EFFECT OF DRYING TEMPERATURE AND RHIZOME SIZE ON BIOACTIVE COMPONENTS OF TURMERIC (*Curcuma Longa* L.)

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Effect of Drying Temperature and Rhizome Size on Bioactive Components of Turmeric (*Curcuma Longa* L.)

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Approval Letter

This dissertation entitled Effect of Drying Temperature and Rhizome Size on Bioactive Components of Turmeric (Curcuma longa L.) presented by Ghanshyam Aryal has been accepted as the partial fulfillment of the requirements for the B.Tech. degree in Food Technology.

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Abstract

The aim of this study was to optimize the rhizome size and drying temperature to produce high quality turmeric powder. The turmeric rhizomes were collected from local farm of Dharan. The rhizomes were cleaned and sorted into primary, secondary and tertiary rhizomes. Secondary rhizomes were taken for the study and cross section area of $2.434 \pm 0.023 \text{ cm}^2$ was maintained. Response surface methodology (randomized, design type D-optimal with quadratic model) was applied using Design Expert version 13 taking factors as rhizome size from 1 to 4 cm and drying temperature from 40 to 70°C which generated sixteen samples. The samples were cut and dried as per the parameters obtained from design expert till the moisture content reached to 10%. The optimum value of rhizome size and drying temperature was obtained by the analysis of five response factors i.e. curcumin, oleoresin, essential oil content, total phenolic content and antioxidant activity. The optimized turmeric powder was compared with the market sun-dried sample.

The optimum value of rhizome size and drying temperature was obtained to be 2.6 cm and 55°C with the value of curcumin, essential oil, oleoresin, TPC and antioxidant activity to be 5.8%, 7.04%, 20.85%, 38.12 mg GAE/ g dry matter and 49.68 %RSA respectively. The optimized turmeric powder was found to be superior in quality than sun-dried market sample in terms of all measured bioactive parameters and antioxidant activity. Thicker rhizome size and lower drying temperature led to the longer drying time which caused the oxidative degradation of bioactive components. Similarly, very high drying temperature and thinner rhizome size led to thermal degradation of bioactive components.

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List of Abbreviations

Abbreviation	Full form
AA	Antioxidant activity
BMC	Bisdemethoxycurcumin
CUR	Curcumin
DMC	Demethoxycurcumin
DPPH	1,1-diphenyl-1-picryl-hydrazyl radical
EO	Essential oil
GAE	Gallic acid equivalent
GLUT	Glucose transporter
HIV	Human immunodeficiency virus
IL	Interleukin
МСР	Monocyte chemoattractant protein
RSM	Response surface methodology
SFE	Supercritical fluid extraction
STM	Secondary thickening meristem
TNF	Tumor necrosis factor
TPC	Total phenolic content
T2D	Type 2 diabetes

Part I

Introduction

1.1 General introduction

Spices have been widely used as condiments for thousands of years because of their flavor, taste and color. Several spices have been used as medicinal plants in folk medicine for the treatment of various diseases because they contain many bioactive compounds and possess a lot of beneficial health effects (Zheng *et al.*, 2016). Spices are dried aromatic plant products used to flavor foods and beverages. They include leaves (rosemary, sage), flowers and flower buds (clove), bulbs (garlic, onion), rhizomes (turmeric), fruit (pepper, cardamom), and other parts of the plant. Frequently, blends of several spices are used (Shelef, 1984).

Curcuma longa, commonly known as turmeric, is a tropical perennial monocotyledonous herbaceous plant widely used and cultivated in South and South-eastern Asia (Nwaekpe *et al.*, 2015). It belongs to the Family *Zingiberaceae*. The term longa refers to the elongated shape of the rhizome, where turmeric is derived from the rhizome of the plant having a characteristic orange-yellow color (Prasad and Aggarwal, 2011). The Latin word *curcuma* is believed to be derived from the Arabic root *kurkum* meaning "saffron," in reference to similar coloring properties (Solymosi *et al.*, 2015). It is an ancient spice and a traditional remedy that has been used as a medicine, condiment and flavoring (Jilani *et al.*, 2012). Turmeric is the golden spice that gives many Asian dishes their yellow color and pungent earthy flavor. It is an essential ingredient of curry powders, accounting for about 10–30% of the blend (Govindarajan and Stahl, 1980). Turmeric has been valued worldwide as a functional food because of its health promoting property (Ammon and Wahl, 1991). Its rhizomes are oblong, ovate, pyriform and often short-branched (Yadav *et al.*, 2013).

Turmeric (*Curcuma longa*) is extensively used as spice, food preservative and coloring material in Nepal, India, China and South East Asia. In the East, turmeric is precious as the therapeutic goldmine inhabits significant position in the psyche of Hindu. It forms an important part of various sanctified Hindu rituals focus its importance for mankind. It is mentioned in Ayurveda, Hindus age old system of medicine, and one encounters its name and use recorded in Sanskrit, the ancient Hindu's language describing the ageless *Vedas* between 1700 and 800 BC during the period known as the *Vedic* age (Nair, 2013).

The main bio-active principles of turmeric, the curcuminoids, can be used as antiinflammatory, anti-oxidant, hypocholestraemic, choleratic, antimicrobial, insect repellent, anti-rheumatic, antivenomous, antiviral, antidiabetic, anti-hepatotoxic, anti-cancerous and anti- helminthic (Singh *et al.*, 2011). Curcuminoids are yellow components comprising of curcumin (96%), desmethoxycurcumin (6%), and bisdesmethoxycurcumin (0.3%) while the volatile oil composes of a number of monoterpenes and sesquiterpenes including zingiberene, curcumene, α - and β -tumerones (Charoenchai *et al.*, 2020). Curcumin (1,7bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is the major active compound with an amount of 2–6% by weight in the rhizome and essential oil comprise of 5.8% (Yadav *et al.*, 2013).

Drying is an important method to preserve a wide variety of products; it reduces water content and water activity to limit growth of spoilage bacteria. It is one of the most effective and widely used methods for food preservation. The main aim of drying is to reduce the moisture present in turmeric, which is 70–80% at the time of harvest to a safe limit of 10% for grinding or 6% for safe storage and to use it during off-seasons (Singh *et al.*, 2010a). Vieira and Jorge (1997), studied the drying of turmeric root where rhizomes were cut into 5 mm slices, treated with saturated steam for 5 min and dried at 45, 55 or 65°C at air flow rate of 1.5 m³/sec. It was observed that, pretreatment with steam and drying at 65°C was advantageous. Another study performed by Singh *et al.* (2010a), found that the optimum drying conditions for best turmeric quality were found to be air temperature of 55–60°C and air velocity of 2 m/sec.

Drying improves the extractability of compounds due to destruction of cell walls which increases solvent migration during extraction. At 40 and 50 °C under LE condition, curcumin content degraded due to longer exposure to heat and light compared to 60 and 70°C. The highest contents of 4.97% curcumin was found during the hot-air drying at 60 °C temperature, and the values decreased at higher temperatures (70 to 90°C) (Komonsing *et al.*, 2021).

1.2 Statement of the problem

In Nepal, among the spice crops, turmeric occupies 9,795 ha area with production of 99,907 tons in 2021. The total area under turmeric production in Sunsari district in 2019/2020 was 415 ha with a total production of 4,314 ton (MoALD, 2021). Drying system of turmeric in

Nepal is still conventional and due to which maximum amount of bioactive components get lost resulting poor quality product. The widely practiced traditional open sun-drying by rural farmers has inherent limitations such as high crop losses due to inadequate drying, fungi attacks, insects, birds, rodent encroachment and unpredictable weather effects (Ekechukwu, 2010). The effort in process modification of turmeric powder, its essential oil and oleoresin is still lacking in Nepal.

The whole rhizome could be dried by direct sunlight for about 43 days to reach the final moisture content of 10% (Raza *et al.*, 2018). Sliced turmeric required shorter time (3–5 days) to reach as low as 7% of moisture content under sun drying at 35–45°C (Chumroenphat *et al.*, 2021). Jose and Joy (2005) suggested that traditional drying method could result in the loss of volatile oil (up to 25%) by evaporation, and in the destruction of some of the light-sensitive oil constituents. Chumroenphat *et al.* (2021) indicated long drying process could adversely affect curcuminoids content and biological properties as well as product qualities. It showed that about 72% of curcuminoids in turmeric slices was lost after sun drying. Although, studies on the drying kinetics of turmeric in various solar dryers have been published (Borah *et al.*, 2015; Prasad and Aggarwal, 2011).

In the present context of Nepal, turmeric processing is done in conventional method without much considering its effect on bioactive components which are most essential components of turmeric. There were only a few studies on the degradation of its curcuminoids and bioactive compounds with the combined effect of drying temperature and size of turmeric rhizome (Jose and Joy, 2009; Sharma *et al.*, 2021). The solution to this problem is to study the bioactive component loss of turmeric varying drying temperature and size of rhizome. The initiation of this research work is longtime felt concept in perspective of Nepal for promoting spice marketing in high level. Thus, in order to achieve turmeric powder of better quality and for better process control, optimum drying temperature and rhizome size must be identified. This dissertation was feasible in Sunsari as it is the major production area of turmeric (Baral *et al.*, 2021).

1.3 Objectives of the study

1.3.1 General Objective

• The general objective was to study the effect of drying temperature and rhizome size on bioactive components of turmeric powder.

1.3.2 Specific Objectives

The specific objectives of this study were as follow:

- To carry out proximate analysis of fresh and dried turmeric rhizomes.
- To optimize drying temperature and rhizome size by measuring quality parameters (curcumin, essential oil, oleoresin and total phenolic content).
- To determine and compare antioxidant activity of dried turmeric powder.
- To compare optimized and traditionally sun-dried turmeric powder.

1.4 Significance of the study

This study determines the bioactive components of turmeric rhizomes for different size dried at different temperature which helps to produce consistent product of high quality which is lacking in present scenario of Nepal. This research will help to find the optimum rhizome size and drying temperature which yield maximum bioactive components in turmeric powder. After the completion, this research work will be more beneficial for commercial production of turmeric power and dried rhizome of higher quality which may encourage more farmer to be involved in turmeric farming.

1.5 Limitations of the study

 Only curcumin, oleoresin, essential oil, total phenolic content and antioxidant activity were determined.

Part II

Literature review

2.1 Historical background

The use of turmeric dates back nearly 4000 years to the Vedic culture in India, where it was used as a culinary spice and had some religious significance. According to Sanskrit medical treatises and Ayurvedic and Unani systems, turmeric has a long history of medicinal use in South Asia. Susruta's Ayurvedic *Compendium*, dating back to 250 BC, recommends an ointment containing turmeric to relieve the effects of poisoned food. The name turmeric derives from the Latin word *terra merita* (meritorious earth), referring to the color of ground turmeric, which resembles a mineral pigment (Prasad and Aggarwal, 2011). It is also known as the "yellow root," "golden spice," "Indian saffron", and has been used for at least 6000 years in traditional medicine and religious practice. It has 55 synonyms in Sanskrit based on its religious or medicinal properties. The oldest reference to turmeric was in the *Atharvaveda* (Gopinath and Karthikeyan, 2018).

The exact geographic origin of turmeric is unknown, but it is a safe bet that it could be Southeast Asia (Velayudhan *et al.*, 1999). Turmeric was mentioned in the writings of Marco Polo concerning his 1280 journey to China and India and it was first introduced to Europe in the 13th century by Arab traders (Rathaur *et al.*, 2012). During his several legendary voyages to India via the "Silk Route," Marco Polo was so impressed by turmeric that he had mentioned it as a vegetable which possesses properties akin to saffron but is not actually saffron (Parry, 1969).

Initially, it was cultivated as a dye because of its brilliant yellow color. With the passage of time, ancient populations came to know of its varied uses and they began introducing it into cosmetics. The plant's roots are used in one of the most popular Indian *Ayurvedic* preparations called "*Dashamularishta*," a concoction prepared from 10 different types of roots, which relieve fatigue, and have been in use since thousands of years. Human breast tumors can be treated with turmeric leaf extracts. The plant's flowers are used as an antidote against worms in the stomach of humans and can also cure jaundice and venereal diseases. Apart from *Curcuma longa*, several species of economic importance are available. About 70–110 species of the genus have been reported throughout tropical Asia (Nair, 2013)

2.2 Botanical profile

It was in 1753 that the genus *Curcuma* was established by Linnaeus in his *Species Plantarum* (Linnaeus, 1799). *Curcuma longa* L. is a perennial rhizomatous herb which grows up to one meter in height. It is the most utilized species of the genus *Curcuma* and family *Zingiberaceae*. It has a short pseudostem and large oblong leaves. The underground rhizome has a mother rhizome, with multiple branching secondary and primary rhizomes. They are ovate, oblong, or pyriform and have a pale yellow, reddish yellow, or orange brown colour. It has pale yellow flowers and does not bear fruits (Gopinath and Karthikeyan, 2018). The family is composed of 47 genera and 1400 species of perennial tropical herbs, found usually in the ground flora of lowland forests (Ravindran *et al.*, 2007).

Turmeric was described as *C. longa* by Linnaeus and its taxonomic position is as follows (Chattopadhyay *et al.*, 2004):

Kingdom: Plantae

Class: Liliopsida

Subclass: Commelinids

Order: Zingiberales

Family: Zingiberaceae

Genus: Curcuma

Species: longa

The wild turmeric is called *C. aromatica* and the domestic species is called *C. longa*.

2.3 Morphology of turmeric

Holt *et al.* (1950) presented the morphological description of turmeric, which was subsequently cited by several other authors (Ravindran *et al.*, 2007).



Fig. 2.1 Turmeric plant



Fig. 2.2 Turmeric rhizome

2.3.1 Habitat

Turmeric is an erect perennial herb, grown as an annual and in certain cases as a biennial as well. It grows to a height of around 120 cm but, significant variations exist in plant height, among varieties as well as in plants grown under different agro climatic conditions (Rao *et al.*, 2006).

2.3.2 Leaves

Leaves are borne in a tuft, alternate, obliquely erect or sub sessile, with long-leaf stalks or sheaths forming a pseudo stem or the aerial shoot. The leafy shoots rarely exceed 1 m in height and are erect. Usually, there will be 6–10 leaves in a leafy shoot (Das *et al.*, 2004).

2.3.3 Epidermis

Uniseriate, thin-walled, barrel-shaped parenchymatous cells. Trichomes are less frequent at the adaxial surface. At the abaxial surface, small, unicellular, hook-like trichomes are present with a slightly bulbous base (Das *et al.*, 2004).

2.3.4 Hypodermis

Multiseriate, mostly one or two layered, composed of irregularly polygonal colorless cells, present interior to both upper and lower epidermis (Das *et al.*, 2004).

2.3.5 Mesophyll

Mesophyll is not differentiated into palisade and spongy tissue according to Das *et al.* (2004). But Jayasree and Sabu (2005) reported one-layered palisade tissue in all species of *Curcuma*.

2.3.6 Vascular bundles

The vascular bundles are arranged in three layers, developing unequally at different levels. Main vascular bundles form a single conspicuous abaxial arc, alternating with air canals, and embedded in chlorenchyma. The abaxial conducting system consists of an arc of vascular bundles of different sizes that are circular in outline. The abaxial conducting system consists of vascular bundles that are similar in appearance to the main vascular bundles, but are sclerenchymatous sheath above the xylem and below the phloem, extruded protoxylem, small mass of metaxylems and phloem tissue. Vascular bundles of accessory arcs have reduced vascular tissues and contracted protoxylem. Abaxial bundles are enveloped within almost a complete fibrous sheath (Das *et al.*, 2004).

The epidermal and stomatal structures of turmeric and *C. amada* were investigated by Raju and Shah (1975). They have reported that the upper epidermis consists of polygonal cells which are predominantly elongated at right angles to the long axis of leaf. Irregular polygonal cells are present on the lower epidermis, except at the vein region, where they are vertically elongated and thick-walled. The epidermal cells in the scale and sheath leaves (the first 2–5 leaves above ground without the leaf blade) are elongated parallel to the axis of the leaf. Oil cells are rectangular thick-walled and suberized and are frequent in the lower epidermis. They observed that the leaves are amphistomatic, with a distinct substomatal cavity and stomata may be diperigenous, tetraperigenous, or anisocytic. Often, two subsidiary cells align completely with guard cells (Das *et al.*, 2004).

2.3.7 Rhizome

The rhizome is the underground stem of turmeric, which can be divided into two parts, the central pear-shaped "mother rhizome" and its lateral axillary branches known as "fingers." Normally, there is only one main axis. Either a complete finger or a mother rhizome is used as planting material. It is also called the "seed rhizome." Normally, the "seed rhizome"

produces only one main axis, which develops into the aerial leafy shoot. The base of the main axis enlarges and becomes the first formed unit of the rhizome which ultimately develops into the mother rhizome. Axillaries buds from the lower nodes of the "mother rhizome" develop and give rise to the first order of branches, often called the "primary fingers." Their number varies from two to five. Primary branches grow to some length and either develops into an aerial shoot or stop growing further. They grow in a haphazard manner in different directions and in some cases grow up to the ground level with one or two, or even no, leaves. Secondary branches developing at higher nodes of primary branches are diageotropic (Raju and Shah, 1975). Some primary branches after hitting ground level do not form any aerial shoot, but, exhibit positive geotropic growth. Primary fingers branch further, resulting in secondary and tertiary branches, and these branches do not produce aerial shoots. The majority of them show positive geotropic growth or obliquely downward growth. The *C. longa* types have more sideward growth, while the *C. aromatica* types have more downward growth (Ravindran *et al.*, 2007).

2.3.8 Nodes and internodes

Mature mother rhizomes may have 7–12 nodes, and the intermodal length varies from 0.3 to 0.6 cm. However, the first few internodes at the proximal end are elongated due to which the mother rhizome reaches the ground level (Shah and Raju, 1975). Primary and secondary fingers have longer internodes of about 2 cm length, compared to mother rhizomes. Except the first one or two, all the other nodes in the mother rhizome as well as fingers have axillaries buds (Ravindran *et al.*, 2007).

2.3.9 Aerial shoot

The foliage leaves emerge from the buds on the axils of the nodes of the underground bulb and sometimes from the primary finger also. The petiole of the foliage leaf is long and has a thick leaf sheath. The long-leaf sheaths overlap and give rise to the aerial shoot (Ravindran *et al.*, 2007).

2.3.10 Shoot apex

The apical meristem of the shoot has the tunicacorpus type configuration. The tunica is twolayered, with cells dividing anticlinally, while in the corpus, which is the region proximal to tunica, the cells divide in all directions. The central region is surrounded by the flank meristem, which produces the procambrium, cortical region, and leaf primordium (Ravindran *et al.*, 2007).

2.3.11 Roots

Roots emerge from the mother rhizomes and often from fingers, not from the secondary and tertiary fingers. Some of the roots enlarge and become fleshy due to storage of food materials. They serve the function of nutrient and water absorption, anchorage, and storage of assimilated food. In certain species, some of the roots terminate in bulbous tubers (Ravindran *et al.*, 2007).

2.3.12 Turmeric rhizome and its developmental anatomy

The turmeric rhizome anatomy and its development has been investigated (Ravindran *et al.*, 1998; Sherlija *et al.*, 1999). These investigators provided a detailed description of the rhizome and its different developmental stages based on histology. Transverse sections of rhizomes show an outer zone and an inner zone, separated by intermediate layers. Both have vascular bundles. The vessels show spiral and scalar form perforation plates. The phloem contains sieve tubes and two or three companion cells. Early in rhizome development, when it is about 4–7 mm in diameter, the outer zone is 1.5–2.5 mm, the inner zone is 2.5–3.5 mm, and the intermediate layer is about 0.5 mm in thickness. A mature mother rhizome measures about 2–3 cm across, having an outer zone of about 6–10 mm, inner zone about 10–12 mm, and the intermediate layer about 1–1.5 mm in thickness. At this stage, the primary finger is about 1–2 cm in diameter, outer zone 4–5 mm, inner zone 9–10 mm, and intermediate layer about 1 mm in thickness (Ravindran *et al.*, 2007).

Rhizome enlargement initiates through the activity of meristematic cells, present below the young primordial of the developing rhizome. These cells develop into primary thickening meristem (PTM), which is responsible for the initial thickening in the width of the developing cortex by producing primary vascular bundles that are collateral. At the lower levels of the developing rhizome, the PTM becomes primarily a root-producing meristem. After the formation of the primary vascular cylinder, some of the pericycle cells at different places undergo one or more periclinal divisions, forming secondary thickening meristems (STMs), which vary from two to six layers. This meristem produces secondary vascular bundles and parenchyma cells on its inner side. These parenchyma cells become packed with starch grains on maturity. The crowded arrangement of the secondary vascular bundles, which are amphicribral, and their distribution clearly distinguishes them from the primary bundles which are collateral and scattered. The ground parenchyma in actively growing regions contains oil canals along with phloem and xylem. Oil canals are formed lysigenously by the disintegration of entire cells (Ravindran *et al.*, 2007).

2.4 Cultivation

The turmeric plant is propagated by division of an underground stem or rhizome which produces erect leafy shoots of height of upto 1m leaves, alternate, obliquely erect or sub sessile, are oblong lanceolate and dark green, surmounting leaf sheaths tapering near the leaf and broadening near the base, enveloping in the succeeding shoot (Govindarajan and Stahl, 1980)

Turmeric is grown at hilly areas of Nepal (hot and moist climate). It is usually grown in regions with an annual rainfall of 1000-2000 mm; below 1000 mm, irrigation is required. Cultivation has been extended into wetter areas with over 2000 mm of rain per annum can be grown up to 1220 mm in Himalayan foothills loamy or alluvial, loose, friable, fertile soils and cannot withstand water logging (Purseglove *et al.*, 1981). Turmeric is grown in mid-July along with finger millet, rice and sugarcane as an alternative crop. Mulching is necessary during growing stage (Govindarajan and Stahl, 1980)

2.5 Harvesting

In Nepal, normally turmeric is harvested in month of December to January. However, its harvested depending upon its prevailing demand for dried turmeric; crop is harvested in between 7 to 9 months after planting (Pande and Yonjan, 1991).

For easy harvesting the leaves are cut close to the ground. The field is irrigated if needed and ploughed in between rows for easy collection. The rhizome bunches are carefully lifted, adhering soil removed by soaking in the water and further cleaning of roots and scales before they are collected at curing yards (Ravindran *et al.*, 2007).

2.6 Processing of turmeric rhizome

The postharvest processing of turmeric involves many units operations such as washing, cleaning, curing or blanching, drying, polishing, size reduction and packaging (Hirko *et al.*, 2020). Processing of raw rhizomes assumes importance from the point of view of the appearance and color of the end product. The processing consists of two major stages i.e. curing and drying (Govindarajan and Stahl, 1980).

2.6.1 Curing

Curing is a process of cooking the raw rhizome in water sufficient to cover the bulk until soft before drying and cleaning. Boiling destroys the vitality of fresh rhizomes, obviates the raw odor, reduces drying time, gelatinized starch and yields a uniformly colored product. The traditional curing process involve boiling in plain water while improved curing involves boiling in alkaline water (0.1% Sodium bicarbonate) till the rhizome become soft (Varshney *et al.*, 2004).

The period at which boiling is stopped largely affects the final colour and aroma of the final product. Over-cooking spoils the colour of the final product while under-cooking renders the dried product brittle. Wet curing methods, viz. hot water or steam blanching have drawbacks such as leaching of nutrients and color and quality deterioration. Some studies reported that high temperatures, such as experienced in blanching cause thermal degradation of curcumin (Chen *et al.*, 2014), while other studies have shown that blanching protects the bioactive ingredients from the effects of drying (Blasco *et al.*, 2006).

2.6.2 Drying

Drying is the simplest and cheapest method for food and agricultural processing. The application of drying in food industries is not only to inactivate enzymatic activity or to convert raw plants to a product that is safe, edible, and suitable for storage but also to improve extractability of the material for industrial extraction by reduction of water content (Komonsing *et al.*, 2021). Different methods used for turmeric drying are sun drying, solar drying, hot-air oven drying, cabinet drying, freeze drying and micro-wave oven drying (Charoenchai *et al.*, 2020; Chumroenphat *et al.*, 2021; Hirko *et al.*, 2020).

The whole rhizome could be dried by direct sunlight for about 43 days to reach the final moisture content of 10% (Raza *et al.*, 2018). Sliced turmeric required shorter time (3–5 days) to reach as low as 7% of moisture content under sun drying at 35–45 °C (Chumroenphat *et al.*, 2021). For freeze drying, turmeric slices were first frozen at -50 °C for 12 h, before drying. The heating plate and cold trap were cooled to -100 °C; vacuum was under 20 Pa absolute pressure. Hot-air oven drying involved an electric thermo-static drying hot-air oven at 50 °C to final moisture content of 7% (Chumroenphat *et al.*, 2021). In cabinet drier, the optimum drying conditions for best turmeric powder quality were found to be air temperature of 55–60°C and air velocity of 2 m/sec (Singh *et al.*, 2010a). Hirko *et al.* (2020), investigated that unsliced rhizome form and use of the poly-tunnel solar dryer give product of highly acceptable quality characteristics as compared to sun-dried sliced rhizome.

2.7 Chemical and nutritional composition

Proximate analysis of turmeric reveals that the turmeric rhizome contains moisture, carbohydrate, protein, fat, minerals (potassium, sodium, calcium, iron, phosphorus), and trace amounts of vitamins (Prasad *et al.*, 2014).

S.N	Parameter	Value (%)
1	Moisture	6-13
2	Carbohydrate	60-70
3	Protein	7-10
4	Fiber	2-7
5	Mineral matter	3-7
6	Fat	5-10
7	Volatile oil	3-7
8	Curcumin	2-6

 Table 2.1 Chemical composition of turmeric

Source: Dahal (2018)

The chemical and nutritional composition of turmeric varies with varieties, climatic condition, soil condition, fertilizer used etc. Further, there is a great effect of maturity, handling and storage, drying and other processing methods on the composition of turmeric (Sasikumar, 2012).

S.N	Parameter	Amount
1	Moisture, g	6
2	Food energy, kcal	390
3	Protein, g	8.5
4	Fat, g	6.9
5	Carbohydrate, g	71.9
6	Ash, g	6.8
7	Calcium, g	0.2
8	Phosphorus, mg	260
9	Sodium, mg	10
10	Potassium, mg	2500
11	Iron, mg	47.5
12	Thiamine, mg	0.09
13	Riboflavin, mg	0.19
14	Niacin, mg	4.8
15	Ascorbic acid, mg	50

Table 2.2 Nutritional composition of turmeric per 100g

Source: Dahal (2018)

2.8 Chemistry of turmeric

There are various products obtained from turmeric, which have tremendous potential as commercial products. Turmeric, like other spices is available in the market as whole, ground, or as oleoresin. The institutional sector in western countries buys turmeric oleoresins, whereas in the industrial sector demand is more for whole turmeric; value-added products from turmeric include dehydrated turmeric powder, turmeric oils, oleoresins, and curcuminoids (Nair, 2013). The main active components in the rhizome are essential oil and curcuminoids. Curcuminoids and essential oils are classified as secondary metabolites produced by *curcuma* plants, with well-defined bioactivity (Lee *et al.*, 2014). The volatile oil is responsible for the turmeric aroma, while the curcuminoids (curcumin and its analogues) are responsible for its bright yellow color (Govindarajan and Stahl, 1980).

2.8.1 Curcuminoids

Curcuminoids, 5-8% of turmeric, are bioactive phenolic compounds and consist of more than 100 individual curcuminoids that have been isolated and identified from genus *Curcuma*, about 50 of which are present in *C. longa* (turmeric).

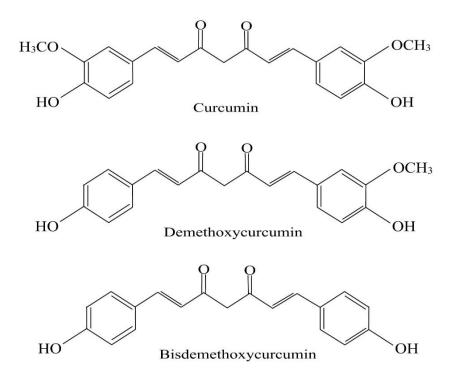


Fig. 2.3 Chemical Structure of Curcuminoids (Poudel et al., 2019)

Turmeric contains three major curcuminoids, curcumin (CUR; 77%), demethoxycurcumin (DMC; 17%), and bisdemethoxycurcumin (BMC; 3–6%) (Kotha and Luthria, 2019). The most valued constituents of turmeric are its yellow pigments, curcumin (Govindarajan and Stahl, 1980). Curcumin is an oil-soluble pigment, practically insoluble in water at acidic and neutral pH, and soluble in alkali (Prasad *et al.*, 2014).

Curcumin is the primary pigment and is generally used in various food industries as a food color (Ratnambal, 1986). Studies showed that curcumin has a very powerful antioxidant effect (Osawa *et al.*, 1995). Several researchers have reported in vitro studies showing that curcuminoids have important medicinal properties: chemo-preventive effects, hepato-protective, nephron-protective, anti-cancer, immune-modulatory, neuro-protective, anti-proliferative, anti-oxidant and anti-inflammatory (Hewlings and Kalman, 2017; Jurenka, 2009).

2.8.2 Essential oil

Turmeric oil is a group of essential oils which mainly consists of more than 250 diverse terpenoids, identified from *Curcuma* species. Turmeric oil represents another major group of bioactive compounds in turmeric. Essential oils obtained by steam distillation represent 3–7% of the turmeric rhizome and mainly consist of terpenoids, including sesquiterpenoids (e.g., α -phellandrene, zingiberene), monoterpenoids (e.g., sabinene, cineol), and norsesquiterpenoids (Meng *et al.*, 2018). Bansal *et al.* (2002), found that essential oils in rhizome were dominated by α - and ar-turmerones (40.8%), myrcene (12.6%), 1,8-cineole (7.7%), and *p*-cymene (3.8%). The characteristic turmeric aroma is imparted by ar-turmerones, the major aroma principle in the oil (Parthasarathy *et al.*, 2008).

Oil of turmeric is an antacid, and in small doses, acts as a carminative, stomachic, appetizer, and tonic. The oil given by vapor inhalations found to have significant effect in removing sputum, relieving coughs, and preventing asthma; it is effective for the treatment of respiratory diseases (Dosoky and Setzer, 2018). Turmeric-leaf EO showed cytotoxic activity against breast-tumor (Hs578T) and prostate-tumor (PC-3) cells. It also showed antibacterial, antifungal, antiaflatoxigenic, and mosquitocidal activities (Essien *et al.*, 2015; Roth *et al.*, 1998; Sindhu *et al.*, 2011).

2.8.3 Turmeric oleoresin

Turmeric oleoresin is a brownish-orange viscous oily product obtained by the solvent extraction of the ground spice with organic solvents such as acetone, ethylene dichloride and ethanol, etc. Supercritical CO_2 extraction and molecular distillation are the latest technologies followed to extract and separate turmeric oleoresin and to get a high-quality product. Supercritical fluid extraction (SFE) does not result in thermal degradation products or contamination of the solvent (Kurmudle *et al.*, 2011).

Turmeric oleoresin is a mixture of curcuminoids, volatile oil, nonvolatile fatty and resinous material, and other active ingredients (Aniesrani Delfiya *et al.*, 2015). Oleoresin turmeric contains 30 to 40% curcumin, 15 to 20% volatile oil, and 20 to 30% fixed oils, depending on the cultivar and extraction medium. Oleoresin of turmeric, obtained by extraction with volatile solvents, contains the aroma as well as the taste principles of turmeric in highly concentrated form. It is a composite of the volatile aroma components and the non-volatile taste and color principles of the turmeric (Balakrishnan, 2016).

2.8.4 Total phenolic components of turmeric

Phytochemical composition of turmeric includes 0.4% saponin, 0.76% alkaloid, 0.03% sterol, 1.08% tannin, 0.40% flavonoid, 0.82% phytic acid, and 0.08% phenol. Phenolic compounds are secondary metabolites of plant metabolism and can chelate metallic ions, scavenge free radicals during oxidative stress, and enhance antimicrobial activities. In addition, phenolic compounds are important for human health because they can reduce the risk of certain diseases, such as cardiovascular, cancer, and chronic disease (Yang *et al.*, 2020). Chumroenphat *et al.* (2021) reported that the highest total phenolic content (TPC) was found in fresh turmeric (58 mg GAE/g DM), followed by freeze dried and hot air dried turmeric powder (35.7 and 30.5 mg GAE/ g DM, respectively).

Phenolic Components	Amount (mg GAE/ 100g dry extract)
Gallic Acid	781 ± 5.18
Protocatechuic acid	33.1 ± 1.87
Catechin	20.3 ± 0.97
Chlorogenic acid	17.9 ± 0.88
Epicatechin	45.5 ± 1.64
Ferulic acid	187 ± 6.78
Coumarin	25.0 ± 1.23
Rutin	19.89 ± 1.57
Curcumin	98.6 ± 3.83
Myricetin	5.32 ± 0.57
Cinnamic acid	10.3 ± 1.35
Genistein	33.1 ± 3.10
Quecercetin	4.78 ± 0.83

 Table 2.3 Phenolic profile of turmeric extract

Source: Yang et al. (2020)

Phenolic compounds are good electron donors because their hydroxyl groups can directly contribute to antioxidant action. According to multiple reports in the literature, phenolic compounds exhibit free radical inhibition, peroxide decomposition, metal inactivation or oxygen scavenging in biological systems and prevent oxidative disease burden (Oberoi and Sandhu, 2015). Total phenolic content of turmeric was measured using Folin-Ciocalteu's phenol regent. Ereifej *et al.* (2016), found that acetone was superior extractant for phenolics from turmeric.

2.8.5 Antioxidant activity

Antioxidants may be defined as substances that, when present in food, delay, control, or inhibit oxidation and deterioration of food quality. In the body, antioxidants reduce the risk of degenerative diseases arising from oxidative stress (Halliwell, 1999). Antioxidants have been reported to prevent oxidative damage caused by free radical, it can interfere with the oxidation process by reacting with free radicals, chelating, and catalytic metals and also by acting as oxygen scavengers (Shahidi *et al.*, 1992). The phenolic compounds, curcuminoids, oleoresins and essential oil contributes to the antioxidant activity of turmeric (Yang *et al.*, 2020).

A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity (Tailor and Goyal, 2014). The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free DPPH radical with an odd electron gives absorbance (purple color) at 517nm. When the antioxidants in plant extract react with DPPH, it is reduced to DPPH-H and results in decolorization to yellow color with respect to the number of electrons captured. The color absorbance corresponds inversely to the radical scavenging activity of the sample extract. The scavenging of DPPH by radical scavengers can be summarized as:

DPPH + A•	 DPPH – A (2)
A• + A•	 A – A (3)

Where FE is a scavenger of the extract and A^{\bullet} is a radical. The newly formed radical (A^{\bullet}) can mainly follow radical-radical interaction to render stable molecules, via radical disproportionate, collision of radicals with abstraction of an atom by one radical from another equations (Tailor and Goyal, 2014).

2.9 Pharmacology and health benefits of turmeric

Several species of the genus *Curcuma* have been employed in Ayurveda and traditional folk medicine for treatment of various diseases and disorders. Turmeric is effective for the treatment of biliary diseases, hepatic disorders, anorexia, coryza, dyspepsia, rheumatism, sinusitis and used as antiseptic, gastro protectant, tonic, stimulant, and blood purifier (Chattopadhyay *et al.*, 2004).

Turmeric has shown several pharmacological effects such as anticarcinogenic and chemopreventive, anti-Alzheimer's disease, antiallergic, antioxidant, α -amylase inhibitor, neurotoxin-inhibitory, anti-inflammatory, antiobesity, antidiabetic, antiproliferative, hypoglycemic, hypolipidemic, antiarthritic, immunomodulating, gastroprotective, antiulcerogenic, larvicidal, angiogenic, antivasoconstrictive, antibacterial, neuroprotective, antifungal and anti-aflatoxigenic (Rajkumari and Sanatombi, 2018).

2.9.1 Antioxidant effect

The antioxidant activity of curcumin was reported as early as 1975. It acts as a scavenger of oxygen free radicals (Sharma, 1976). The ability of the antioxidants in turmeric to decrease free radicals is similar to that in vitamins C and E. Since the antioxidant activities of turmeric are not degraded by heat (unlike most vitamins), even using the spice in cooking provides benefit. Unnikrishnan and Rao (1995), found that it can protect hemoglobin from oxidation. In vitro, curcumin can significantly inhibit the generation of reactive oxygen species (ROS) like superoxide anions, H₂O₂ and nitrite radical generation by activated macrophages, which play an important role in inflammation. Curcumin also lowers the production of ROS *in vivo* (Joe and Lokesh, 1994). Since ROS have been implicated in the development of various pathological conditions (Bandyopadhyay *et al.*, 1999), curcumin has the potential to control these diseases through its potent antioxidant activity.

Similarly several studies had shown remarkable antioxidant activity of essential oil. The potent antioxidant activity of turmeric EO is thought to be responsible for inhibiting brainedema formation, one of the most dangerous consequences of ischemic brain injury (Dohare *et al.*, 2008). Turmeric EO prevented oxidative stress via reducing the synthesis or release of cortisol and increasing the activity of antioxidant enzymes, and thereby protecting from the formation of reactive oxygen species excess (Singh *et al.*, 2010b).

2.9.2 Antimicrobial effect

Bioactive components of turmeric showed potent antibacterial activity against *Helicobacter pylori, Bacillus cereus, B. coagulans, B. subtilis, Staphylococcus aureus, Escherichia coli, Vibrio parahaemolyticus, Proteus mirabilis* and *Pseudomonas aeruginosa.* It also showed strong antifungal effects against *Aspergillus flavus, A. niger, A. parasiticum, Rhizoctonia solani, Helminthosporium oryzae, Trichoconis padwickii, Curvularia lunata, C. pallescens, C. trifolii, Fusarium verticillioides, F. moniliforme, F. oxysporum, Penicillium digitatum, Alternaria dianthi, Trichophyton longifusus,* and *Colletotrichum falcatum* (Dosoky and Setzer, 2018).

In addition, *C. longa* oil was reported to have antiaflatoxigenic and antiprotozoan activities and curcumin has shown to have antiviral activity. Most importantly, curcumin also shows anti-HIV (human immunodeficiency virus) activity by inhibiting the HIV-1 integrase needed for viral replication. It also inhibits UV light induced HIV gene expression. Thus curcumin and its analogues may have the potential for novel drug development against HIV (Mazumder *et al.*, 1995; Taher *et al.*, 2003).

2.9.3 Anticarcinogenic effect

Crude organic extracts of turmeric inhibited lipopolysaccharide (LPS)-induced production of tumor necrosis factor (TNF)- α and prostaglandin E2 (PGE2) in human leukemia (HL-60) cells (Lantz *et al.*, 2005). Turmeric EO demonstrated strong protective effect against benzo[a]pyrene-induced increase in micronuclei in circulating lymphocytes and protected against cytogenetic damage in patients suffering from oral submucous fibrosis, a precancerous condition for oral cancer (Hastak *et al.*, 1997).

It was also cytotoxic to the pancreatic cancer (PANC-1), melanoma (B16), prostatecancer (LNCaP), and human cervical adenocarcinoma (HeLa) cell lines due to the presence of arturmerone, α -turmerone, β -turmerone, curlone, ar-curcumene, zingiberene, and β sesquiphellandrene (Zhang and Kitts, 2021).

2.9.4 Anti-inflammatory activity

The volatile oil and also the petroleum ether, alcohol and water extracts of *C. longa* show anti-inflammatory effects (Yegnanarayan *et al.*, 1976). Chainani-Wu (2003) studies have

identified a number of different molecules involved in inflammation that are inhibited by curcumin including phospholipase, lipooxygenase, cyclooxygenase, leukotrienes, thromboxane, prostaglandins, nitric oxide, collagenase, elastase, hyaluronidase, monocyte chemoattractant protein-1 (MCP-1), interferon-inducible protein, tumor necrosis factor (TNF), and interleukin-12 (IL-12).

2.9.5 Hypoglycemic and antidiabetic activity

Curcumin and other related bioactive compounds present in turmeric have been proposed to protect against type 2 diabetes (T2D) through different mechanisms that involve a hypoglycemic effect attributed to upregulation of insulin, enhanced insulin sensitivity, and lower cellular uptake of glucose. The mechanism of which curcumin evokes hypoglycemic and antidiabetic effects involves the pancreatic β -cells (Zhang and Kitts, 2021).

In adipocytes and hepatocytes, curcumin reduces glucose uptake by inhibiting the translocation of glucose transporter-4 (GLUT4) from the cytosol to plasma membrane, and by interfering with the insulin receptor substrate/phosphatidylinositol-3-kinase/protein kinase B (IRS/PI3K/Akt) signaling pathway. The in vitro antidiabetic potentials of the turmeric extract, BMC, EO, and its major component ar-turmerone have in common a capacity to inhibit the activities of α -amylase and α -glucosidase, two key enzymes involved in glucose digestion and also linked to T2D.

2.9.6 Anti-obesity

Obesity is a major co-morbidity of T2D, and strategies that have been developed to treat this disorder by inhibiting the sterol regulatory element-binding protein (SREBP) pathway, important for regulating gene expressions that stimulate fatty acid, triacylglyceride, and cholesterol biosynthesis. Curcumin is an active inhibitor of triacylglyceride and cholesterol synthesis by downregulating expressions of both SREBP-1 and SREBP-2, respectively (Ding *et al.*, 2016).

2.9.7 Dermatological use of turmeric

Turmeric is useful as an external antibiotic in preventing bacterial infection in wounds. It is known that turmeric, and especially the curcumin, inhibits skin cancer, likely due to decreasing the expression of proto-oncogenes. External application stops pain and swelling, heals wounds, and treats many skin diseases ranging from acne to leprosy (Brouet and Ohshima, 1995). The itching and inflammation that accompanies hemorrhoids and anal fissures can reduce by use of turmeric. Turmeric can also benefit skin conditions including: eczema, psoriasis and acne, for those it is potent detoxifier (Fournier and Gordan, 2000).

Natural plant products like turmeric have been formulated to heal and prevent dry skin, treat skin conditions such as eczema and acne, and retard the aging process. It is eminent for accelerated healing of both septic and non-septic wounds (Natarajan and Bright, 2002). A fresh juice from rhizome or a paste prepared from turmeric is often used as a local application as well as internally in the treatment of leprosy skin disease. In case of smallpox and chickenpox, turmeric is applied as a powder or as a paste to facilitate the process of scabbing (Phan *et al.*, 2001).

2.10 Cosmetology of turmeric

Turmeric may be the first known cosmetic as it has been traditionally smeared on the skin by women. It is believed to reduce facial hair growth, reduce acne and improve complexion (Shaffrathul *et al.*, 2007). Tetrahydrocurcumin is an off-white hydrogenated form of curcumin that is used topically as a cutaneous antioxidant. It may prevent rancidity of lipids when added to moisturizers. Curcuminoids have potential in cosmeceuticals as antioxidant, anti-inflammatory and skin lightening agents (Gopinath and Karthikeyan, 2018). Topical gel preparation of 1% curcumin inhibited phosphorylase kinase and improved the lesions in chronic plaque psoriasis. It may also promote healing and prevent scarring in acute injuries such as burns, by inhibiting phosphorylase kinase and subsequent NF-kB/TGF- β signalling pathway (Heng, 2010).

The essential oils may have potential in the perfume, cosmetic and soap industry (Sasikumar, 2005). Curcumin gel has been reported to improve the appearance of photodamaged skin conditions such as pigmentary changes, solar elastoses, actinic poikiloderma, solar lentigines and actinic keratosis when applied for prolonged period such as six months. It may promote apoptosis of cells with DNA damage (Heng, 2010). For the treatment of dandruff, and as hair colorants and dyes, plant extracts are used as hair growth stimulators, the mechanism of action appears to be an acceleration of blood circulation or increased nutrition to the hair follicles (Philip, 1983).

2.11 Safety of turmeric and curcumin

The average intake of turmeric by Asians varies from 0.5 to 1.5 g/day/person, which produces no toxic symptoms (Eigner and Scholz, 1999). Detailed studies have been reported on the safety evaluation of the rhizomes of *C. longa* and its alcohol extract, curcumin. Male and female Wistar rats, guinea pigs and monkeys were fed with turmeric at much higher doses (2.5 g/kg body wt.) than normally consumed by humans. No changes were observed in the appearance and weight of kidney, liver and heart (Bhavanishankar *et al.*, 1980).

Human clinical trials performed by Aggarwal *et al.* (2003) indicate that curcumin has no toxicity when administered orally at doses of 10 g/day. Acute toxicity to oral curcumin is unlikely due to poor bioavailability, and is well tolerated for short-term use at a dose of up to 10 g per day. However, some adverse effect such as nausea, diarrhoea and dyspepsia have been reported (Gopinath and Karthikeyan, 2018).

2.12 Factors affecting the yield and quality of turmeric

The yield of fresh turmeric rhizome and its quality depends upon on different factors; some of them are listed below.

2.12.1 Soil

Turmeric thrives well on loose and friable, well-drained, loamy or alluvial soils. Coarse or heavy soils hinder rhizome development. The crop is raised without irrigation, where rainfall is bimodal and ample and with irrigation in plains where rainfall is low and unimodal. The crop is sensitive to saline soils as well as saline water irrigation. It grows in soils with a pH range of 4.3 to 7.5. The crop is grown up to 1200 m above the mean sea level. The rhizome yield and quality varied greatly with the types of soil texture (Sarma *et al.*, 2003).

2.12.2 Plantation period

Though turmeric is a perennial rhizomatous crop, it is domesticated and grown as a rain fed or irrigated annual crop. Adjusting the time of planting to coincide with the rainfall pattern has been a major consideration as it determines the rhizome yield to a large extent. The yield will be maximum at first fortnight of March but the plantation period for different places depends upon the altitude and climate, the oleoresin content and crude fiber content of turmeric is found to increase with the increase of days of plantation (Monteith, 1981).

2.12.3 Depth of planting

Turmeric rhizomes planted at the depths of 8, 12, and 16 cm emerged earlier and more evenly than those planted at a shallower depth (4 cm) in both glasshouse and field experiments conducted in dark red soils of southern Japan. The overall results suggested that rhizomes of turmeric should be planted at a depth of 8 to 12 cm in dark red soil for higher yield and lower weed competition (Ishimine *et al.*, 2003).

2.12.4 Seed rate

The seed rate for turmeric generally ranges between 1000 and 1200 kg/ha, differing slightly for differences in the size and types of rhizomes and plant spacing. Rao *et al.* (1975), observed that usually 1800 kg of mother rhizomes or 1200 kg of fingers are used for planting 1 ha. Attarde *et al.* (2003), recommended 2000 to 2300 kg/ha of mother rhizome for planting.

2.12.5 Mulching

Mulch is a cover to the soil surface. It may comprise plant residues from the previous crop or imported for the purpose, e.g., straw, wood dust, saw dust, gravel, or plastic sheeting. The effect of mulch is complex. Reduction in soil water loss occurs not only because the mulch acts as a barrier preventing loss, but also because the soil radiation balance and its thermal regime are usually altered, thus influencing the evaporation rate at the surface. The effect of the mulch may therefore be beneficial only where frequent wetting occurs. Mulch should be applied soon after turmeric is planted to encourage early sprouting and to control weed growth (Rao *et al.*, 1975).The quality of mulch was more effective in conserving soil moisture and increasing the growth and yield of turmeric (Kumar and Savithri, 2003).

2.12.6 Nutrition

Plants suffer nutrient deficiency stress when the availability of soil nutrients (and/or the amount of nutrients taken up) is lower than that required for sustaining metabolic processes in a particular growth stage. Deficiency may occur as a result of: an inherently low amount of nutrients in the soil, low mobility of nutrients in the soil, or poor solubility of given

chemical forms of the nutrients (Mengel *et al.*, 2001). Turmeric generally responds to increased soil fertility by producing higher yields, but the quantity of fertilizers (inorganic or organic) required to produce a crop varies with variety, soil, and weather conditions prevailing during crop growth. It has been observed that the uptake of nutrients was higher up to the third month for potassium, up to the fourth month for nitrogen, and up to the fifth month for phosphorus with subsequent decrease. The crop attains maximum vegetative growth during the fourth and fifth month, suggesting the need for earlier application of N, P, and K for increasing the plant growth (Rao and Rao, 1988). Turmeric responds to heavy dressings of organic matter, and many experimental evidences are available on the beneficial effects of organic manures either alone or in combination with inorganic fertilizers on the growth and productivity of turmeric (Rao *et al.*, 2004).

2.12.7 Harvesting

Turmeric crop is usually ready for harvest after about 6 to 9 months of growth, depending upon the variety. At maturity, the leaves turn yellow, fade, and, subsequently wither and dry. Maximum rhizome yield and dry rhizomes are obtained at this stage. At harvest, the leaves and stems are cut close to the ground to maximize the removal of vegetative material. Because the rhizomes are dug by manual labor, the fields are irrigated prior to digging to ease and speed the harvesting process. Rhizomes are subsequently cleaned, and fingers are separated from mother rhizomes. Although the harvest period varies for different areas (Kathirvel and Manian, 2002).

2.12.8 Disease and pest

There are several diseases known to infect turmeric which affects its yield and quality of turmeric. There is different most serious disease known to infect turmeric. Firstly rhizome rot, rhizomes of turmeric affected by rhizome rot and wilt which is caused by *Pythium sp.* and foliage symptoms of turmeric affected by Rhizome rot caused by *Fusariumsp* (Shankaraiah *et al.*, 1991). Secondly leaf blotch, leaves of turmeric affected by leaf blotch caused by *Taphrinamaculans* and leaf spot (*Colletotrichum capsici*). Leaf spot, leaves of turmeric affected by *Colletotrichum* leaf spot (*Colletotrichum capsici*) (Ramakrishnan and Soumini, 1954).

2.12.9 Crop environment

Growth and development of turmeric rhizome and leaves are dependent on several factors, such as nutrition, cultivation practices, genotype, and environmental factors. The crop endures an annual average rainfall of 640 to 4200 mm and optimum annual mean temperatures of 18.2 to 27.4°C. The seed rhizomes planted in the field take about a month to produce new shoots. The Weather during this period had no significant effect on yield, and it is probably significant only after emergence of the crop (Kandiannan *et al.*, 2002). However a temperature range of 25 to 35°C optimum for the sprouting of turmeric rhizome buds, and sprouting does not occur below 10 or above 40°C. Seedlings elongate well in the temperature range between 25 and 30°C, but do not survive above 40°C (Ishimine *et al.*, 2003).

Turmeric grows luxuriantly in shades, but it produces larger and better rhizomes in the open ground exposed to sun (Ridley, 1912). Turmeric comes up well under partial shaded conditions, but thick shade affects yield adversely. Growth parameters showed a positive beneficial effect up to 25 and 50% shade, respectively. The yields at 25, 50, and 70% shade levels expressed as percentage of that in the open were 74, 55, and 30% on fresh weight basis, respectively (Sundararaj and Thulasidas, 1976).

Part III

Materials and methods

3.1 Materials

3.1.1 Chemicals and apparatus

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

3.1.2 Sample collection

12 kg of fresh turmeric rhizome harvested after eight months of cultivation was brought from the local farm of Bijayapur, Dharan-14 at the rate Rs. 90 per kg. Turmeric rhizomes were classified as primary, secondary and tertiary based on the morphology of turmeric rhizome. Secondary rhizomes were taken for study. The rhizomes were cleaned to make it dirt-free. For comparative study of optimized turmeric powder with traditionally sun-dried turmeric powder, dried turmeric rhizome was bought from local market of Dharan which is then grinded to make powder.

3.2 Methods

3.2.1 Experimental Design

A statistical software Design expert® version 13.0 from Statease Inc; USA, was used for experimental design, model building and data analysis. The turmeric powder was prepared with variation in: (a) rhizome size and (b) drying temperature. The range for temperature is 40-70°C while for size is 1-4 cm. The ranges were selected based on different literature (Singh *et al.*, 2010a; Komonsing *et al.*, 2021) and several laboratory trial study. A total of 16 runs were given by design expert version 13.0 (response surface methodology, randomized, design type D-optimal with quadratic design model). The responses selected for the study were curcumin content, oleoresin content, essential oil content, total phenolic content (TPC) and antioxidant activity (AA).

The responses for different experimental combinations were related to coded variables (A and B) by second-degree polynomial equation:

$$Y = \alpha_0 + \alpha_1 A + \alpha_2 B + \alpha_{11} A^2 + \alpha_{22} B^2 + \alpha_{12} A B + \beta$$

The coefficients of the polynomial were represented by α_0 (constant); α_1 , α_2 (linear effects); α_{11} , α_{22} (quadratic effects); α_{12} (interaction effects) and β (random error).

	-	-
Name	Goal	Range
Drying Temperature	To be in range	40-70°C
Rhizome size	To be in range	1-4 cm
Curcumin	To be maximized	To be determined
Oleoresin content	To be maximized	To be determined
Essential oil content	To be maximized	To be determined
Total Phenolic content	To be in range	30-40 mg GAE/g d.m.
Antioxidant activity	To be maximized	To be determined

Table 3.1 Different constraints for optimization of turmeric powder

3.2.2 Preparation of turmeric powder

A general process for the preparation of turmeric powder is shown in Fig. 3.1.

Turmeric rhizome (raw and fresh, 8 months old) Cleaning (to remove dirt) Sorting (into primary, secondary and tertiary rhizome) based on morphology Secondary rhizomes Making uniform cross-sectional area (2.434 ± 0.023 cm²) for all rhizome using steel pipe Cutting using knife into sizes as obtain from design expert measuring with vernier caliper Cabinet drying at different temperature till moisture content 10% Cooling Grinding in grinder Turmeric powder Analysis of response factors

Fig. 3.1 General steps of turmeric powder preparation

3.2.3 Analytical methods

3.2.3.1 Determination of physical parameter

The weight and length-diameter of the turmeric rhizomes were measured using weighing balance and vernier caliper respectively.

3.2.3.2 Determination of moisture

The moisture content of fresh and dried turmeric powder was determined by immiscible solvent distillation method using toluene as described in Rangana (1986).

3.2.3.3 Determination of crude protein

Crude protein of fresh turmeric rhizome was determined by using micro-Kjeldahl method with conversion factor 6.25 as described in Rangana (1986).

3.2.3.4 Determination of crude fiber

Crude fiber of fresh turmeric rhizome was determined as described in Rangana (1986).

3.2.3.5 Determination of crude fat

Crude fat of fresh turmeric rhizome was determined by solvent extraction using petroleum benzine as described in Rangana (1986).

3.2.3.6 Determination of total ash

Total ash of fresh turmeric rhizome was determined by dry ashing as described in Rangana (1986).

3.2.3.7 Determination of total carbohydrate

Total carbohydrate of fresh turmeric rhizome was determined by difference method.

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

3.2.3.8 Determination of essential oil

Essential oil of fresh rhizome and dried turmeric powder was determined by steam distillation using clevenger's apparatus as described in Rangana (1986).

3.2.3.9 Determination of oleoresin

Oleoresin of turmeric powder was determined by solvent extraction using acetone as described in Rangana (1986).

3.2.3.10 Determination of curcumin content

Determination of curcumin content was done spectrophotometrically with standard curcumin according to FSSAI (2016).

3.2.3.11 Preparation of extract for total phenolic content

The turmeric extract was prepared as per Ereifej *et al.* (2016). One gram of turmeric powder was weighed and extracted with 50 ml methanol. Extraction was carried out under stirring for 60 min at 60°C followed by filtration using whatman filter paper 41 into 50 ml volumetric flask, the volume was made upto the mark using acetone and kept in the dark at refrigerated temperature until the time of analysis.

3.2.3.11.1 Determination of total phenolic content

The total phenolic content of turmeric powder was determined by Folin-Ciocalteu assay as described in Ereifej *et al.* (2016). The total phenolic compounds were assayed spectrophotometrically, 0.2 ml of turmeric extract were transferred into a test tube, then mixed with 0.4 ml of 10% diluted Folin-Ciocalteu's phenol reagent, after three minutes 0.8 ml of a 10% solution of sodium carbonate as a buffer was added and mixed well, all the tubes were allowed to stand for one hour at room temperature. The absorbance was measured at 725 nm using the spectrophotometer. A mixture of reagents without the sample was used as a blank. The phenolic compounds content was expressed as gallic acid equivalents (mg GAE/g) on dry weight basis; the gallic acid was used to prepare a calibration curve. Gallic acid standard was used to prepare the standard curve as follow; 200 mg of gallic acid were dissolved in 20 ml of distilled water, 0, 10, 25, 50, 100, 200 and 400 ppm concentrations, were prepared, all reagents were added as above and the absorbance was read at 725 nm.

3.2.3.12 Preparation of extract for antioxidant activity determination

The ethanolic extract of turmeric was prepared as per Singh *et al.* (2010b). 1 g of turmeric powder is extracted with 30 ml of methanol which was centrifuged at 4000 rpm for 15 min at room temperature. Supernatant obtained was filtered using whatman filter paper. The volume was made upto 50 ml with methanol.

3.2.3.12.1 Determination of antioxidant activity

Antioxidant activity was measured as radical scavenging activity against 1, 1-diphenyl-1picryl-hydrazyl radical (DPPH) as described by Singh *et al.* (2010b) with slight modification. Ethanolic extract was prepared for the determination of antioxidant activity. 0.1 mM DPPH solution was prepared by dissolving 4 mg of DPPH in 100 ml of methanol. Freshly prepared sample of 0.5 ml was taken in test tubes and 4 ml of DPPH of added. After incubation in dark for 30 min, the absorbance of the solution was read spectrophotometrically at 517 nm. The absorbance of DPPH solution without sample addition was read. The difference in absorbance of DPPH solution and DPPH solution + sample was calculated. The decrease in absorbance with sample addition was used for calculation of the antioxidant activity. Finally percentage radical scavenging activity (%RSA) was determined using following equation:

DPPH radical scavenging activity (%RSA) = $[(A0-A1)/(A0)] \times 100$

Where A0 is the absorbance of DPPH radical + methanol;

A1 is the absorbance of DPPH radical + sample or standard

3.2.4 Statistical analysis

Response surface methodology was used for the optimization of rhizome size and drying temperature in Design Expert version 13. The independent process variables: rhizome size and drying temperature, were correlated using a second order quadratic model. Data were modelled by multiple regression analysis to determine the second order polynomial coefficient for each term of the equation. The statistical significance of the term was determined using analysis of variance for each response after the data were fitted to the selected model. Coefficient of determination R^2 measures the degree of fit as it is the ratio of explained variation to the total variation. A better model fits the actual data when R^2 approaches unity. R^2 , adjusted R^2 , predicted R^2 and p-values were used to test the adequacy of the model. Then the effect of independent variables was interpreted using the best fit model.

The analysis of variance (ANOVA) tables were generated and the significance of all terms in the polynomial equation was judged by computing the F-value and p-values at 5% level of significance. Data was statistically processed using paired t-test in MS-Excel 2013 at 5% level of significance.

Part IV

Results and discussion

The present study was conducted to prepare a superior quality turmeric powder from turmeric rhizome by optimizing drying temperature and rhizome size. The various combination of rhizome size and drying temperature were obtained by design expert software. The effect of rhizome size and drying temperature on curcumin, oleoresin, essential oil, total phenolic content (TPC) and antioxidant activity were analyzed by response surface methodology (RSM). The fresh turmeric rhizome, optimized turmeric powder and market turmeric powder were analyzed for the physiochemical properties. Results and discussion of the overall study are described in the following headings.

4.1 Proximate and chemical composition of fresh turmeric rhizome

The proximate and chemical composition of fresh turmeric rhizome is given in Table 4.3 and Table 4.4 respectively.

Parameters	Values*
Moisture (%)	78.05 ± 0.08
Crude protein (%,db)	8.23 ± 0.1
Crude fat (%,db)	6.53 ± 0.05
Total ash (%,db)	3.87 ± 0.07
Crude fiber (%,db)	4.75 ± 0.06
Carbohydrate (%,db)	76.63 ± 0.15

Table 4.1 Proximate composition of fresh turmeric rhizome

* Values were the mean of three determinations \pm standard deviation.

The proximate values of the of fresh turmeric rhizome found in our study was in line with the study performed by Rajkumari and Sanatombi (2018), though the values of protein and fat were slightly higher. The slight high value of protein and fat is may be due to difference in location of turmeric production and time of harvest. The values for the proximate of turmeric rhizome were in range with other several studies (Dahal, 2018; Prasad and Aggarwal, 2011; Yadav *et al.*, 2013).

Parameters	Values*
Curcumin content (%,db)	7.39 ± 0.08
Oleoresin content (%,db)	21.08 ± 0.13
Essential oil content (%,db)	8.24 ± 0.14
Total phenolic content (mg GAE/g DM)	58.6 ± 0.37
Antioxidant activity (%RSA)	52.02 ± 0.16

 Table 4.2 Chemical composition of fresh turmeric rhizome

* Values were the mean of three determinations \pm standard deviation.

The values for all parameter was found in consistent with several studies (Charoenchai *et al.*, 2020; Chumroenphat *et al.*, 2021; Priyanka *et al.*, 2017). Chumroenphat *et al.* (2021), reported that the TPC of fresh turmeric is 58 mg GAE/g dry matter which is similar to the value of TPC obtained from this study. Priyanka *et al.* (2017), found that the antioxidant activity of fresh turmeric is 52.927 %RSA. According to Charoenchai *et al.* (2020), the values for curcumin, essential oil and oleoresin of fresh turmeric rhizome were 7.01%, 7.57% and 18.23% respectively. Slight variation in the values is may be due to location, season, harvesting time and maturity.

4.2 Effect of rhizome size and drying temperature on response factors

Observed values of the responses (curcumin, oleoresin, essential oil, TPC and antioxidant activity) were shown in Table B.1.

4.2.1 Effect on curcumin content

The curcumin content of the turmeric powder varied from 3.95 to 5.78% (Table B.1). Table C.1 and C.2 show the coefficient of the model and other statistical attributes of curcumin content. A quadratic regression model was best fitted to describe the effect of rhizome size

and drying temperature on curcumin content of turmeric powder. The model F value was found significant (p<0.0001). The lack of fit test was not significant (p>0.05). The lack of fit F-value of 0.75 implies the lack of fit is not significant relative to the pure error. Nonsignificant lack of fit is desired to fit the model. The coefficient of determination R^2 was 0.9852, which indicated that 98.52% of the variability of the response could be explained by the model. The predicted R^2 of 0.9629 is a reasonable agreement with the adjusted R^2 of 0.9779. The best fitted quadratic model for the effect of variables on curcumin content of the product was given best described by coded equation 4.1

Curcumin content = $+5.75 + 0.3199A + 0.1794B + 0.3624AB - 1.16 A^2 - 0.3783B^2 \dots 4.1$

Where A and B are the coded values of rhizome size and drying temperature. A, B, A^2 , B^2 and AB are model terms.

In the quadratic equation 4.1, curcumin content had significant (P<0.05) positive effect of both the rhizome size (A) and drying temperature (B) at 95 % level of confidence. The quadratic term of both rhizome size (A²) and drying temperature (B²) had significant (P<0.05) negative effect on curcumin content as given in Table C.2. The interaction term of rhizome size and drying temperature (AB) had significant (P<0.05) positive effect on curcumin content.

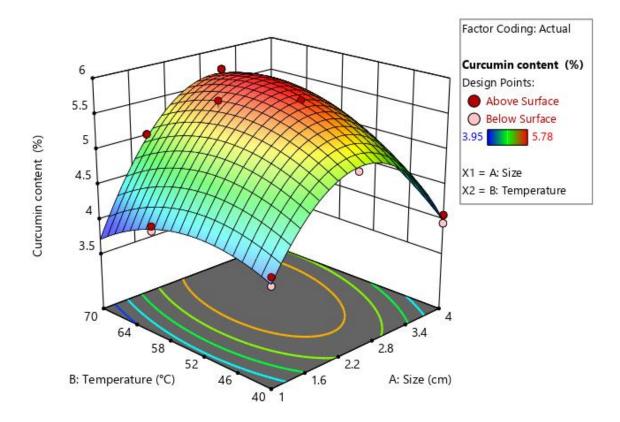


Fig. 4.1 Response surface plot of curcumin content of turmeric as a function of rhizome size and drying temperature.

With the combined effect of rhizome size and drying temperature, the curcumin content of turmeric powder increased and reached maximum until further increases leads to decrease in curcumin content (Fig. 4.1).

The low value of curcumin in turmeric powder dried with of larger rhizome size and at lower temperature is may be due to longer drying time. Similarly the lower value of curcumin in turmeric powder prepared by drying at higher temperature and with smaller rhizome size is may be due to increase in exposure area at a very higher temperature. Increase in exposed area and longer drying time both led to the degradation of curcumin (Chumroenphat *et al.*, 2021). Similarly, the study of Kadam *et al.* (2013) stated that as the thickness of sample is reduced the curcumin content goes on decreasing for different drying temperatures.

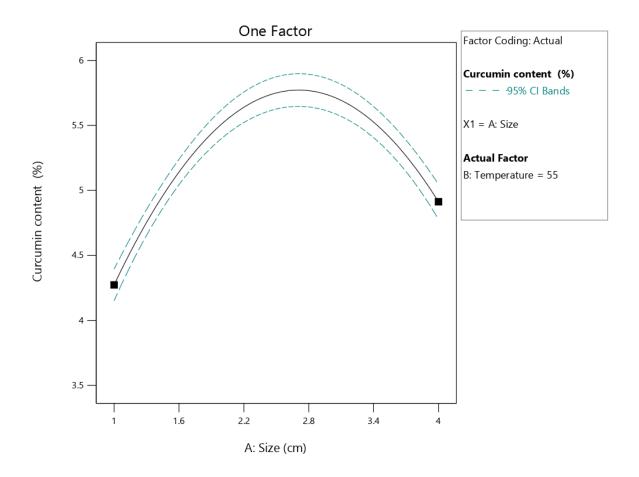


Fig. 4.2 Single factor interaction graph of curcumin content for A: Size

From Fig. 4.2, it can concluded that increase in rhizome size led to gradual increase in curcumin content upto certain point and further increase in size resulted in the decline in curcumin content.

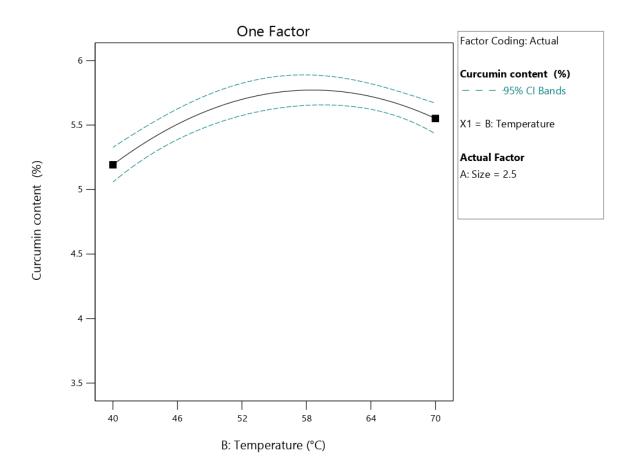


Fig. 4.3 Single factor interaction graph of curcumin content for B: Temperature

Fig. 4.3 showed that increase in drying temperature led to increase in curcumin content upto certain point and further increase in temperature resulted in slow decline of curcumin content.

4.2.2 Effect on oleoresin content

The oleoresin content of the turmeric powder varied from 16.17 to 20.74% (Table B.1). Table C.3 and C.4 show the coefficient of the model and other statistical attributes of oleoresin content. A quadratic regression model was best fitted to describe the effect of rhizome size and drying temperature on oleoresin content of turmeric powder. The model F value was found significant (p<0.0001). The lack of fit test was not significant (p>0.05). The lack of fit F-value of 1.02 implies the lack of fit is not significant relative to the pure error. Non-significant lack of fit is desired to fit the model. The coefficient of determination R^2 was 0.9983, which indicated that 99.83% of the variability of the response could be explained by the model. The predicted R^2 of 0.9958 is a reasonable agreement with the

adjusted R^2 of 0.9974. The best fitted quadratic model for the effect of variables on oleoresin content of the product was given best described by coded equation 4.2

Oleoresin content =
$$+20.88 + 0.3743$$
A - 0.0424 B + 0.1186 AB - 3.4 A² - 1.03 B²4.2

Where A and B are the coded values of rhizome size and drying temperature. A, B, A^2 , B^2 and AB are model terms.

In the quadratic equation 4.2, oleoresin content had significant (P<0.05) positive effect of the rhizome size (A) while non-significant (P>0.05) negative effect of the drying temperature (B) at 95 % level of confidence. The quadratic term of both the rhizome size (A²) and drying temperature (B²) had significant (P<0.05) negative effect on oleoresin content as given in Table C.4. The interaction term of rhizome size and drying temperature (AB) had significant (P<0.05) positive effect on oleoresin content.

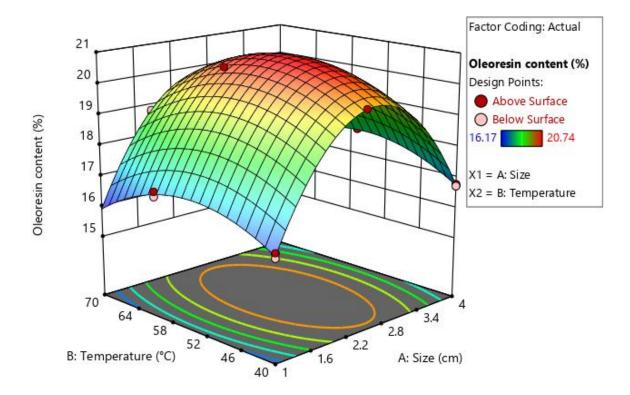


Fig. 4.4 Response surface plot of oleoresin content of turmeric as a function of rhizome size and drying temperature.

With the combined effect of rhizome size and drying temperature, the oleoresin content of turmeric powder increased and reached maximum until further increases leads to decrease in oleoresin content (Fig. 4.4).

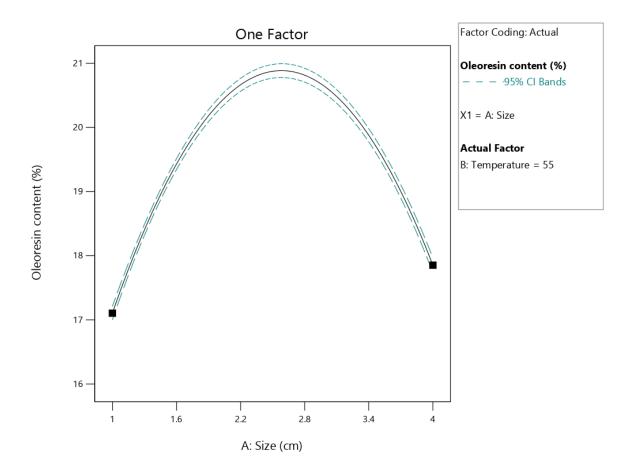


Fig. 4.5 Single factor interaction graph of oleoresin content for A: Size

From Fig. 4.5, it can concluded that increase in rhizome size led to gradual increase in oleoresin content upto certain point and further increase in size resulted in the gradual decline in oleoresin content.

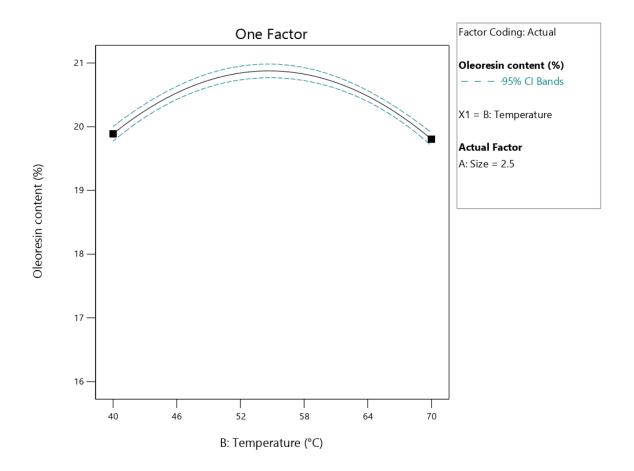


Fig. 4.6 Single factor interaction graph of oleoresin content for B: Temperature

Fig. 4.6 reveled that increase in drying temperature led to increase in oleoresin content upto certain point and further increase in temperature resulted in the decline of oleoresin content. The study performed by Singh *et al.* (2010a) reported that oleoresin content first increased with increase in temperature and then decreased with further increase in temperature after reaching a peak value for both finger and mother rhizomes. The maximum value of oleoresin content obtained in their study was 19.54%.

4.2.3 Effect on essential oil (EO) content

The essential oil content of the turmeric powder varied from 5.47 to 7.35% (Table B.1). Table C.5 and C.6 show the coefficient of the model and other statistical attributes of essential oil content. A quadratic regression model was best fitted to describe the effect of rhizome size and drying temperature on essential oil content of turmeric powder. The model F value was found significant (p<0.0001). The lack of fit test was not significant (p>0.05). The lack of fit F-value of 1.21 implies the lack of fit is not significant relative to the pure

error. Non-significant lack of fit is desired to fit the model. The coefficient of determination R^2 was 0.9714, which indicated that 97.14% of the variability of the response could be explained by the model. The predicted R^2 of 0.9275 is a reasonable agreement with the adjusted R^2 of 0.9571. The best fitted quadratic model for the effect of variables on EO content of the product was given best described by coded equation 4.3

EO content =
$$+7.25 + 0.058A - 0.2502B + 0.4065AB - 0.9594A^2 - 0.5882B^2 \dots 4.3$$

Where A and B are the coded values of rhizome size and drying temperature. A, B, A^2 , B^2 and AB are model terms.

In the quadratic equation 4.3, EO content had non-significant (P>0.05) positive effect of the rhizome size (A) while highly significant (P>0.05) negative effect of the drying temperature (B) at 95 % level of confidence. The quadratic term of both the rhizome size (A^2) and drying temperature (B^2) had significant (P<0.05) negative effect on EO content as given in Table C.6. The interaction term of rhizome size and drying temperature (AB) had significant (P<0.05) positive effect on EO content.

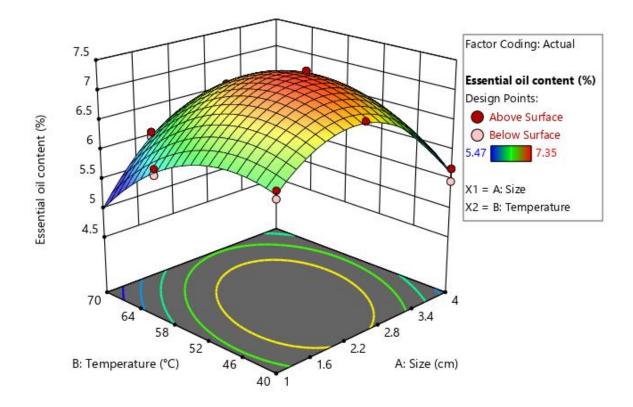


Fig. 4.7 Response surface plot of essential oil content of turmeric as a function of rhizome size and drying temperature.

Fig. 4.7 revealed that the essential oil content increased with combined increase in rhizome size and drying temperature upto certain point. Further increase led to decline in EO content of the turmeric powder.

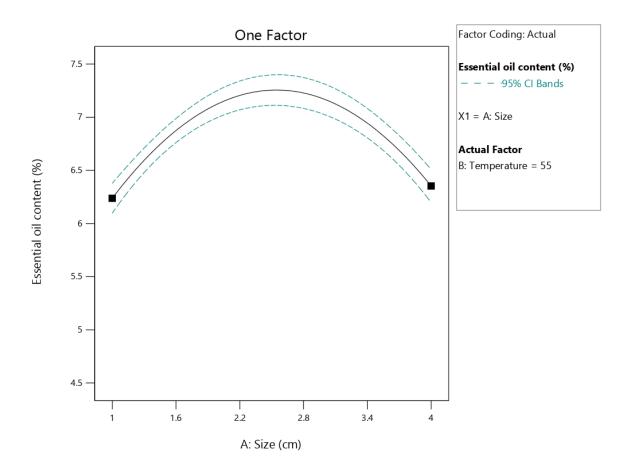


Fig. 4.8 Single factor interaction graph of essential oil content for A: Size

From Fig. 4.8, it can be concluded with the increase of rhizome size, EO content of turmeric powder increased and reached maximum until further increase led to decrease in EO content.

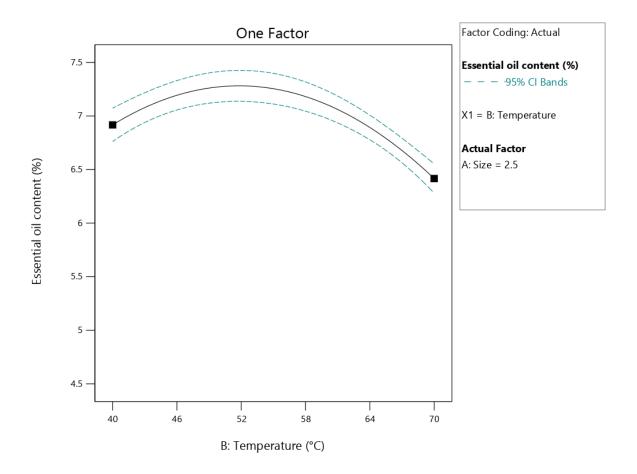


Fig. 4.9 Single factor interaction graph of essential oil content for B: Temperature

Fig. 4.9 revealed that slight increase in temperature led to small increase in EO content of turmeric powder. Further increase in drying temperature led to drastic decline in EO content.

4.2.4 Effect on Total Phenolic Content (TPC)

The TPC of the turmeric powder varied from 29.78 to 39.13 mg GAE/g dry matter (Table B.1). Table C.7 and C.8 show the coefficient of the model and other statistical attributes of total phenolic content. A quadratic regression model was considered as best fitted to describe the effect of rhizome size and drying temperature on TPC of turmeric powder. The model F value was found significant (p<0.0001). The lack of fit test was not significant (p>0.05). The lack of fit F-value of 1.62 implies the lack of fit is not significant relative to the pure error. Non-significant lack of fit is desired to fit the model. The coefficient of determination R^2 was 0.9926, which indicated that 99.26% of the variability of the response could be explained by the model. The predicted R^2 of 0.9813 is a reasonable agreement with the

adjusted R^2 of 0.9889. The best fitted quadratic model for the effect of variables on total phenolic content of the product was given best described by coded equation 4.4

$$TPC = +38.42 + 1.07A - 2.85B - 0.0444AB - 0.866A^2 - 5.24B^2 \dots 4.4$$

Where A and B are the coded values of rhizome size and drying temperature. A, B, A^2 , B^2 and AB are model terms.

In the quadratic equation 4.4, TPC had highly significant (P<0.05) positive effect of the rhizome size (A) but significant (P<0.05) negative effect of the drying temperature (B) at 95 % level of confidence. The quadratic term of both the size of rhizome (A^2) and drying temperature (B^2) had significant (P<0.05) negative effect on TPC as given in Table C.8. The interaction term of rhizome size and drying temperature (AB) had non-significant (P>0.05) negative effect on TPC of the turmeric powder.

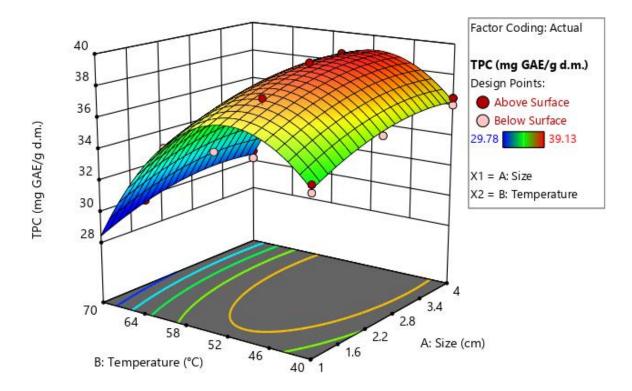


Fig. 4.10 Response surface plot of TPC of turmeric as a function of rhizome size and drying temperature.

From Fig. 4.10, it can be concluded that combined slight increase in size and temperature caused increase in TPC until it reached maximum and further increase led to drastic decrease in TPC of the turmeric powder.

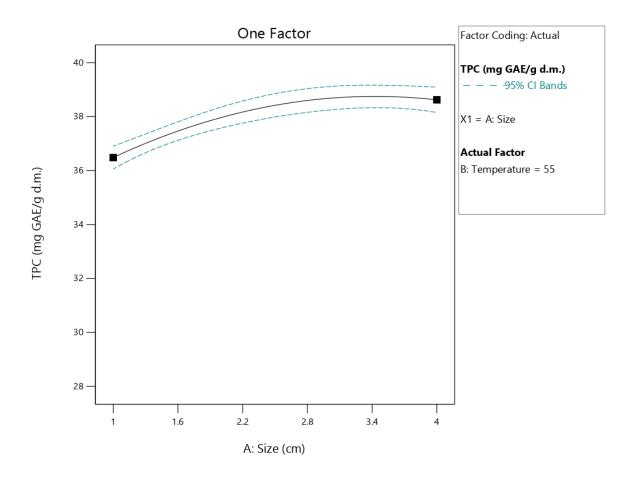


Fig. 4.11 Single factor interaction graph of TPC for A: Size

Fig. 4.11 showed that with increase in rhizome size, the TPC of turmeric powder increases. The increase in TPC was non-linear.

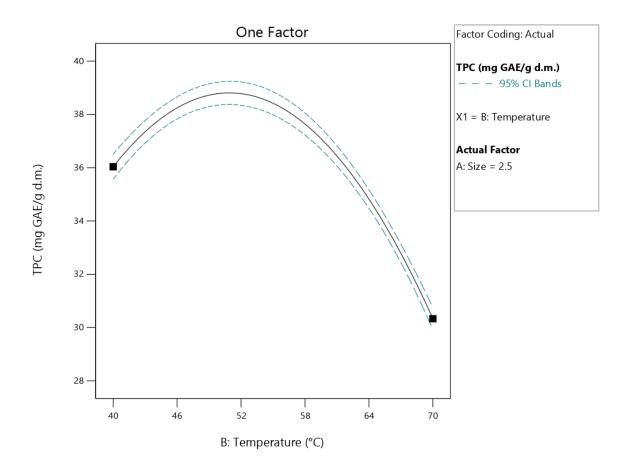


Fig. 4.12 Single factor interaction graph of TPC for B: Temperature

Fig. 4.12 revealed that increase in slight increase in temperature caused increase in TPC upto maximum until further increase led to drastic decline in TPC of turmeric powder.

The initial lower value of TPC is may be due to the activation of enzyme polyphenol oxidase (PPO) at a temperature range of 35-45°C which causes oxidative degradation of polyphenols. The rise in TPC value with the slight rise in temperature was due to the inactivation of enzyme PPO, as PPO are thermally unstable and lose activity after 60°C (Prathapan *et al.*, 2009). The drastic reduction in values of TPC at higher temperature is may be due to the nonenzymatic oxidation of polyphenols. Thermal processing in general might be expected to reduce TPC (Wang *et al.*, 2013).

4.2.5 Effect on antioxidant activity (AA)

The antioxidant activity measured as % DPPH radical scavenging activity of the turmeric powder varied from 38.37 to 49.17 %RSA (Table B.1). Table C.9 and C.10 show the coefficient of the model and other statistical attributes of antioxidant activity. A quadratic

regression model was best fitted to describe the effect of rhizome size and drying temperature on antioxidant activity of turmeric powder. The model F value was found significant (p<0.0001). The lack of fit test was not significant (p>0.05). The lack of fit F-value of 2.55 implies the lack of fit is not significant relative to the pure error. Non-significant lack of fit is desired to fit the model. The coefficient of determination R² was 0.9509, which indicated that 95.09% of the variability of the response could be explained by the model. The predicted R² of 0.8912 is a reasonable agreement with the adjusted R² of 0.9263. The best fitted quadratic model for the effect of variables on total phenolic content of the product was given best described by coded equation 4.5.

Antioxidant activity = $+49.58 + 1.31A + 1.19B + 1.54AB - 5.03A^2 - 4.65B^2$ 4.5

Where A and B are the coded values of rhizome size and drying temperature. A, B, A^2 , B^2 and AB are model terms.

In the quadratic equation 4.5, antioxidant activity had highly significant (P<0.05) positive effect of both the rhizome size (A) and the drying temperature (B) at 95 % level of confidence. Similarly, the quadratic terms of both size of rhizome (A^2) and drying temperature (B^2) had highly significant (P<0.05) negative effect on antioxidant activity as given in Table C.10. The interaction term of rhizome size and drying temperature (AB) had significant (P<0.05) positive effect on antioxidant activity.

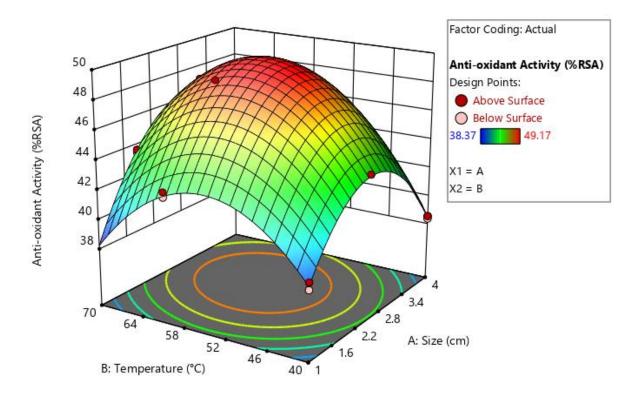


Fig. 4.13 Response surface plot of antioxidant activity of turmeric as a function of rhizome size and drying temperature.

From Fig. 4.13, it can be concluded that, with the combined increase in size and temperature, the antioxidant activity of turmeric powder increased upto certain limit and further increase led to decline in antioxidant activity.

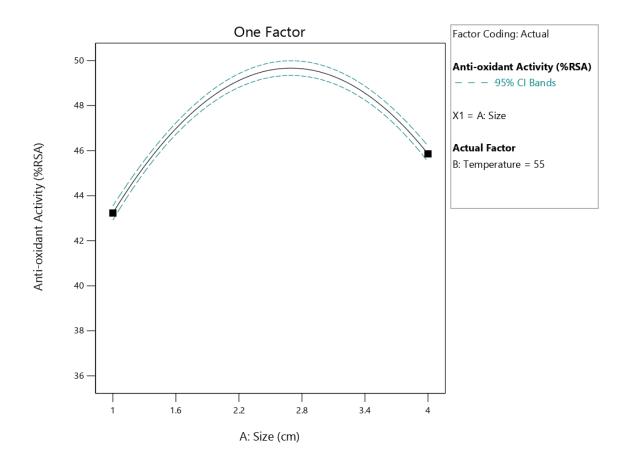


Fig. 4.14 Single factor interaction graph of antioxidant activity for A: Size

Fig 4.14 showed that with the increase in rhizome size antioxidant activity of turmeric powder increased and reached maximum point and further increase in size caused decrease in antioxidant activity.

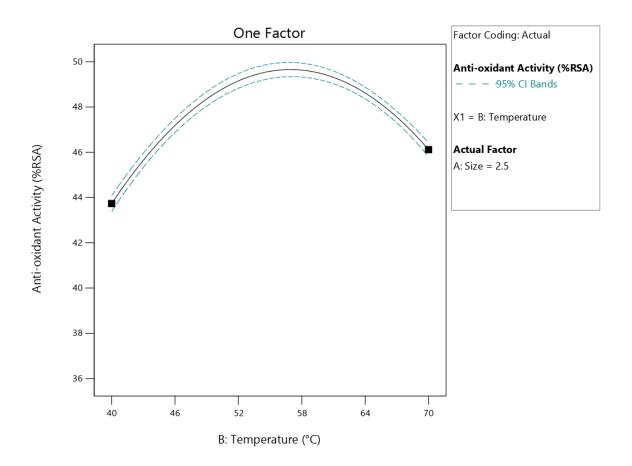


Fig. 4.15 Single factor interaction graph of antioxidant activity for B: Temperature

Fig 4.15 showed that increase in temperature of drying caused increase in antioxidant activity upto certain point. Further increase in temperature led to decline in antioxidant activity of turmeric powder.

4.3 Optimization of rhizome size and drying temperature

The effect of rhizome size and drying temperature on curcumin content, oleoresin content, essential oil content, total phenolic content and antioxidant activity of the final turmeric powder was observed. To determine the optimum combination of rhizome size and drying temperature, numerical response optimization technique was carried out. The assumptions were to develop a suitable combination of size and temperature for turmeric rhizome to develop turmeric powder with higher curcumin content, oleoresin content, EO content, TPC and antioxidant activity. Constraints of setting goal for optimization was shown in Table 4.3.

Name	Goal	Lower limit	Upper limit
Rhizome size (cm)	is in range	1	4
Drying temperature (°C)	is in range	40	70
Curcumin content (%)	maximize	3.95	5.78
Oleoresin content (%)	maximize	16.17	20.74
Essential oil content (%)	maximize	5.47	7.35
Total phenolic content (mg GAE/g DM)	is in range	30	40
Antioxidant Activity (%RSA)	maximize	38.37	49.17

 Table 4.3 Different constraints for optimization of turmeric powder

Based on the constraints in Table 4.3 and under the assumption by design expert (version 13), one optimized solution with desirability 0.985 was obtained. The optimized conditions for maximum curcumin, oleoresin, EO, TPC and AA were found to be 2.63 cm (~2.6 cm) of rhizome size and $54.95^{\circ}C$ (~ $55^{\circ}C$) temperature of cabinet dryer as shown in Fig. D.1. Singh *et al.* (2010a), found that the optimum drying conditions for best product quality of turmeric powder was air temperature of 55–60°C and air velocity of 2 m/sec. Similarly, Komonsing *et al.* (2021), suggested that drying at 60°C without light exposure was the best condition to preserve curcumin, color, total phenolic contents, and antioxidant capacity of the turmeric powder.

4.4 Verification of the model

The suitability of the model developed for predicting the optimum response values was tested using the recommended optimum conditions of the variables and was also used to validate the experimental and predicted values of the response. For this, the turmeric rhizome was dried with suggested combination of rhizome size (2.6 cm) and drying temperature (55°C). The result for the expected responses and experimental responses following the optimized condition was compared and found as shown in Table 4.4. The R² value for the plot was found to be 0.9999 with regression equation as y = 0.9997x - 0.0795, where y is mean observed value and x is predicted value. The plot is shown in fig D.2 in appendix D.

Responses	Rhizome size (cm)	Drying temperature (°C)	Predicted value	Mean observed value
Curcumin content (%)	2.6	55	5.78671	5.795
Oleoresin content (%)	2.6	55	20.8628	20.8467
Essential oil content (%)	2.6	55	7.2142	7.06633
TPC (mg GAE/g DM)	2.6	55	38.4875	38.1203
Antioxidant activity (%RSA)	2.6	55	49.734	49.8247

Table 4.4 Predicted and actual values of the responses at the optimized condition

4.5 Physicochemical properties

4.5.1 Chemical composition of optimized and sun-dried market turmeric powder

The chemical composition of optimized turmeric powder and sun-dried turmeric rhizome bought for market was determined and compared. The dried rhizome bought from market was grinded to make turmeric powder. The chemical composition of optimized and sundried market turmeric powder is given in Table 4.5.

	Turmeric powder		
Parameters	Optimized	Market	
Moisture (%)	$10.04^{a} \pm \ 0.07$	$9.97^{a}\pm0.06$	
Crude protein (%,db)	$8.05^{a}\pm0.24$	$8.06^{a}\pm0.31$	
Crude fat (%,db)	$6.32^{a}\pm0.19$	$5.72^{b}\pm0.19$	
Crude fiber (%,db)	$4.58^{a}\pm0.25$	$5.06^{a}\pm0.12$	
Total ash (%,db)	$3.82^{a}\pm0.06$	$4.12^{b}\pm0.07$	
Carbohydrate (%,db)	$77.23^a \pm 0.67$	$77.04^{a}\pm0.17$	
Curcumin (%,db)	$5.8^{a}\pm0.2$	$4.15^b \pm 0.15$	
Oleoresin (%,db)	$20.85^{a}\pm0.06$	$17.97^b \pm 0.07$	
Essential oil (%,db)	$7.04^{a}\pm0.08$	$6.65^b \pm 0.07$	
Total Phenolic content (mg GAE/g DM)	$38.12^{a}\pm0.58$	$32.86^b \pm 0.95$	
Antioxidant activity (%RSA)	$49.68^{a}\pm0.28$	$34.89^b \pm 0.13$	

Table 4.5 Chemical composition of optimized and sun-dried market turmeric powder

Note: Values are the means of three determinations. Figures after \pm are the standard deviation. Values in the row having same superscript are not significantly different at 5% level of significance.

The values for moisture content, crude protein, crude fiber and carbohydrate content of optimized and market turmeric powder did not have significance difference at 5% level of significance. However, the crude fat content of optimized turmeric powder showed higher value as compared to that of market sample. The higher value of fat in optimized powder is may be due to the lesser time of drying. Longer drying time result in the oxidation of lipids (Liu *et al.*, 2019). Similarly, total ash content showed significant difference. The total ash of market sample showed higher value of 3.12% than that of optimized sample which was 2.82%. The higher content of total ash in market sample is may be due to the poor processing

and open drying area. The market sample was dried by the farmer in an open place without much considering the cleanliness of drying area because of which the market rhizome may had some of the adhere sand or dust.

The curcumin, oleoresin, essential oil, total phenolic content and antioxidant activity all showed significant difference between optimized and market sample at 5% level of significance. The lower value of curcumin content in the market sample is may be due to the longer time of drying occurs during sun drying which causes degradation of curcumin.

The lower value of oleoresin content of market sample can be explained from the study of Hirko *et al.* (2020). Hirko *et al.* (2020), reported that the effect of direct sun light and high temperature during drying and form of rhizome causes the breakup of oleoresin cells and facilitates the rapid release of oleoresin containing curcuminoids to the surrounding during processing. Similarly, this study showed the value of essential oil in optimized turmeric powder i.e. 7.04% was higher than that of market sample i.e. 6.65%. This can be explained from the study of Jose and Joy (2009) who reported traditional drying method could result in the loss of volatile oil of up to 25% by evaporation, and in the destruction of some of the light-sensitive oil constituents.

The lower value of total phenolic content in market sample i.e. 32.86 mg GAE/g dry matter as compared to that of optimized sample the value of which was 38.12 mg GAE /g dry matter was may be due to the degradation of polyphenols by enzymes polyphenol oxidase (PPO) which is activated at the temperature of sun drying (35-40°C) and the higher value of TPC in optimized turmeric powder was due to the inactivation of PPO at a temperature range of 55-60°C which was reported by Prathapan *et al.* (2009).

Similarly, the antioxidant activity of market sample was found to be 34.89 %RSA which was much lower as compared to the value optimized turmeric powder which was 49.68 %RSA. As the bioactive components such as curcumin, essential oil and total phenolic content are the components responsible for the antioxidant activity of the turmeric powder (Jayaprakasha *et al.*, 2006), the decrease in the value of these components of the market sample resulted to the decrease in value of antioxidant activity.

Part V

Conclusions and recommendations

5.1 Conclusions

Based on the results of present study, the following conclusions are drawn:

- 1) The proximate composition of fresh turmeric was found to be 78.05% moisture with the value of crude protein, crude fat, crude fiber, total ash and carbohydrate to be 8.23%, 6.53%, 4.75%, 3.87% and 76.63% on dry weight basis respectively.
- 2) The optimum rhizome size and drying temperature was found to be 2.6 cm and 55°C which produce best quality of turmeric powder having value of curcumin, oleoresin, essential oil, TPC and antioxidant activity to be 5.8%, 20.85%, 7.04%, 38.12 mg GAE/ g dry matter and 49.68 %RSA
- 3) The optimized turmeric powder was found to be superior in all bioactive parameters and antioxidant activity as compared to traditionally sun-dried turmeric powder.
- 4) Thicker rhizome size and lower drying temperature led to the longer drying time which caused the oxidative degradation of bioactive components.
- 5) Similarly, very high drying temperature and thinner rhizome size led to thermal degradation of bioactive components.

5.2 Recommendations

- 1) The turmeric rhizome should be dried at 55°C with 2.6 cm of rhizome size.
- 2) Turmeric from different location can be studied for various pretreatment along with variation in rhizome size and drying temperature.

Part VI

Summary

Turmeric (*Curcuma longa*) is extensively used as spice, food preservative and coloring material in Nepal, India, China and South East Asia. The bioactive components of turmeric such as curcuminoids, essential oil and oleoresin contributes to the antioxidant, antimicrobial, anti-allergic, inflammatory, hypocholestraemic, choleratic, insect repellent, anti-rheumatic, antivenomous, antiviral, antidiabetic, anti-hepatotoxic and anti-cancerous. Improper drying caused the degradations of these bioactive components.

In this study, turmeric rhizome was taken from local market of Dharan. The study was done to produce higher quality of turmeric powder varying rhizome size and drying temperature of cabinet dryer. Design expert version 13 was used to produce samples. The range for rhizome size was 1 to 4 cm while for drying temperature was 40 to 70°C. Sixteen sample were obtained. The turmeric rhizomes were cleaned and made dirt free and was sorted in primary, secondary and tertiary rhizome from which secondary rhizomes were taken for the study. The cross-sectional area was maintain to be 2.434 ± 0.023 cm² for all sample and the samples were dried to moisture content of 10%. The analysis was done for both proximate and chemical composition of fresh as well as dried turmeric rhizome. The chemical analysis include measurement of curcumin, oleoresin, essential oil content, TPC and antioxidant activity. The optimized value of rhizome size and temperature of cabinet dryer was obtained on the basis of the values of curcumin, oleoresin, EO content, TPC and antioxidant activity.

The optimized rhizome size and drying temperature was found to be 2.6 cm and 55°C with the value of curcumin, oleoresin, EO content, TPC and antioxidant activity to be 5.8%, 20.85%, 7.04%, 38.12 mg GAE/ g dry matter and 49.68 %RSA respectively. The drying of turmeric rhizome caused decrease in the values of measured bioactive components. The optimized turmeric powder was compared with sun dried market sample which was bought as dried rhizome. The market sample was grinded to make powder and was compared with optimized turmeric powder. The optimized turmeric powder was found to be of higher quality in terms of all bioactive components and antioxidant activity.

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Appendices

Appendix A

A.1 Apparatus required

- Cabinet Dryer
- Spectrophotometer
- Soxhlet apparatus
- Clevenger Apparatus
- Dean and stark distillation flask
- Digestion flask and Kjeldahl distillation set
- Balance
- Heating arrangement
- Buchner's filter assembly
- Water bath
- Muffle furnace
- Balance
- Glasswares

A.2 Chemicals required

- Standard curcumin
- 1, 1-diphenyl-1-picryl-hydrazyl radical (DPPH)
- Folin-Ciocalteu's reagent
- Gallic acid
- Indicators (Phenolphthalein, Methylene blue)
- Acetone
- Boric acid
- Toulene
- Ethyl alcohol: 50% and 95% alcohol
- Other basic laboratory chemicals

Appendix B

	F1	F2	R1	R2	R3	R4	R5
	A:Size	B:Temp-	Curcumin	Oleoresin	Essential	TPC	AA
Run	cm	erature	content	content	oil content	mg GAE/g	%RSA
		°C	%	%	%	DM	
1	4	56	4.92	17.88	6.44	38.54	46.01
2	1.8	70	4.87	18.78	6.07	29.78	43.52
3	1.5	50	4.89	18.97	6.89	38.08	45.51
4	2.7	52	5.72	20.74	7.35	39.13	49.17
5	4	40	3.95	16.72	5.69	36.06	38.54
6	4	70	4.96	16.85	5.97	30.21	43.69
7	2.4	40	5.13	19.87	6.97	35.67	43.85
8	1	40	4.05	16.33	6.24	34.34	39.2
9	4	40	4.07	16.78	5.47	36.51	38.37
10	2.1	60	5.67	20.5	6.78	36.24	48.84
11	1	40	4.17	16.17	6.37	33.85	38.7
12	4	70	5.16	16.91	5.83	30.73	44.19
13	2.9	68	5.78	20.02	6.66	31.98	47.51
14	1	60	4.21	17.03	6.02	34.87	42.86
15	1.8	70	5.03	18.83	5.98	30.01	43.69
16	1	60	4.14	16.86	5.91	34.65	42.52

 Table B.1 Responses for different rhizome size and drying temperature

Appendix C

Source	Std. Dev.	R ²	Adjusted R ²	Predicted R ²	PRESS	
Linear	0.5686	0.2883	0.1788	-0.0075	5.95	
2FI	0.5818	0.3122	0.1402	-0.1042	6.52	
Quadratic	0.0934	0.9852	0.9779	0.9629	0.2193	Suggested
Cubic	0.0959	0.9907	0.9766	0.9135	0.5106	
Quartic	0.0996	0.9916	0.9748		*	Aliased

 Table C.1 Model summary statistics for curcumin content

Source	Sum of	df	Mean	F-value	p-value	
	Squares		Square			
Model	5.82	5	1.16	133.53	< 0.0001	significant
A-Size	0.9039	1	0.9039	103.71	< 0.0001	
B-Temperature	0.2963	1	0.2963	34.00	0.0002	
AB	0.7909	1	0.7909	90.75	< 0.0001	
A²	3.54	1	3.54	406.07	< 0.0001	
B ²	0.3947	1	0.3947	45.29	< 0.0001	
Residual	0.0872	10	0.0087			
Lack of Fit	0.0375	5	0.0075	0.7554	0.6171	not significant
Pure Error	0.0497	5	0.0099			
Cor Total	5.91	15				

 Table C.2 Analysis of variance (ANOVA) for Quadratic Model of curcumin content

Source	Std. Dev.	R ²	Adjusted R ²	Predicted R ²	PRESS	
Linear	1.65	0.0542	-0.0914	-0.3510	50.67	
2FI	1.68	0.0987	-0.1266	-0.4616	54.82	
Quadratic	0.0806	0.9983	0.9974	0.9958	0.1568	Suggested
Cubic	0.0739	0.9991	0.9978	0.9956	0.1664	
Quartic	0.0801	0.9991	0.9974		*	Aliased

Table C.3 Model summary statistics for oleoresin content

Source	Sum of	df	Mean	F-value	p-value	
	Squares		Square			
Model	37.44	5	7.49	1153.38	< 0.0001	significant
A-Size	1.24	1	1.24	190.57	< 0.0001	
B-Temperature	0.0166	1	0.0166	2.55	0.1411	
AB	0.0847	1	0.0847	13.04	0.0048	
A ²	30.49	1	30.49	4695.47	< 0.0001	
B ²	2.92	1	2.92	450.25	< 0.0001	
Residual	0.0649	10	0.0065			
Lack of Fit	0.0328	5	0.0066	1.02	0.4905	not significant
Pure Error	0.0321	5	0.0064			
Cor Total	37.51	15				

Source	Std. Dev.	R ²	Adjusted R ²	Predicted R ²	PRESS	
Linear	0.5404	0.0654	-0.0784	-0.3597	5.52	
2FI	0.5443	0.1248	-0.0939	-0.4750	5.99	
Quadratic	0.1078	0.9714	0.9571	0.9275	0.2947	Suggested
Cubic	0.0972	0.9861	0.9652	0.8917	0.4400	
Quartic	0.1025	0.9871	0.9612		*	Aliased

Table C.5 Model summary statistics for essential oil content

Table C.6 Analysis of variance (ANOVA) for Quadratic Model of essential oil cor	ntent

Source	Sum of	df	Mean	F-value	p-value	
	Squares		Square			
Model	3.95	5	0.7893	67.95	< 0.0001	Significant
A-Size	0.0297	1	0.0297	2.56	0.1408	
B-Temperature	0.5766	1	0.5766	49.64	< 0.0001	
AB	0.9954	1	0.9954	85.69	< 0.0001	
A²	2.43	1	2.43	209.37	< 0.0001	
B ²	0.9543	1	0.9543	82.15	< 0.0001	
Residual	0.1162	10	0.0116			
Lack of Fit	0.0636	5	0.0127	1.21	0.4195	not significant
Pure Error	0.0526	5	0.0105			
Cor Total	4.06	15				

Source	Std. Dev.	R ²	Adjusted R ²	Predicted R ²	PRESS	
Linear	2.54	0.4148	0.3248	0.1596	120.47	
2FI	2.57	0.4481	0.3101	0.1022	128.69	
Quadratic	0.3264	0.9926	0.9889	0.9813	2.68	Suggested
Cubic	0.2606	0.9972	0.9929	0.9888	1.61	
Quartic	0.2854	0.9972	0.9915		*	Aliased

 Table C.7 Model summary statistics for Total Phenolic Content

Table C.8 Analysis of variance (ANOVA) for Quadratic Model of Total Phenolic Content

Source	Sum of	df	Mean	F-value	p-value	
	Squares		Square			
Model	142.28	5	28.46	267.07	< 0.0001	significant
A-Size	10.12	1	10.12	95.03	< 0.0001	
B-Temperature	74.91	1	74.91	703.07	< 0.0001	
AB	0.0119	1	0.0119	0.1113	0.7455	
A ²	1.98	1	1.98	18.60	0.0015	
B ²	75.63	1	75.63	709.84	< 0.0001	
Residual	1.07	10	0.1065			
Lack of Fit	0.6583	5	0.1317	1.62	0.3054	not significant
Pure Error	0.4071	5	0.0814			
Cor Total	143.34	15				

Source	Std. Dev.	R ²	Adjusted R ²	Predicted R ²	PRESS	
Linear	3.16	0.2854	0.1755	-0.0101	183.40	
2FI	3.28	0.2871	0.1088	-0.1837	214.94	
Quadratic	0.2421	0.9968	0.9952	0.9920	1.44	Suggested
Cubic	0.2479	0.9980	0.9949	0.9828	3.13	
Quartic	0.2595	0.9981	0.9944		*	Aliased

Table C.9 Model summary statistics for antioxidant activity

Source	Sum of	df	Mean	F-value	p-value	
	Squares		Square			
Model	180.99	5	36.20	617.56	< 0.0001	significant
A-Size	15.25	1	15.25	260.16	< 0.0001	
B-Temperature	13.10	1	13.10	223.50	< 0.0001	
AB	14.34	1	14.34	244.69	< 0.0001	
A ²	66.97	1	66.97	1142.61	< 0.0001	
B ²	59.70	1	59.70	1018.56	< 0.0001	
Residual	0.5861	10	0.0586			
Lack of Fit	0.2494	5	0.0499	0.7408	0.6250	not significant
Pure Error	0.3367	5	0.0673			
Cor Total	181.57	15				

Table C.10 Analysis of variance (ANOVA) for Quadratic Model of antioxidant activity

Appendix D

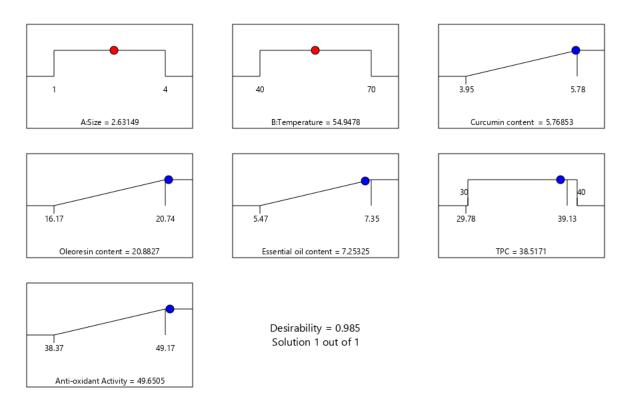


Fig. D.1 Numerical optimized solutions for turmeric

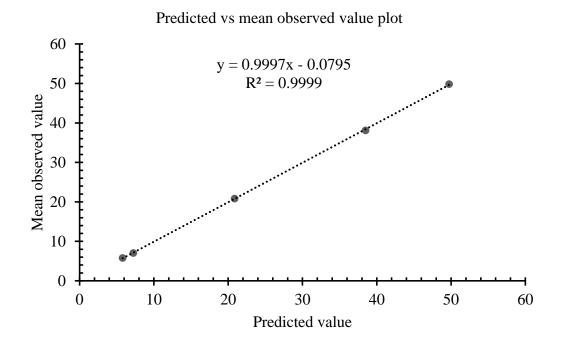


Fig D.2 Predicted value vs. mean observed of optimized product

Appendix E

	Variable 1	Variable 2
Mean	10.04	9.96667
Variance	0.0049	0.00413
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	1.3364048	
P(T<=t) one-tail	0.1261856	
t Critical one-tail	2.1318468	
P(T<=t) two-tail	0.2523712	
t Critical two-tail	2.7764451	

Table E.1 t-Test: Two-Sample Assuming Unequal Variances for moisture contentVariate: Moisture content

	Variable 1	Variable 2
Mean	8.05	8.056667
Variance	0.0567	0.096633
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	-0.029488391	
P(T<=t) one-tail	0.488943856	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.977887712	
t Critical two-tail	2.776445105	

Table E.2 t-Test: Two-Sample Assuming Unequal Variances for crude proteinVariate: Crude protein

	Variable 1	Variable 2
Mean	6.32	5.72
Variance	0.0367	0.0351
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	3.8783737	
P(T<=t) one-tail	0.008933	
t Critical one-tail	2.1318468	
P(T<=t) two-tail	0.0178659	
t Critical two-tail	2.7764451	

Table E.3 t-Test: Two-Sample Assuming Unequal Variances for crude fatVariate: Crude fat

	Variable 1	Variable 2
Mean	4.58	5.0633333
Variance	0.0601	0.0132333
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-3.0914104	
P(T<=t) one-tail	0.02682722	
t Critical one-tail	2.35336343	
P(T<=t) two-tail	0.05365444	
t Critical two-tail	3.18244631	

Table E.4 t-Test: Two-Sample Assuming Unequal Variances for crude fiber.

Variate: Crude fiber

	Variable 1	Variable 2
Mean	3.82333333	4.12333
Variance	0.00363333	0.00503
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	-5.5815631	
P(T<=t) one-tail	0.00252605	
t Critical one-tail	2.13184679	
P(T<=t) two-tail	0.00505209	
t Critical two-tail	2.77644511	

Table E.5 t-Test: Two-Sample Assuming Unequal Variances for total ashVariate: Total ash

	Variable 1	Variable 2
Mean	77.226667	77.0367
Variance	0.4532333	0.02723
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	0.4747693	
P(T<=t) one-tail	0.3408715	
t Critical one-tail	2.9199856	
P(T<=t) two-tail	0.681743	
t Critical two-tail	4.3026527	

Table E.6 t-Test: Two-Sample Assuming Unequal Variances for carbohydrateVariate: Carbohydrate

	Variable 1	Variable 2
Mean	5.795	4.1523333
Variance	0.039628	0.0233613
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	11.336437	
P(T<=t) one-tail	0.0001726	
t Critical one-tail	2.1318468	
P(T<=t) two-tail	0.0003452	
t Critical two-tail	2.7764451	

Table E.7 t-Test: Two-Sample Assuming Unequal Variances for curcumin content.Variate: Curcumin content

	Variable 1	Variable 2
Mean	7.0396667	6.6546667
Variance	0.0067693	0.0045013
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	6.2812569	
P(T<=t) one-tail	0.0016402	
t Critical one-tail	2.1318468	
P(T<=t) two-tail	0.0032804	
t Critical two-tail	2.7764451	

Table E.8 t-Test: Two-Sample Assuming Unequal Variances for essential oil content.Variate: Essential oil content

	Variable 1	Variable 2
Mean	20.846667	17.967
Variance	0.0037613	0.004561
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	54.67393	
P(T<=t) one-tail	3.35E-07	
t Critical one-tail	2.1318468	
P(T<=t) two-tail	6.7E-07	
t Critical two-tail	2.7764451	

Table E.9 t-Test: Two-Sample Assuming Unequal Variances for oleoresin content.Variate: Oleoresin content

	Variable 1	Variable 2
Mean	38.120333	32.856333
Variance	0.3333053	0.8996303
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	8.2111938	
P(T<=t) one-tail	0.0018902	
t Critical one-tail	2.3533634	
P(T<=t) two-tail	0.0037804	
t Critical two-tail	3.1824463	

 Table E.10 t-Test: Two-Sample Assuming Unequal Variances for TPC.

Variate: Total phenolic content

	Variable 1	Variable 2
Mean	49.676667	34.888333
Variance	0.0793303	0.0165163
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	82.735396	
P(T<=t) one-tail	1.946E-06	
t Critical one-tail	2.3533634	
P(T<=t) two-tail	3.892E-06	
t Critical two-tail	3.1824463	

Table E.11 t-Test: Two-Sample Assuming Unequal Variances for antioxidant activity.Variate: Antioxidant activity

Color plates



P1 Cleaning of turmeric rhizome



P2 Drying of turmeric rhizome



P3 Dried turmeric rhizomes



P4 Turmeric powder



P5 Preparation of sample for analysis



P6 Analysis of sample