, 2002).

2.3.6 Flower

The blooming stem emerges from the meristem through the pseudostem after the production of a predetermined number of leaves. A complex inflorescence of flower clusters arises as the flowering stem breaks through the leaf crown after 6-9 months (in tropics) and 10-11 months (in subtropics) of plantation. On the fluorescence, three different types flowers are formed; female (pistillate) flowers that give rise to the fruit, male (staminate) flowers present at male bud that may or may not fertile and hermaphrodites or neuters these are situated between female flowers and male bud on the inflorescence axis or rachis The first flowers to bloom are female, and they have big ovaries that eventually turn into fruits. A male bud in the shape of a bulb with tiny flowers grows at the end of the inflorescence as it matures. However, in most cultivated bananas, the fruit develops by parthenocarpy preventing formation of seeds (Pillay and Tripathi, 2007; Simmonds, 1959).

2.3.7 Fruit

Banana "fruit" is actually a berry and is referred to as a "finger." The fleshy pulp, which primarily comes from the endocarp (pericarp's innermost layer), is separated from the skin or peel by the hypanthium (the fruit's floral receptacle) and outer layer (exocarp), which are both parts of the pericarp (the ovary wall-derived fruit wall). Tepals, styles, and staminodes all separate from the ovary throughout the maturation of the fruit, creating a distinctive calloused scar at the fruit's tip. To distinguish various kinds, look for the shape of the fruit's apex, which might be tapering, rounded, or blunt (Lobo and Rojas, 2020).

Common *Musa* bananas vary according on the cultivar in terms of color, size, shape, texture, and flavor. Typically, they are elongate-cylindrical, straight to curved, and measure 3 to 40 cm in length and 2 to 8 cm in diameter. The skin can be green, yellow, or red and has varying degrees of fibrousness. Peel thickness also vary with types of bananas. The flesh is starchy to sweet and can range from white to cream, yellow, or yellow-orange to orange (Lobo and Rojas, 2020).

2.4 Nutritional composition of banana

Millions of people worldwide eat banana fruit every day for a variety of nutritional benefits and as part of their regular diet. Ripe banana has become a staple diet in most of the African nations, it is being used as green vegetables and for medicinal purpose in some Eastern countries (Kaur *et al.*, 2016). Due to their high vitamin A and B6 concentration, bananas are also helpful in reducing heart disease and cancer. Its elevated potassium level lowers blood pressure and can prevent cardiovascular disease. Sometimes, banana is used for the treatment of gastric ulcer and diarrhea (Othman *et al.*, 2021). Banana is often ingested by people who are intolerant to salt because of low sodium and fat content. It is rich in carbohydrate, minerals including calcium and potassium and antioxidants like dopamine, carotenoid, retinol, ascorbic acid (Englberger *et al.*, 2003; Kanazawa and Sakakibara, 2000; Wall, 2006). Nutritional composition of banana is shown in table 2.1 below.

Nutrient	Amount per 100 g
Water (g)	74.91
Carbohydrate (g)	22.84
Protein (g)	1.09
Dietary fiber (g)	2.6
Total sugar (g)	12.23
Minerals (mg)	467
Vit. C (mg)	8.7
Vit. A (µg)	3
Thiamine (mg)	0.031
Riboflavin (mg)	0.073
Niacin (mg)	0.665
Vit. K (µg)	0.5

 Table 2.1 Nutritional composition of banana

Source: USDA (2019)

2.5 Proximate composition of banana

Proximate analysis reveals that banana contains moisture, protein, fat, ash, carbohydrate, and fiber (Kookal and Thimmaiah, 2018). The chemical and nutritional composition of bananas vary with varieties, climatic condition, soil condition, fertilizer used etc. Further, there is a great effect of maturity, handling, and method of ripening. According to USDA (2019) proximate composition of banana is tabulated in table 2.2 as follow:

Parameters	Amount (g/ 100g)
Moisture	74.91
Protein	1.09
Fat	0.33
Ash	0.82
Carbohydrate	22.84
Fiber	2.6

Table 2.2 Proximate composition of banana

Source: USDA (2019)

2.6 Banana production

In Nepal, bananas are a valuable agricultural product and a significant fruit crop. It is grown on 18,329 ha of land throughout the nation, covering 68 of Nepal's 77 districts, with a total production of 318,338 tons and a productivity of 17.37 t/ha (knoema, 2021). Chitwan district is regarded as the primary hub for banana production among them, followed by Saptari, Jhapa, Morang, and Rupandehi districts (Pant *et al.*, 2023). 300-500 cultivars of banana are growing in more than 100 countries, mainly in Brazil, India, and Philippines with an annual production of approximately 114 million metric tons of bananas are produced annually worldwide from 5.6 million hectares of land (FAO 2018) (Ruwali *et al.*, 2022).

2.7 Banana plantation

Banana is perennial herb grown in humid tropical region. At the plant's base, fresh offsets develop on the rhizome. The offsets (suckers) are then removed and used in vegetative propagation to establish new plants. A commercial plant can be productive up to 10 years. Since plants multiply parthenogenetically rather than by producing seeds, vegetative propagation is crucial (Morton, 1987). Conventionally, the plant is vegetatively propagated using corms, large and small suckers and sword suckers. Only breeding initiatives use seeds. Suckers of any size can be used for the propagation avoiding tiny and sick suckers. The ideal

size for transplanting is a sucker with its first mature-type leaves. Corms are used to establish new plantings as they are disinfected to reduce soilborne illness. Since tissue grown plantlets are fully pest-free, they are occasionally utilized to launch new plantations (Arvanitoyannis and Mavromatis, 2009).

2.8 Soil type and climate

Although they may be grown in most types of soil, bananas need rich, well-drained soil for maximum growth and development. The ideal soils are deep, well-drained loams with excellent water-holding capacity, humus content, and an optimal pH of 5-7. Maintaining an optimal pH is crucial for mineral absorption, as higher or lower values can create deficiencies. Stagnant water can induce diseases like Panama disease and Fusarium wilt (FAO, 2019; Lobo and Rojas, 2020).

Depending upon type of banana, it can be grown up to the height of 2000m from sea level but it is difficult to grow above 1000m. (Nelson *et al.*, 2006). Temperature of 26–28°C is optimum for banana shooting and 29-30°C is for fruit development. Temperatures below 13°C inhibit root growth, below 6°C destroys leaf chlorophyll, and frost temperatures (0°C or lower) kill the plant. Rainfall of 200 cm per year is abundant for banana cultivation (Arvanitoyannis and Mavromatis, 2009; Lobo and Rojas, 2020).

2.9 Banana fruit development

Each bloom grows into a finger, which is subsequently grouped together into a node (called a "hand"), with several nodes making up a bunch. For crops produced in the tropics and subtropics, the time between inflorescence (bunch) emergence and harvest varies depending on the season, ranging from 90 to 110 days in the summer to 200 days in the fall. The Cavendish subgroup's typical bunch mass fluctuates between 30 and 35 kg in the tropics, although it can exceed 50 kg for ratio crops in the subtropics (Carr, 2009; Robinson and Nel, 1989).

2.10 Harvesting

Being climacteric fruit, bananas are harvested when green in color but at physiological maturity or pre-climacteric stage to ensure appropriate ripening (Lobo and Calderón, 2020). Harvesting entails cutting of banana bunches and transport them to storage area. Different

kinds of indices are used to determine the suitable time to harvest banana such as length, size, diameter, color, age of bunches, growth degree days etc. Bananas that must be transported over great distances are harvested while still green and refrigerated until they reach their destination because they have a limited shelf life after ripening. The importer or distributor then allows them to ripen under carefully monitored circumstances (Thompson and Burden, 1995).

2.11 Banana ripening

The ripening process is a complicated, irreversible chain of events (change in physiological, biochemical and organoleptic changes) that is started by several phytohormones, chiefly ethylene but also abscisic acid and others (Iqbal *et al.*, 2017). A number of genetic and metabolic pathways are coordinately activated during the intricate process of ripening. A brief, sharp rise in the rate of respiration and ethylene production occurs during the ripening of climacteric fruit (Thompson and Burden, 1995).

Under natural fruit ripening conditions, ethylene gas is auto stimulated by the ripening fruits. However, the fruits ripen slowly and unevenly, leading to high weight loss and failure to develop good color and aroma. Thus unripe bananas need to be exposed to an exogenous source of ethylene gas for the assurance of firm pulp texture, good flavor, and bright peel color (Abraham *et al.*, 2022).

2.11.1 Changes during banana ripening

A series of physiological and physical changes occur throughout the ripening process, resulting in an edible fruit (Gowen, 2012). An increase in sweetness, a decrease in astringency, a yellowing of the peel, and a softening of the texture are the main organoleptic changes that occur when bananas ripen. The modifications arise from a sequence of metabolic alterations in the banana fruit's skin and flesh (Maduwanthi and Marapana, 2017). Some of the main biochemical changes that occur during fruit ripening include increased respiration, chlorophyll degradation, biosynthesis of carotenoids, anthocyanin, essential oil components, aroma, increased activity of the enzyme that breaks down cell walls, and a brief increase in ethylene production (Gamage and Rahman, 1999).

Fruit softening is caused by turgor loss, starch breakdown, and enzyme-catalyzed alterations to the composition and structure of the wall (Ali *et al.*, 2004). Ripeness causes

solubilization, de-esterification, and depolymerization of cell wall polysaccharides such cellulose, hemicellulose, and pectin (Brummell *et al.*, 2004). The increase in sweetness is due to the conversion of starch to simple sugars; sucrose, fructose, and glucose (Adao and Gloria, 2005). Peel is changed from green to yellow as chlorophyll vanishes (Subagio *et al.*, 1996). A variety of volatile substances, such as esters, alcohols, ketones, aldehydes, and phenol esters, give bananas their distinct aroma. There is a tremendous loss of tannin as ripening proceeds, resulting in a loss of astringency (Ding and Ikram, 2010).

2.12 Ethylene as a ripening hormone

Ethylene (C_2H_2) is a simple gaseous hydrocarbon and is regarded as a multipurpose phytohormone, ethylene controls both growth and senescence. It is one of the first plant hormones identified in the 19th and early 20th centuries using coal-based illuminating gas (Abeles *et al.*, 2012). Depending on the plant type, application timing, and concentration, it either stimulates or inhibits growth and senescence processes (Khan *et al.*, 2008; Mattoo, 2018). Many climacteric fruits, some of which are significant sources of fiber and nutrition for human diets, are regulated in their ripening process by the gaseous plant hormone ethylene (Barry and Giovannoni, 2007). Ethylene functions across the whole life of the plant, despite being most commonly linked to ripening. The processes of seed germination, seedling growth, abscission of leaves and petals, organ senescence, stress reactions, and disease responses are all regulated by ethylene (Schaller and Kieber, 2002). Ethylene is a basic gaseous hydrocarbon that can enter and exit plant tissues through both endogenous and external means. Depending on the product and how it is used, it may be advantageous or detrimental (Watkins, 2002).

It is widely acknowledged that ethylene is crucial to the ripening of climacteric fruits since it starts a vast production of the gas at the beginning of the respiratory climacteric period and can be given exogenously to both stimulate ripening and endogenous ethylene production. In response to various stimuli such as physical wounding, cold injury, pathogen infection, and chemical inducers, as well as during specific periods of growth and development including fruit ripening, seed germination, and leaf and flower senescence and abscission, the body produces more ethylene naturally (Yang and Hoffman, 1984). When bananas grow from fingerlings to full maturity, the ethylene level remains surprisingly stable until the fruit starts to ripen (Burg and Burg, 1965).

It is common practice to use exogenous ethylene to start the uniform ripening of bananas. On the other side, commercial methods of handling, storing, and transporting banana fruit are predicated on limiting ethylene production and activity or avoiding exposure to it. Controlling the atmosphere and temperature is one of these tactics. One relatively new method for managing fruit ripening and senescence related to ethylene binding is 1-methylcyclopropene (1-MCP) therapy. 1-MCP successfully postponed the ripening of banana fruits, prolonging their green life (Duan *et al.*, 2007). For climacteric fruits that have already ripened on the plant, the respiratory climacteric may not be necessary (Lelievre *et al.*, 1997).

2.12.1 Biosynthesis of ethylene

In plants, methionine is the precursor of ethylene. Methionine is first transformed into Sadenosyl-L methionine (SAM), a process that is aided by SAM synthetase. Next, 1aminocyclopropane-1-carboxylic acid (ACC) is produced from SAM. The enzyme ACC synthase catalyzes this conversion, and ACC oxidase catalyzes the subsequent conversion of ACC to ethylene (Straeten and Montagu, 1991).

 $MET \xrightarrow{SAM \text{ synthetase}} SAM \xrightarrow{ACC \text{ synthase}} ACC \xrightarrow{ACC \text{ oxidase}} C_2H_2$

Source : Straeten and Montagu (1991)

2.12.2 Mode of action

Ethylene is a key plant hormone that influences plants through a complex signaling system that is essential for orchestrating a number of physiological functions (Abeles, 1972). The process begins with the binding of ethylene to its receptor. The ethylene is perceived by receptor proteins (ETR) in the endoplasmic reticulum (ER). The copper cofactor needed for ethylene binding is provided by RAN1. GR mediates the production of the receptor signal and is most likely connected to the receptor. According to some theories, TPR attaches to ethylene receptors and causes the degradation of the receptors. As negative regulators of ethylene signaling, the receptors trigger Constitutive Triple-Response1 (CTR1) when ethylene is not present. This inactivates Ethylene Insensitive2 (EIN2), suppressing the

ethylene response. The degradation mechanism of the transcription factors EIN3/Ethylene Insensitive3-Like1 (EIL1) is mediated by the Ethylene Insensitive3-binding F-box (EBF) proteins. The transcription of ethylene response genes stops when EIL is not present. When ethylene binds to the receptors, it inactivates them, which stops CTR1 phosphorylation activity. By binding to primary ethylene response elements, active EIN2 stabilizes EIL transcription factors, which in turn can trigger the expression of target genes, including those encoding the ERF transcription factors. These genes encode proteins essential for a variety of cellular reactions, such as senescence, ripening of fruit, cell elongation, and reactions to environmental stressors (Ju *et al.*, 2012; Liu *et al.*, 2015; Solano *et al.*, 1998).

2.13 Induced ripening with ethylene Gas

According to Burg and Burg (1965), banana ripening can be hastened up by as little as 0.1 ppm of ethylene. Ethylene gas can be used to artificially ripen fruits at concentrations of up to 100 ppm (100μ l/L), depending on the crop, type, and maturity (Himani, 2020).

Commercially, bananas are ripened by being exposed to 100-150 ppm of ethylene gas for 24 hours at $25^{\circ}C-27^{\circ}C$ with 90–95% relative humidity. Sufficient ventilation is necessary to avoid CO₂ buildup above 1%, which diminishes the efficiency of ethylene (Kader, 1997).

2.14 Induced ripening with calcium carbide

Calcium Carbide (CaC₂), also known as carbide or calcium acetylide, is primarily used for producing acetylene and calcium cyanamide. CaC₂ of technical grade is grey or brown in color and contains around 85% CaC₂ as well as other chemicals. When exposed to moisture, CaC₂ creates acetylene gas, an analog of ethylene, and that mimics its actions as a natural plant hormone that promotes fruit ripening (Farouq and Fashoranti, 2022; Okeke *et al.*, 2022). Because of low cost and availability calcium carbide is commonly used for artificial ripening of bananas in developing nations, including Sri Lanka, despite official rules prohibiting it (Hartshorn, 1931).

 $CaC_2 + H_2O \longrightarrow Ca(OH)_2 + C_2H_2$

It entails the use of ventilated containers at an initial temperature of 27 °C, a relative humidity of 90-95%, and 2 g of calcium carbide per kg of fruit (Sy and Wainwright, 1990).

2.14.1 Hazards associated with calcium carbide

Calcium carbide, a highly reactive compound, has carcinogenic and neurological qualities. When compared to ethylene, acetylene produced from carbide is combustible and explosive even at low concentrations and also has a disagreeable odor (Lewis, 2016). The use of calcium carbide is not only toxic to the consumers but also harmful to those who handle it (Dhembare, 2013). When an excessive amount of carbide is applied to fresh fruit for ripening, the fruit loses flavor and may become poisonous. It is also considered exceedingly dangerous due to the presence of arsenic and phosphorus Hydride traces (Asif, 2012). Vomiting, diarrhea with or without blood, a burning sensation in the chest and belly, thirst, exhaustion, and difficulty swallowing and speaking are early symptoms of arsenic and phosphorus poisoning. Other symptoms include numbness in the legs and hands, cold and moist skin, and low blood pressure, which can be fatal if not treated promptly. It affects the neurological system, causing headaches, dizziness, mood problems, tiredness, mental confusion, and seizures in brief periods, and memory loss and cerebral edema in over time (Tamal *et al.*, 2008).

2.15 Induced ripening with natural leaves

In different areas of the Nepal different indigenous methods are applied to ripen the bananas. Indigenous methods include use of ripened fruit (African bush mango, ripened tomato etc.), paddy straw, smoke, plant leaves etc. Leaves of different plants like *siris (Delonix regia)*, *raj briksha (Casia fistula)*, *asuro (Adhatoda vassica)*, *jatropha curcas*, papaya, new *bouldia*, *dhurshilo* etc. are being used as natural ripening agents for ripening of fruits like bananas depending upon their availability in different regions (Ruwali *et al.*, 2022). These leaves are collected during day time to avoid the dew and excessive relative humidity. Inside the jute sack, leaves are placed on the ground, then bananas are placed and again the bananas are covered with a layer of leaves and left to ripen in suitable storage condition (Anonymous).

Ethylene is produced form the leaf with the degradation of chlorophyll and with the process of senescence and abscission. The both leaf disc and petioles are responsible for the ethylene production and respiration (Aharoni *et al.*, 1979). Numerous environmental and

developmental factors control the biosynthesis of ethylene. Young leaves produce almost no ethylene but mature and senescing leaves produce significant amount of the ethylene. Ethylene production is higher in the wounded leaves. The ability of plant hormones to control the production of ethylene is well established. It is commonly known in the study of ethylene biosynthesis that auxin-induced ethylene production results from the auxin's activation of ACC synthase. Contrary to auxin, ethylene has a more subtle effect on ethylene biosynthesis. However, ethylene can control its own biosynthesis, either boosting or inhibiting it (Riov *et al.*, 1990). Riov *et al.* (1990) observed that, the abscisic acid also induces ethylene biosynthesis.

2.15.1 Siris (Delonix regia)

Delonix regia is a leguminous plant, belongs to *Caesalpiniaceae* family. It is commonly found in nations like Brazil, Mexico, China, Nigeria, Senegal, Ghana, Nepal, India, Pakistan, Afghanistan, Cameroon, Togo, Congo, Sudan, Kenya, Tanzania, Rwanda, and Madagascar. The tree can reach a height of 9.1 to 18.0 meters and has a large canopy that spreads out like an umbrella. The leaves have long stalks that are adorned with many, approximately 12-mm-long flowers, and the pods are flat, woody, dark brown, and measure 61 cm in length and 5.1 cm in width. The plant's leaves, flowers, bark, and roots are rich in essential minerals, fatty acids amino acids, phytochemicals including: saponins, alkaloids, carotene, hydrocarbons, phytotoxins, flavonoids, tannins, steroids, carotenoids, galactomannon, lupeol, β -sitosterol, terpenoids, glycosides, and carbohydrates. In the past this plant's parts like leaves, stem and bark had been used for the prevention and cure of fever, constipation, inflammation, arthritis, hemoplagia, piles, boils, pyorrhea, scorpion bites, bronchitis, asthma, and dysmenorrhea (Alagbe *et al.*, 2020). In some areas of Nepal Siris's leaves are used as natural banana ripening agent (Ruwali *et al.*, 2022).

Traditionally, different parts of *D. regia* have been utilized for various medicinal purposes. The plant itself is used for treating rheumatism, flatulence, anemia and fever also has spasmogenic properties, and is employed as a cathartic, as an emetic. The flowes have anthelmintic and insecticidal properties, also used to treat gynecological disorders such as dysmonorrhea, as well as febrile conditions, inflammation, and diarrhea. The leaves are used to treat bronchitis and pneumonia in infants, have anti-diabetic properties, and are also used for gastic problems, body pain, and rheumatic joint pain. The bark is used as an antiperiodic and febrifuge. The root is used to reduce abdominal pain (Modi *et al.*, 2016).

Hydrochloric acid extracts from the leaves and seeds suppress aluminum corrosion in hydrochloric acid solutions. Seed gum is used as a binder and has medicinal applications and lower concentration improves the qualities of fast disintegration tablets, while greater concentrations are used in sustained-release formulations. To improve patient compliance, quetiapine fumarate sustained-release tablets were made with seed polysaccharides. Seed polysaccharides are also utilized in floating drug delivery systems for a longer-lasting effect, pulsatile drug release systems for colonic delivery, and microencapsulation of papain, which has various release qualities depending on pH level. Decolorization is achieved using activated carbon derived from fruit husk, which has equivalent adsorptive capacity to commercial goods. The fruit shell acts as a biosorbent, reducing the toxicity of chromium in electroplating effluent. Pods include activated carbon, which can absorb food pigments and heavy metals like nickel and lead from aqueous solutions. The flowers' aqueous extract is used to dye clothes, creating a fade-resistant reddish-brown hue, as well as a component in herbal sunscreen formulas. The bark of D. regia is used to monitor and accumulate ambient trace metals, as well as to remove nickel from aqueous solutions by adsorption. Furthermore, a chloroform extract of the bark has shown termiticidal effects, killing 80% of termites within 48 hours (Modi et al., 2016).

2.15.2 Asuro (Adhatoda vasica)

Adhatoda vasica commonly called *adosa* or *asuro*, is a tiny, evergreen shrub that grows in Asia, throughout India, Sri Lanka, Nepal, Pakistan, Indonesia, Malasia, Panama and China. It has height of 1-3 feet and many long opposite branches. It is a member of the *Acanthaceae* family and has numerous traditional ayurvedic uses. It has stimulating impact on respiratory system. Because of its antispasmodic and expectorant properties, it has been used successfully for the treatment of respiratory disorders such as chronic bronchitis and asthma. In some areas of India. it is also used to stimulate uterine contraction. The *vasica* leaves are also used to treat rheumatic pain and urinary tract infections (Gangwar and Ghosh, 2014). It is also utilized for a cough, breathing difficulties, nasal congestion, bleeding disorders, allergic conditions, upper respiratory infections, excessive uterine bleeding, heavy menstruation, and epistaxis (nosebleed) *Adhatoda vasica* (vasaka) contains mucolytic, expectorant, and bronchodilator properties, hence it is often utilized in respiratory disorders. It reduces a cough, combats respiratory infections, and aids in the control of asthma. (Singh,

2017). At eastern part of Nepal, it has been used for the ripening of bananas as natural ripening agent (Ruwali *et al.*, 2022).

Adhatoda vasica (vasaka) contains numerous active principles, which are most likely responsible for its medicinal benefits. These include vasicine, a quinazoline alkaloid; vasicine acetate; vasicinone, vasicinolone; and 2-acetyl benzyl. It has a wide range of therapeutic characteristics that make it extremely useful in treating a variety of health disorders. It contains powerful antitussive effects. It relieves a cough. Animal tests revealed that the effects on cough suppression were equivalent to codeine. It is used in ayurvedic medicine when a patient coughs up yellow, thick sputum while also having a fever, wheezing, or trouble coughing up mucus. It is also known to have anti-inflammatory properties. It eases asthma and lessens lung and airway irritation. Moreover, the vasicine ingredient in this plant is a bronchodilator, which facilitates breathing and lessens asthmatic wheezing. It possesses antimicrobial and antibacterial properties. As a result, it helps with a range of respiratory system bacterial infections. It is useful in treating viral illnesses because of its antiviral therapeutic properties. When nasal discharge is heavy and yellow during the common cold, ayurvedic practitioners prescribe it (Singh, 2017).

Adhatoda vasica contains bronchial tube-cleaning properties. Because of its expectorant properties, it lessens mucus thickening and bronchial tube irritation. It unclogs the bronchial tubes and lessens sputum production, coughing, exhaustion, dyspnea, and chest pain related to bronchitis. It is found useful in treating sore throat, throat pain and tonsillitis. It decreases redness, pain, and inflammation of soft palate. It possesses anti-ulcer properties. It helps with ulcerations and bleeding issues. It works well for acidity, gastritis, and dyspepsia. It lessens the stomach's production of acid. Researchers have found that in patients with gastritis, hyperacidity, and non-ulcer dyspepsia, it lowers both free and total HCL. It also lessens the inflammation in joints. When combined with other herbs, it helps to lower elevated uric acid as well as gout discomfort and tenderness (Singh, 2017).

2.16 Sensory characteristics of ripe bananas

When bananas are ripe, they usually have a bright yellow peel, however certain varieties may have green color and brown spots depending upon varieties and how the ripe. The peel is smooth and may be slightly waxy. Ripe bananas chew easily because of their smooth, and soft texture. The fruits are smooth with a slight firmness, but it becomes softer as it ripens further. The flavor of ripe bananas is sweet and tropical, sometimes characterized as fruity or floral. As the bananas ripen its aroma intensifies. Bananas have a mostly sweet taste with a subtle acidity that gives the flavor profile more depth. Additionally, ripe bananas may have faint caramel or vanilla flavors. Bananas are a sweet fruit that naturally leaves a little sticky aftertaste on the palate. When eaten, they have a smooth and creamy mouthfeel that is gratifying and easily digested (Sampiano and Durban, 2022).

2.17 Shelf-life of the banana

After being harvested, bananas cannot be stored for extended period of time because it is fragile and perishable (Othman *et al.*, 2021). Bananas may be kept for approximately three weeks at a temperature of just above 55° F (13° C) with a relative humidity of 85 to 95% (Ikisan, 2018). Ruwali *et al.* (2022) has mentioned that storage life of banana not only depends on the post-harvest handling of the bananas but also on the ripening agents those are used for banana ripening. Artificial chemical ripening agents like CaC_2 induce ripening of fruits at faster rate but the fruits ripened with such chemical agents have shorter storage life (Hossain *et al.*, 2015; Roy *et al.*, 2021).

Part III

Materials and methods

3.1 Materials

3.1.1 Banana

The green mature banana (*Musa cavendishii*) which was locally available in Dharan was selected for ripening during ashad month.

3.1.1.1 Sampling of banana

Triplicate sample was prepared from one or two adjacent combs of a bunch, and the base of each was covered with plastic tape. Each sample possessed 6 fingers. From the triplicate sample, out of three one was used for ripening with the leaf of *siris*, one was used for ripening with the leaf of *asuro*, and another was used for ripening with carbide.

3.1.2 Leaf of siris

Leaf of *siris (Delonix regia)* was brought from local area of Dharan-14. Leaf from all parts of plant was used.

3.1.3 Leaf of asuro

Leaf of *asuro (Adhatoda vasica)* was brought from local area of Dharan-14. Leaf of all parts of plant was used.

3.1.4 Chemicals and apparatus

All the chemicals, laboratory glassware and equipment used for study were analytical grade quality and obtained from Central Campus of Technology laboratory. The major apparatus and chemicals required are listed in Appendix D.

3.2 Methods

3.2.1 Experimental Procedure

Optimum banana to leaf ratio (on the basis of weight) was determined by trial-and-error method. The banana to leaf ratio that was able to ripe the banana in the shortest period of time was considered optimal. Banana to leaf ratio of 3:1, 2:1, 1:1, 1:2, 1:3 was used. The mature green banana samples, which were free from damage, were taken. The samples were ripened with different ripening methods (Ripening with natural leaves and ripening with carbide). Analysis of ripened bananas was carried out along with respiration rate and shelf life.

3.2.2 Ripening method

3.2.2.1 Ripening using calcium carbide

The sample was placed inside the jute sack at a temperature of 29°C–31°C and a relative humidity of 90%–95%. The carbide at the rate of 2 g per kg of fruit was wrapped in newspaper and placed with the sample inside the jute sack.

3.2.2.2 Ripening using leaves

The sample was kept between the layers of leaves inside the jute sack at a temperature of $29^{\circ}C-31^{\circ}C$ and relative humidity of 90%-95%.

3.2.3 Analytical method

3.2.3.1 Determination of moisture

Moisture content of the samples was determined according to Ranganna (1986) by hot air oven method by using the gravimetric method.

3.2.3.2 Determination of crude protein

Crude protein of raw and ripened protein was determined by using micro-Kjeldahl method with conversion factor 6.25 as described in Ranganna (1986).

3.2.3.3 Determination of crude fiber

Crude fiber of raw and ripened banana was determined as described in Ranganna (1986).

3.2.3.4 Determination of crude fat

Crude fat of raw and ripened banana was determined by solvent extraction method using petroleum ether as mentioned by Ranganna (1986).

3.2.3.5 Determination of total ash

Total ash of raw and ripened banana was determined by dry ashing method as described in Ranganna (1986).

3.2.3.6 Determination of carbohydrate

Carbohydrate content of the banana was determined by difference method.

Carbohydrate (%) = 100 - (moisture + crude protein + crude fat + crude fiber + total ash)

3.2.3.7 Determination of reducing sugar

Reducing sugar was determined by the Lane and Eynon method mentioned by Ranganna (1986).

3.2.3.8 Determination of total sugar

Total sugar was determined by the Lane and Eynon method mentioned by Ranganna (1986).

3.2.3.9 Determination of pH

pH of the sample was determined by using pH meter. pH meter was calibrated using buffer solution of pH 7 and pH 4 at the required temperature.

3.2.3.10 Determination of titratable acidity

Titratable acidity of the banana was determined as mentioned in Ranganna (1986).

3.2.3.11 Determination of respiration rate

Initially, air was made free of CO_2 by passing the air through 40% potassium hydroxide solution. Then it was passed through the respiring bananas where CO_2 respired by the produce was collected in calcium hydroxide solution. Thus collected CO_2 concentration was determined by gravimetric method (i.e. by taking weight of calcium carbonate precipitated) which was finally related with the total CO_2 produced (Schippers, 1977).

3.2.3.12 Determination of shelf-life

Shelf life was assessed by color inspection of fruits at alternate days. Bananas ripened with different techniques were placed in a room (separate location) with the identical condition of a temperature of 29-32° C and an RH of 90-95 % without using packaging materials.

3.2.4 Sensory analysis

For sensory analysis 9 points hedonic rating (1= dislike extremely, 9= like extremely) method as mentioned by Ranganna (1986) was used. The semi-trained panelist members consisted of research students and faculties of Central Campus of Technology, Dharan who had some previous experience in sensory analysis. Using the ANOVA (no blocking) and LSD at 5% level of significance with the help of software Genstat 12th edition, version 12.1.0, 2009, the difference in sensory characteristics was determined. Sensory table obtained is presented in appendix B.

3.2.5 Statistical analysis

The data were examined using a two-way ANOVA with no blocking at the 5% level of significance. The LSD method was to compare the treatment means (Genstat 5 Version 12.1, Lawes Agricultural Trust, Rothamsted Experimental 35 Station, 2009). Tukey test was used for the post hoc test. All the referencing was done using Endnote X9.

Part IV

Results and discussion

The present study was conducted to optimize the banana to leaf ratio for ripening of the bananas, and to study the ripening of bananas using leaf of *siris*, and *asuro* and carbide under similar conditions. Respiration rate along with changes in physiological properties; acidity, pH, and TSS of bananas ripened with different methods during storage were also measured. Results and discussion of the overall study are described in the following headings.

4.1 Proximate composition of matured unripe banana

Proximate composition of matured unripe banana is shown in table 4.1

Parameters	Values*
Moisture %	65.79 ± 0.39
Protein % (db)	3.59 ± 0.23
Fat % (db)	1.69 ± 0.03
Ash % (db)	1.64 ± 0.02
Fiber % (db)	11.78 ± 0.17
Carbohydrate %(db)	80.01 ± 0.46

Table 4.1 Proximate composition of matured unripe banana

Values are the means of three determinations \pm standard deviations.

The value for moisture, protein, fat, ash, carbohydrate and fiber of banana in dry basis (except for moisture content) was found to be 65.79%, 3.59%, 1.69%, 1.64%, 80.01% and 11.78% respectively. The proximate values of the fresh bananas found in our study were in line with the study performed by Kookal and Thimmaiah (2018), though the values of moisture content is slightly higher. Difference in moisture content and crude fat may be due to the difference in variety of bananas, location of cultivation, altitude, and climatic

condition. The values determined are in line with USDA (2019) and Kookal and Thimmaiah (2018).

4.2 Optimization of banana to leaf ratio

The banana to leaf ratio used for the ripening of bananas under similar conditions of a temperature of 29-31°C and a relative humidity of 90-95% is given in table 4.2 below.

Leaf used	Banana: leaf (weight basis)	Time (hours)
	3:1	-
	2:1	66
Asuro	1:1	76
	1:2	-
	1:3	-
	3:1	-
	2:1	60
Siris	1:1	69
	1:2	-
	1:3	-

Table 4.2 Banana to leaf ratio that was used for ripening of banana

"-" sign indicates fail to ripen before 4 days.

For both leaves; *siris* and *asuro*, the banana to leaf ratio of 2:1 was found to be optimal ratio for the ripening of bananas. It took 60 hours and 66 hours to ripen the banana for the leaves of *siris* and *asuro* respectively. Table 4.1 shows that leaves lower than or equal to 1/3 part and more than or equal to 2 parts of the bananas were not able to ripen the bananas before four days. From this, it can be said that bananas do not ripen by the warmth produced by a larger number of leaves.

 $CO_2>1\%$ reduces the effectiveness of ethylene (Kader, 1997). From this study, we can say that the use of an excess number of leaves produces $CO_2>1\%$ as a result the effectiveness of ethylene towards ripening has ceased.

4.3 Ripening of banana by the leaf of siris and asuro and carbide

Time period required to ripen the bananas by using different three ripening methods at a temperature of 29-31°C and a relative humidity of 90-95% is tabulated in table 4.3 below.

Ripening agent	Time (hours)
Asuro	66
Siris	60
Carbide	49
Control	171

Table 4.3 Time required to ripen bananas by different methods

The time required to ripen the bananas for CaC_2 (i.e. 49 hours) was lower than that of leaves of *asuro* and *siris*, whereas banana ripened with *asuro* and *siris* leaves ripened faster than the control sample.

The sample treated with CaC_2 was ripened by the action of acetylene gas from carbide (Okeke *et al.*, 2022), whereas the control was ripened by ethylene gas from fruit under favorable conditions of temperature and relative humidity (Burg and Burg, 1965). The samples ripened with leaves were ripened by the ripening factor from leaves, not only due to the combination of temperature, relative humidity, and ethylene produced by the fruit, because the control took 171 hours to ripen, but the samples ripened with *siris* and *asuro* took 60 hours and 66 hours, respectively. So, there were some factors that were responsible for the ripening of bananas with the leaves. The long time for the ripening of the control was due to the lack of an adequate amount of ethylene produced by the fruits, and the short time for the ripening of the bananas with the leaves might be due to the production of adequate ethylene from the leaves and fruit since leaves produce ethylene gas at senescence (Aharoni *et al.*, 1979).

4.4 Proximate and physiochemical composition of ripened banana

Proximate and physiochemical composition of ripened banana is given in table 4.4 and 4.5 respectively.

Parameters	Banana treated with <i>siris</i>	Banana treated with <i>asuro</i>	Banana treated with carbide
Moisture %	$70.44^{a} \pm 0.73$	$72.08^b \pm 0.36$	$75.78^{\circ} \pm 0.22$
Protein %	$1.32^{\text{b}} \pm 0.02$	$1.31^b\pm0.04$	$1.02^{a} \pm 0.06$
Fat %	$0.58^{a}\pm0.02$	$0.53^{a}\pm0.07$	$0.48^{a} \pm 0.04$
Ash %	$0.47^{ab}\pm0.04$	$0.54^b \pm 0.06$	$0.42^{a}\pm0.04$
Fiber %	$2.69^b \pm 0.02$	$2.50^b \pm 0.06$	$2.09^{a}\pm0.12$
Carbohydrate %	$24.7^{c}\pm0.96$	$23.09^b\pm0.31$	$20.13^{a}\pm0.42$

Table 4.4 Proximate composition of ripened banana

Values are the means of three determinations \pm standard deviations.

The value of moisture, protein, fat, ash, carbohydrate, and fiber in wet basis was found to be 70.44%, 1.32%, 0.58%, 0.47%, 24.7%, and 2.69% respectively for the banana ripened with the leaf of *siris*, those values for the bananas ripened with the leaf of *asuro* were found to be 72.08%, 1.31%, 0.48%, 0.54%, 23.09%, and 2.50% respectively, and the values of the same parameters for the bananas ripened with carbide were found to be 75.78%, 1.02%, 0.53%, 0.42%, 20.13%, and 2.09% respectively. These results are in line with Nuhu *et al.* (2020) and USDA (2019). Ash content was slightly lower than that reported by Nuhu *et al.* (2020) that might be due to difference in cultivars and location.

The sample ripened with carbide had the lowest protein, fat, ash, fiber, and carbohydrate as compared to others that agrees with the Oguntade and Fatumbi (2019) and also with the statement that, the ripening agent such as carbide has poor nutritional quality as mentioned by Temi *et al.* (2014).

There was significant difference in moisture content, between all three samples at P<0.05. The moisture content was the highest in the banana ripened with carbide i.e. 75.78% and lowest in banana ripened with *siris* i.e. 70.44%. The higher amount of the moisture content in the banana ripened with the carbide is due to the chemical weakens the peel's fiber, making moisture more easily absorbed. Moisture content affects the shelf life and storage stability of fruit samples (Nuhu *et al.*, 2020).

At P<0.05, there was no significant difference in protein, ash, and fiber content of the bananas ripened with the leaf of *asuro* and *siris*. But the values were significantly different from the bananas ripened with carbide.

At P<0.05, there was no significant difference in the crude fat content of bananas ripened with leaf of *siris* and *asuro* and carbide. Though, the value for fat content was higher in banana ripened with leaf of *siris* followed by leaf of *asuro* and carbide.

Physiochemical composition of the samples (immediate after ripening) ripened with different three methods in the same condition is shown in table 4.5 below.

Parameters	Banana treated with <i>siris</i>	Banana treated with <i>asuro</i>	Banana treated with carbide
Acidity %	$0.36^{c} \pm 0.01$	$0.34^b\pm0.01$	$0.28^{a}\pm0.01$
Reducing sugar %	$10.95^b \pm 0.58$	$10.99^{b} \pm 0.62$	$6.11^{a}\pm0.32$
Total sugar %	$16.89^b \pm 0.78$	$16.44^b\pm0.43$	$10.79^{a}\pm0.41$
Non reducing sugar %	$5.94^{a}\pm0.69$	$5.45^{a}\pm0.52$	$4.68^{a}\pm0.13$
Respiration rate (mgCO ₂ /Kg/hour)	$71.89^{a} \pm 3.23$	$68.09^{a} \pm 2.68$	$80.26^{b} \pm 1.77$
pH	$4.48^{a}\pm0.01$	$4.51^b\pm0.02$	$4.92^{c}\pm0.01$
TSS (°Bx)	$22^b \pm 0.25$	$22.25^b\pm0.25$	$17.22^{a} \pm 0.28$

 Table 4.5 Physiochemical composition of ripened banana

Values are the means of three determinations \pm standard deviations.

There was significant difference in acidity of three samples at P<0.05. Acidity was the highest for the sample ripened with leaf of *siris* (0.35%) and the lowest for the sample ripened with carbide (0.28%). Acidity of the sample ripened with leaf of *asuro* was found to be 0.34%. Carbide treated fruit showed highest titratable acidity and this might be due to faster ripening leading to incomplete hydrolysis of starch during the ripening of fruits. This result was in conformity with Das and Balamohan (2013).

At P<0.05, there was no significant difference in reducing sugar and total sugar between the bananas ripened with the leaf of *siris* and *asuro*, but was different significantly with the sample treated with carbide. Reducing sugar and total sugar were found to be 10.95% and 16.89% for the sample ripened with the leaf of *Siris*, 10.99% and 16.44% for the sample ripened with the leaf of *asuro* and 6.11% and 10.79% for the sample treated with the carbide. Reducing sugar as well as total sugar were lowest in the carbide treated sample as mentioned by Roy *et al.* (2021). There was no significant difference in the non-reducing sugar content between the samples ripened with the leaf of *siris* and *asuro* and the carbide though the values were 5.94%, 5.49%, and 4.68% respectively.

There was no significant difference in TSS between samples treated with leaves of *siris* and *asuro* at P<0.05. TSS of the bananas ripened with the leaf of *siris* and *asuro* was found to be 22°Bx and 22.25°Bx respectively. The results are in line with the values reported by Roy *et al.* (2021). The TSS of the bananas ripened with carbide was found to be 17.22°Bx which is lower than that of the bananas ripened with natural ripening agents, this result agrees with the report mentioned by Roy *et al.* (2021).

There was no significant difference (P<0.05) in respiration rate between the samples ripened with the leaf of *siris* and *asuro* but was different significantly with the carbide treated sample. The respiration rate was found to be 71.89 mgCO₂/Kg/hour, 68.09 mgCO₂/Kg/hour and 80.26 mgCO₂/Kg/hour for samples ripened with the leaf of *siris* and *asuro* and carbide respectively. The respiration rate of the banana ripened with the CaC₂ was highest that agrees with the report reported by Temi *et al.* (2014). The CaC₂ treated sample had the highest respiration rate and lower the hours of ripening that is due to the acetylene produced form the carbide which mimics natural ripening hormone ethylene. These values obtained are

greater than that was reported by Gane (1936) (i.e. 61 mg CO₂/Kg/hr. at 31°C.) That might be due to the varietal difference and also the use of ripening agents.

4.5 Sensory evaluation of samples

Statistical analysis of sensory scores obtained from 9 semi-trained panelist using 9-point hedonic rating scale (9= like extremely, 1= dislike extremely) for bananas ripened with *Siris* and *Asuro* leaves and carbide. Statistical analysis using ANOVA (No blocking) followed by LSD (5%) was done to find out the difference between the samples with respect to color, flavor, taste, texture, and overall acceptance which is shown in appendix C. The ANOVA and LSD table for sensory evaluation are presented in Appendix B.



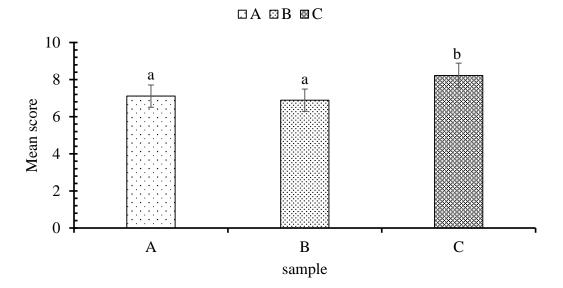
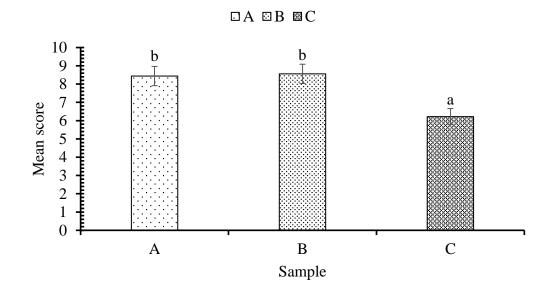


Fig. 4.1 Mean Score of samples in terms of color

The sensory score values of color for all three samples were subjected to two-way ANOVA (No blocking) at 5% level of significance using Genstat 5 version 12.1. The mean scores for color were found to be 7.11, 6.89, and 8.22 for banana ripened with *asuro* (A) and *siris* (B) leaves and carbide (C) respectively. The obtained mean scores are represented as bas diagram in fig. 4.1. Same letters on the top of bar diagram in Fig. 4.1 indicate that there is no significant difference between the samples, whereas different letters on the top indicate that the samples are significantly different with respect to color at P<0.05.

The banana ripened with the CaC_2 had the highest mean score for color as compared to that of banana ripened with *asuro* and *siris* leaves and also was significantly different with them, but there was no significant difference in color between the sample ripened with the *asuro* and *siris leaves*. According to the score given by the panelists, the bananas ripened with the CaC₂ had good peel color development as mentioned by Roy *et al.* (2021) and Hossain *et al.* (2015).



4.5.2 Flavor

Fig. 4.2 Mean Score of samples in term of flavor

The sensory score values of flavor for all three samples were subjected to two-way ANOVA (No blocking) at 5% level of significance using Genstat 5 version 12.1. The mean scores for flavor were found to be 8.44, 8.56, and 6.22 for banana ripened with *asuro* (A) and *siris* (B) leaves and carbide (C) respectively. The obtained mean scores are represented as bar diagram in Fig. 4.2. Same letters on the top of bar diagram in Fig. 4.2 indicate that there is no significant difference between the samples, whereas different letters on the top indicate that the samples are significantly different with respect to flavor at P<0.05.

Though, the mean score of flavors for the bananas ripened with the *siris* leaves was the highest, there was no significant difference between the samples ripened with *siris* leaves and *asuro* leaves, but that was significantly different with the bananas ripened with carbide

at P<0.05. The bananas ripened with CaC_2 had poorer flavor than others that agrees with (Singal *et al.*, 2012)

4.5.3 Taste

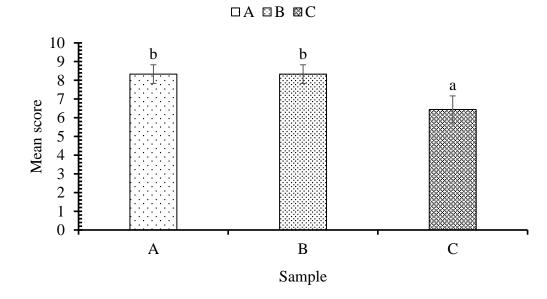


Fig. 4.3 Mean score of samples in terms of taste

The sensory score values of taste for all three samples were subjected to two-way ANOVA (No blocking) at 5% level of significance using Genstat 5 version 12.1. The mean scores for taste were found to be 8.33, 8,33, and 6.44 for banana ripened with *asuro* (A) and *siris* (B) leaves and carbide (C) respectively. The obtained mean scores are represented as bas diagram in Fig. 4.3. Same letters on the top of bar diagram in Fig. 4.3 indicate that there is no significant difference between the samples, whereas different letters on the top indicate that the samples are significantly different with respect to taste at P<0.05.

In terms of taste, there was no significant difference (P<0.05) between the samples ripened with the leaves of *asuro* and *siris*, but those were significantly different with the sample ripened with carbide. Bananas ripened with carbide had the poorest value for taste, as reported by Roy *et al.* (2021)

4.5.4 Texture

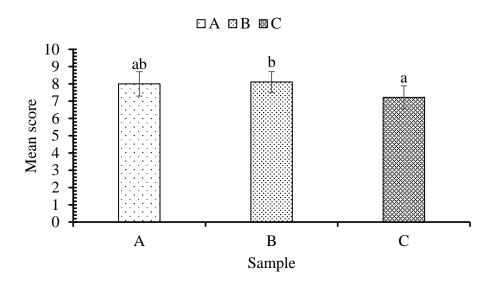


Fig. 4.4 Mean score of samples in terms of texture

The sensory score values of texture for all three samples were subjected to two-way ANOVA (No blocking) at 5% level of significance using Genstat 5 version 12.1. The mean scores for texture were found to be 8, 8.11, and 7.22 for banana ripened with *asuro* (A) and *siris* (B) leaves and carbide (C) respectively. The obtained mean scores are represented as bas diagram in Fig. 4.4. Same letters on the top of bar diagram in Fig. 4.4 indicate that there is no significant difference between the samples, whereas different letters on the top indicate that the samples are significantly different with respect to texture at P<0.05.

At 5% level of significance, sample ripened with leaf of *siris* and sample ripened with carbide were significantly different in terms of texture, but there was no significant difference between sample ripened with *asuro* leaves and *siris* leaves. The mean score value of texture was the lowest for the sample ripened with carbide, which is because chemical ripening agents trigger the cellular fragmentation of the insoluble protopectin and result in gradual decrease in fruit firmness (Farouq and Fashoranti, 2022).

4.5.5 Overall acceptance

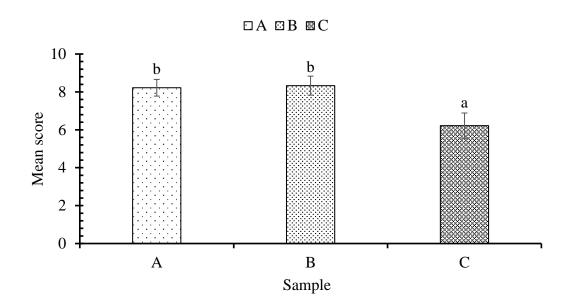


Fig. 4.5 Mean score in terms of overall acceptance

The sensory score values of overall acceptance for all three samples were subjected to twoway ANOVA (No blocking) at 5% level of significance using Genstat 5 version 12.1. The mean scores for overall acceptance were found to be 8.22, 8.33, and 6.22 for banana ripened with *asuro* (A) and *siris* (B) leaves and carbide (C) respectively. The obtained mean scores are represented as bas diagram in Fig. 4.5. Same letters on the top of bar diagram in Fig. 4.5 indicate that there is no significant difference between the samples, whereas different letters on the top indicate that the samples are significantly different with respect to overall acceptance at P<0.05.

Although, the mean sensory score of overall acceptability for the banana ripened with *siris* leaves has the highest value, there was no significant difference between the sample ripened with leaves of *asuro* and *siris*. But the samples ripened with leaves were significantly different with the sample treated with CaC₂. Consumer's acceptability of banana fruits depends on color, aroma, and taste (Roy *et al.*, 2021). From this study it was observed that although the bananas ripened with carbide had good peel color, it had poor flavor.

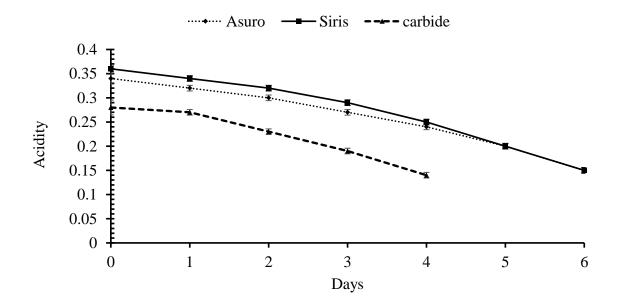
4.6 Storage-life of the bananas ripened with different methods

Sample	Storage-life (days)
Ripened with asuro	6
Ripened with siris	6
Ripened with carbide	4

Table 4.6 Storage-life of the bananas ripened with different methods

Table 4.6 shows that bananas ripened with carbide had the lowest storage life of 4 days and bananas ripened with natural ripening agents (leaves of *asuro* and *siris*) had storage-life of 6 days when stored at similar condition of a temperature of 29-32° C and a relative humidity of 90-95%. Lower storage life of the bananas treated with the carbide in this study is in conformity with the report provided by Roy *et al.* (2021). When calcium carbide is applied, the banana fruits' respiration rate increases and more heat is released, causing rapid ripening and, ultimately, faster degradation or a shorter storage life (Roy *et al.*, 2021).

4.7 Change in physicochemical properties of different samples during storage



4.7.1 Acidity

Fig. 4.6 Change in acidity of the samples during storage

Acidity of the banana ripened with *asuro* leaves and *siris leaves* gradually decreased from 0.34% to 0.15% and 0.36% to 0.15% respectively during six days of storage. There was decrease in acidity of sample ripened with carbide from 0.3% to 0.14% during 4 days of storage. Zomo *et al.* (2014) also reported, titratable acidity decreased gradually with the passage of the storage period and this statement also agrees with Ruwali *et al.* (2022). The decrease in acidity is attributed to utilization of organic acids in various bio-degradable reactions (Zomo *et al.*, 2014).

4.7.2 TSS

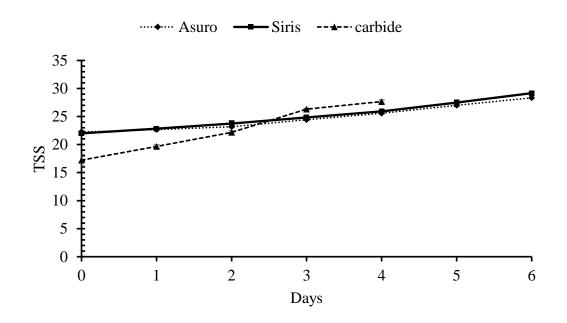


Fig. 4.7 Change in TSS of the samples during storage

TSS of the banana ripened with *asuro* leaves and *siris leaves* gradually increased from 22.25 °Bx to 28.33 °Bx and 22 °Bx to 29.17 °Bx respectively during six days of storage. There was increase in TSS of sample ripened with carbide from 17.22 °Bx to 27.67 °Bx during 4 days of storage. The highest TSS 29.17 °Bx was observed in the banana ripened with *siris* leaves in 6th day of storage. The increase in TSS observed in this study agrees with the report provided by Akter *et al.* (2013). The increase in TSS of bananas during storage is a result of continued metabolic processes, including starch conversion, respiration etc.

4.7.3 pH

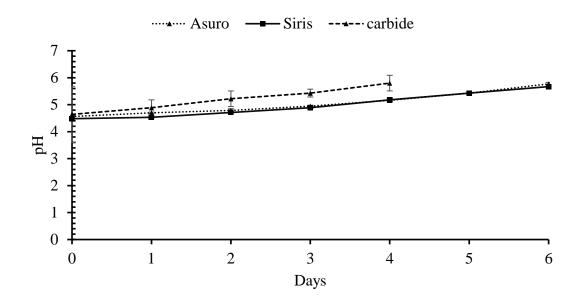


Fig. 4.8 Change in pH of the samples during storage

There was slight increase in pH from 4.56 to 5.77 and 4.48 to 5.67 during the 6 days of storage life for the bananas ripened with leaves of *asuro* and *siris* respectively. The pH of the carbide treated sample increased from 4.64 to 5.8 in fourth days of storage. Zomo *et al.* (2014) has also reported that, pH of the bananas increases during the storage period which is the result of decrease in acidity.

Part V

Conclusion and recommendation

5.1 Conclusions

On the basis of work done following conclusions can be drawn.

- 1. Best banana to leaf ratio for ripening of banana was found to be 2:1 for optimal ripening.
- 2. From analysis, it was found that, banana ripening using leaf of *asuro* and *siris* is an efficient method for banana ripening.
- 3. From the analysis, it was found that, the bananas ripened with the leaves are superior in terms of nutrition as carbide treated bananas have lowest value for protein, carbohydrate, fat, ash, fiber, reducing sugar and total sugar.
- 4. According to sensory analysis, except for color, bananas ripened with the leaves are superior in terms of taste, flavor, texture, and overall acceptability.
- 5. Shelf-life of bananas ripened with carbide is lower than that of bananas ripened with *asuro* and *siris* leaves.

5.2 Recommendations

Based on this study, following recommendations have been made

- 1. Leaves of *dhursilo*, *raj brikshya* can be used for the ripening of banana.
- 2. Study on ripening of banana using different indigenous methods like smoking, ripe tomato, straw can be done.
- 3. Further analysis on study of storage life of banana using different packaging materials can be done.

Part VI

Summary

Bananas are one of the most popular and widely consumed fruits globally; they have both nutritional and medicinal importance. Ripening is a complex process by which fruits undergo physiological, biochemical and structural changes that make them more palatable, edible and attractive. For the purpose of uniform and timely ripening and minimizing loss during ripening of banana, different kinds of ripening agents are used. Mostly, chemical ripening agent carbide is used for the ripening of bananas though, it is prohibited to use because of its health and environmental hazards.

In this study, bananas were taken from a local market in Dharan. *Asuro* and *siris* leaves were collected from the periphery of CCT at Dharan-14. This study was carried out with the purpose of determining the optimum banana-leaf ratio to ripen the bananas and differences in physiochemical and sensory properties and storage life of samples ripened with different ripening agents. From the same bunch of bananas, samples were prepared and subjected for ripening using leaves and carbide under similar conditions of a temperature of 29-32°C and a Rh of 90-95%. Two-way ANOVA (no blocking) at 5% level of significance was used to determine if there was any significant difference between the samples. *Asuro* and *siris* leaves and carbide required 66, 60, 49 hours respectively to ripen the bananas and banana-leaf ratio of 2:1 was found to be optimal.

The value for moisture content of bananas was the highest (75.78%) for the bananas ripened with carbide among three samples. Protein%, fat%, ash%, carbohydrate% and fiber% were maximum in the sample treated with leaves with the value of 70.44, 1.32, 0.58, 0.4, 24.7 and 2.69 for bananas ripened with *Siris* leaves and 1.31, 0.53, 0.54, 23.09 and 2.50 for the bananas ripened with *asuro* leaves and those values for carbide treated bananas was found to be 1.02, 0.48, 0.42, 20.13, and 2.09 respectively. From the sensory analysis, bananas ripened with natural leaves were found to be superior in terms of sensory quality too. Storage life of bananas was found to be 4, 6, 6 days for bananas treated with carbide, *asuro* and *siris* leaves respectively. The natural ripening agents like leaves of *asruro* and *siris* which are non-toxic, cheap and ecofriendly are efficient for the ripening of banana and also give product with better quality in terms of physiochemical, sensory, and storage life.

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Appendices

Appendix A

Sensory evaluation card

Sensory evaluation of banana

Name.....

Date:/.../...

Observe the product by testing. Use appropriate scale to show your attitude by checking at the point that best describes feeling of the products. Write any of defects present described below. An honest expression of your personnel feeling will help me.

Sensory attributes	Sample A	Sample B	Sample C
Color			
Flavor			
Taste			
Texture			
Overall Acceptance			

Judge the above characteristics on the 1-9 scale described as follows:

Like extremely-9	Like slightly-6	Dislike moderately-3
Like very much-8	Neither like nor dislike-5	Dislike very much-2
Like moderately-7	Dislike slightly-4	Dislike extremely-1

Any

comments.....

.....

.....

Signature

Appendix B

ANOVA results of sensory analysis

Table B.1: Two-way ANOVA (no interaction) for color of bananas ripened with *asuro* and *siris* leaves and carbide

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.	l.s.d.
Sample	2	9.1852	4.5926	11.95	<.001	0.619
Panelist	8	3.1852	0.3981	1.04	0.450	1.073
Residual	16	6.1481	0.3843			
Total	26	18.5185				

Table B.2: Two-way ANOVA (no interaction) for flavor of bananas ripened with *asuro* and *siris* leaves and carbide

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.	l.s.d.
Sample	2	31.1852	15.5926	60.14	<.001	0.2400
Panelist	8	1.8519	0.2315	0.89	0.544	0.4157
Residual	16	4.1481	0.2593			
Total	26	37.1852				

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d.
Sample	2	21.4074	10.7037	23.59	<.001	0.673
Panelist	8	0.9630	0.1204	0.27	0.969	1.166
Residual	16	7.2593	0.4537			
Total	26	29.6296				

Table B.3: Two-way ANOVA (no interaction) for taste of bananas ripened with *asuro* and *siris* leaves and carbide

Table B.4: Two-way ANOVA (no interaction) for texture of bananas ripened with *asuro* and *siris* leaves and carbide

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.	l.s.d.
Sample	2	4.2222	2.1111	4.34	0.031	0.697
Panelist	8	2.6667	0.3333	0.69	0.399	1.207
Residual	16	7.7778	0.4861			
Total	26	14.6667				

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.	l.s.d.
Sample	2	25.4074	12.7037	44.26	<.001	0.535
Panelist	8	2.5185	0.3148	1.10	0.414	0.927
Residual	16	4.5926	0.2870			
Total	26	32.5185				

Table B.5: Two-way ANOVA (no interaction) for overall acceptance of bananas ripened

 with *asuro* and *siris* leaves and carbide

Appendix C

Table C.1 Mean score value of sensory parameters of bananas ripened with the *asuro* and *siris* leaves and carbide.

Sample	Mean value for color	Mean value for flavor	Mean value for taste	Mean value for texture	Mean value for overall acceptance
А	7.11 ^a (0.60)	8.44 ^b (0.53)	8.33 ^b (0.5)	8 ^{ab} (0.71)	8.22 ^b (0.44)
В	6.89 ^b (0.60)	8.56 ^b (0.52)	8.33 ^b (0.5)	8.11 ^b (0.60)	8.33 ^b (0.5)
С	8.22 ^c (0.67)	6.22 ^a (0.44)	6.44 ^a (0.72)	7.22 ^a (0.71)	6.22 ^a (0.67)

A = Banana ripened with *asuro* leaves

B = Banana ripened with *siris* leaves

C = Banana ripened with carbide

Figures are arithmetic means of scores in the above table. Figures in parenthesis are standard deviations. Figures in the column having the same superscript are not significantly different and having different superscript are significantly different at 5% level of significance.

Appendix D

Apparatus

- 1 Mortar and pestle
- 2 Knives
- 3 Chopping board
- 4 Bunsen burner
- 5 Thermometer
- 6 Digital electronic balance
- 7 Beaker
- 8 Volumetric flask
- 9 Measuring cylinder
- 10 Conical flask, funnel, test tube
- 11 Soxhlet assembly
- 12 Bushner filter assembly
- 13 Petri plate
- 14 Hot air oven
- 15 Filter paper
- 16 Respiration rate assembly
- 17 Micro-Kjeldahl assembly

Chemical required

- 1 Petroleum ether
- 2 Sulfuric acid
- 3 Sodium hydroxide
- 4 Oxalic acid
- 5 Hydrochloric acid
- 6 Boric acid
- 7 Catalyst mixture
- 8 Phenolphthalein
- 9 Methylene blue
- 10 Methyl red
- 11 Acetic acid

- 12 Distilled water
- 13 Buffer solution
- 14 Fehling's solution
- 15 Carrez solution I and II

Appendix E

ANOVA result for physiochemical properties of bananas ripened with different methods

Table E.1: One-way ANOVA (no blocking) for Respiration rate of bananas ripened with

 asuro and *siris* leaves and carbide

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d.
Sample	2	232.469	116.234	16.78	0.003	5.258
Residual	6	41.565	6.928			
Total	8	274.034				

Table E.2: One-way ANOVA (no blocking) for TSS of bananas ripened with *asuro* and *siris*

 leaves and carbide

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.	l.s.d.
Sample	2	0.2639	0.1319	0.33	0.733	1.268
Residual	6	2.4167	0.4028			
Total	8	2.6806				

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.	l.s.d.
Sample	2	2.4188	1.2092	4.68	0.060	1.015
Residual	6	1.5493	0.2582			
Total	8	3.9681				

Table E.3: One-way ANOVA (no blocking) for non-reducing sugar of bananas ripened with

 asuro and *siris* leaves and carbide

Table E.4: One-way ANOVA (no blocking) for pH of bananas ripened with *asuro* and *siris*

 leaves and carbide

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.	l.s.d.
Sample	2	0.04002	0.020	66.70	<.001	0.035
Residual	6	0.0018	0.0003			
Total	8	0.0418				

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.	l.s.d.
Sample	2	47.3064	23.6532	87.11	<.001	1.041
Residual	6	1.6292	0.2715			
Total	8	48.935				

 Table E.5: One-way ANOVA (no blocking) for reducing sugar of bananas ripened with

 asuro and *siris* leaves and carbide

Table E.6: One-way ANOVA (no blocking) for total sugar of bananas ripened with *asuro* and *siris* leaves and carbide

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.	l.s.d.
Sample	2	69.3787	34.689	108.57	<.001	1.129
Residual	6	1.917	0.3195			
Total	8	71.2958				

Appendix F

ANOVA results for proximate composition of the bananas ripened with different methods

Table F.1: One-way ANOVA (no blocking) for ash content of the bananas ripened with

 asuro and *siris* leaves and carbide

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.	l.s.d.
Sample	2	0.02295	0.011478	5.77	0.04	0.0891
Residual	6	0.01193	0.001989			
Total	8	0.03488				

Table F.2: One-way ANOVA (no blocking) for Carbohydrate content of the bananas

 ripened with *asuro* and *siris* leaves and carbide

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.	l.s.d.
Sample	2	32.2798	16.1399	40.18	<.001	1.266
Residual	6	2.4099	0.4017			
Total	8	34.6898				

Source of variation	d.f.	S.S.	m.s.	V.ſ.	F pr.	l.s.d.
Sample	2	0.558956	0.279478	46.93	<.001	0.1542
Residual	6	0.035733	0.005956			
Total	8	0.594689				

Table F.3: One-way ANOVA (no blocking) for crude fiber content of bananas ripened with

 asuro and *siris* leaves and carbide

Table F.4: One-way ANOVA (no blocking) for crude fat content of bananas ripened with

 asuro and *siris* leaves and carbide

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.	l.s.d.
Sample	2	0.013267	0.006633	3.34	0.106	0.0891
Residual	6	0.011933	0.001989			
Total	8	0.025200				

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.	l.s.d.
Sample	2	44.8625	22.4312	94.45	<.001	0.974
Residual	6	1.4249	0.2375			
Total	8	46.2874				

Table F.5: One-way ANOVA (no blocking) for moisture content of bananas ripened with

 asuro and *siris* leaves and carbide

Table F.5: One-way ANOVA (no blocking) for crude protein content of bananas ripened

 with *asuro* and *siris* leaves and carbide

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.	l.s.d.
Sample	2	0.168467	0.084233	51.92	<.001	0.0805
Residual	6	0.009733	0.001622			
Total	8	0.178200				

Color Plates



P1: Raw banana



P2: Preparation of banana for ripening



P3: Banana ripened with *asuro* leaves



P4: Banana ripened with *siris* leaves



P5: Banana



P6: Analysis of sample