DISSERTATION ON

ULTRASOUND ASSISTED REDUCTION IN OXALATE OF ELEPHANT FOOT YAM– AN EFFICIENT AND NOVEL APPROACH FOR MANKIND

SUBMITTED TO THE

DEPARTMENT OF BIOENGINEERING

FACULTY OF ENGINEERING

INTEGRAL UNIVERSITY, LUCKNOW

IN PARTIAL FULFILMENT

FOR THE

DEGREE OF MASTER OF TECHNOLOGY

IN FOOD TECHNOLOGY

BY

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UNDER THE SUPERVISION OF

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DECLARATION FORM

I, **Shivangi Srivastava**, a student of **M.Tech Food Technology** (II Year/ IV Semester), Integral University have completed my six months dissertation work entitled **"Ultrasound assisted oxalate reduction in Elephant foot yam – an efficient and novel approach for mankind"** successfully from **Department of Bioengineering, Integral University** under the able guidance of **Dr. Rahul Singh, Assistant Professor, Integral University, Lucknow.**I, hereby, affirm that the work has been done by me in all aspects. I have sincerely prepared this project report and the results reported in this study are genuine and authentic.

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CERTIFICATE BY SUPERVISOR

Certificate that Mr./Ms. **Shivangi Srivastava** (1600100378) has carried out the research work presented in this thesis entitled **"Ultrasound assisted oxalate reduction in Elephant foot yam – an efficient and novel approach for mankind"** for the award of **M.Tech Food Technology** from Integral University, Lucknow under my supervision. The thesis embodies results of original work and studies carried out by the student himself/herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution. The dissertation was a compulsory part of her **M.Tech Food Technology.**

I wish her good luck and bright future.

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TO WHOM IT MAY CONCERN

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ACKNOWLEDGEMENT

I would like to thank Almighty God for blessing me with His wisdom, understanding and knowledge. God's guidance and strength helped me to achieve success in every area of my life. This thesis appears in its current form due to assistance and guidance of several people. It gives me great pleasure to express my gratitude to all those who supported me and have contributed in making this thesis possible.

I am thankful to my guide Dr. Rahul Singh, Assistant professor, Integral University, Lucknow who had shown his keen interest in guiding me to carry out my work and above all his overwhelming attitude to help his students. He is solely and mainly responsible for completing my work. I am also thankful to Mr. Vinay Kumar Pandey and Ms. Tripti Singh, Research Scholars, Integral University, Lucknow, Ms Sufia Aziz, Lab Assistant, Food technology Lab and my classmates, Ms Zainab Sultan, Ms. Poornima Singh, Mr. Tanzeel Hasan, Mr. Vishal Singh for their continuous support, motivation and untiring help during this project.

I would like to express my gratitude towards Dr. Alvina Farooqui, Head, Department of Bio-engineering, Integral University, Lucknow for her support, suggestions and encouragement.

I would like to express my sincere gratitude to my teachers Dr. Kaiser Younis, Dr. Owais Yousuf, Er. Poonam Sharma, Assistant Professors, Department of Bio-engineering for their constant guidance, cooperation and support during my work. I thank all my friends for their valuable support which helped me to finish my work within the stipulated period and also thank all the people who are directly or indirectly associated with the successful completion of my work.

Last but the most important, I would like to express my sincere gratitude from the bottom of my heart to my father Mr Chandra Prakash Srivastava, my mother Mrs Amita Srivastava, my sister Ms Ayushi Srivastava, and my brother Mr. Harsh Srivastava for their love, support, encouragement, guidance, motivation and support to my decision that helped me to get success in every area of my life.

Place: Lucknow **Shivangi Srivastava**

Dated:

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ACRONYM

S. NO	ACRONYMS	FULL FORMS
1	TPC	Total phenol content
2	TFC	Total flavonoid content
3	TTC	Total tannin content
4	DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate
5	UAE	Ultrasound-assisted extraction
6	RSM	Response surface methodology
7	ANOVA	Analysis of variance

ABSTRACT

Elephant foot yam includes a high degree of nutritious fibre, carbohydrates, glucose, protein, and sugars. It also has plenty of calcium, as well as sodium, potassium, and vitamin C. Elephant foot yam possess anti-diabetic, analgesic, anti-bacterial, antimicrobial, antifungal, and anthelmintic properties, as well as anti-cancer properties. The various investigations on yam oxalate concentrations conducted on various treatments were described. Due to the presence of oxalate and acridity, elephant foot yam has been extensively underused. Researchers have employed a variety of ways to decrease calcium oxalate, including boiling, NaCl treatment, ultrasonication, and microwave processing. The various investigations on yam oxalate concentrations conducted by various treatments were described in this project work. The principles of ultrasonic generation and their applications in yam processing are discussed. This will help researchers and the food industry find more effective strategies to reduce the antinutrient factor using frequency-controlled power ultrasound. In this present project work, **"Ultrasound assisted oxalate reduction in Elephant foot yam – an efficient and novel approach for mankind"** Yam was treated with conventional as well as novel method and a comparative study is done. Oxalate reduction is targeted mainly as it is an anti-nutritional factor of yam. Various phytochemicals analysis was done such as, Extract yield, Oxalate reduction, Total phenol content, Total Flavonoid content, Total Tannin content, Antioxidant by DPPH.

Keywords: Elephant Foot Yam: Nutrition: Anticancerous: Calcium Oxalate: Ultrasound

CHAPTER 1
INTRODUCTION

The elephant foot yam, or "King of Tubers," Amorphophallus paeoniifolius, is a member of the Araceae family and is indigenous to Asia **(Jogi et al., 2020)**. It is a medical and therapeutically valuable tuber that is underutilised but beneficial to humankind's food industry **(R S Sreerag et al., 2014)**. It is a tropical tuber crop that has a good chance of being adopted as a commercial cash crop in tropical countries because of its high yield potential and acceptability as a vegetable in a range of delicious cuisines and delicacies **(Abiodun et al., 2014).** It is a crop native to Southeast Asia that grows untamed in the Philippines, Malaysia, Indonesia, and other countries in the area. It is also known in India as Suran or Jimmikand, and it has historically been farmed for commercial purposes throughout. Suran or Jimmikand is the prevalent name for it in India, and it is traditionally grown commercially in the states of Tamil Nadu, Andhra Pradesh, Kerala and West Bengal **(S Anuradha et al., 2012).**

Elephant foot Yam (Dioscorea spp.), a tuber regarded as a famine food, is an important part of marginal, small rural communities and households diets during times of food scarcity **(Abraham et al., 2021**). It ranks at fourth most significant tuber crop, behind sweet potatoes, cassava and potatoes and also produces roughly 10% of the global production of roots and tubers **(Afoakwah et al., 2012)**. Dioscorea tubers are superior to other root crops in terms of nutrients **(Santosa et al., 2014)**. It provides a good amount of necessary nutritional supplements such protein, vital amino acids in a balanced ratio, and a variety of dietary minerals **(Barua et al., 2022)**. Dioscorea species are monocotyledonous tuber crops that belong to the family Dioscorea. More than 600 different species of Dioscorea species are found in the world **(Ansil et al., 2014).** The majority of species have special culinary, medical, and economic qualities, but their widespread use is constrained by the existence of anti-nutritional components. The wild and domesticated species that tribal societies use as traditional food are incredibly diverse. However, a crucial requirement for widespread consumption and production is the comprehensive food quality characteristic features in wild yam species. The morphological and molecular characterization of wild yam species is necessary for the identification of superior yam genotypes with improved traits to be used in future yam breeding programmes **(Barua et al., 2021)**. For bio-prospecting the tuber in the food sectors, understanding the chemical properties of wild tubers may be helpful. Despite its cultural and economic significance, there are currently insufficient wild species that have been sufficiently characterised at the

morphological and molecular levels to allow for the selection and breeding of genotypes of yam with better features. Population structure knowledge gaps have been a crucial factor in the genetic degradation of yams. In order to address future food and nutritional security, the ethnobotany of elephant foot yam species in connection to their anti-nutritional, nutritional, and also emphasises recent advancements in nutraceutical and pharmacological qualities of yam species.

Elephant foot yam has gained popularity among tropical aroid tuber crops as a result of its high output in a limited growing season and good net returns **(Barua et al., 2021).** It has medical and therapeutic properties and is rich in energy, vitamins, and minerals **(Behera et al., 2016).**

Due to palatability issues brought on by trypsin inhibitors, oxalate, and acridity, which are antinutritional factors, edible aroids have not reached their full potential. Calcium oxalate crystals and an unknown chemical irritant are thought to be the cause of the acridity **(Behera et al., 2018)**. The chemical irritant is either thought to be a Di glucoside of 3,4-dihydroxybenzaldehyde **(Bimakr et al., 2011)**, an unidentified proteinase **(Rahaman et al., 2021)**, a hormone, or a (**Rao et al., 2020)**. Chronic effects of eating foods high in oxalate include calcium oxalate clusters building up in the kidneys, the development of kidney stones, and a decrease in the bioavailability of calcium **(Santosa et al., 2016).** Humans excrete calcium oxalate crystals in their urine. Only a very minor portion of urinary oxalate is believed to be produced from dietary oxalate, with the majority coming from ascorbic acid and glycine **(Sheikh et al., 2013)**. Urinary oxalate, cystine levels rise, uric acid, and calcium, increasing the risk of kidney stones, consuming high doses of calcium or oxalate may hasten stone development **(Xu et al., 2014)**. The nutritional cost of a new, highly productive elephant foot yam cultivars may be unknown to people who are accustomed to eating foods high in starch **(Ravi et al., 2009).** Elephant foot yam buyers frequently choose the types with the best flavour, texture, and colour instead of those with the best nutritional profiles. There is a paucity of, nutritional characterization, systemic morphological and horticultural of yam varieties **(Yadu et al., 2017).** The findings of **Wang et al., 2020** qualitative assessment of this crop were primarily based on examinations of a small number of cultivars. To help breeders create desirable kinds with high yield and a better nutritional profile, cultivars were assessed for horticultural, nutritional, and antinutritional characteristics for this study.

In the eastern and northern parts of India, local cultivation of wild form are frequently used to produce indigenous ayurvedic treatments, pickles and vegetables for a

variety of ailments **(Srikanth et al., 2019)**. Elephant foot yam has been farmed commercially in the states of, Tamil Nadu, Andhra Pradesh, West Bengal and Kerala for a long time **(S Garima et al., 2017)**. This article assessed the crop's, farm income measures, resource-use efficiency in these states and cost of cultivation, with the exception of West Bengal, as there are no figures on the crop's acreage, production, or yield in the literature **(Umoh et al., 2013).** The tuber's dry matter content varies between 17.50 and 24 percent, starch between 13.93 and 21.53 percent, sugar between 0.55 and 1.77 percent, protein between 0.84 and 2.60 percent, and fat between 0.07 and 0.37 percent **(Nagar et al., 2019).**

According to research, elephant foot yam has a high nutritious fibre, carbohydrate, glucose, protein, and sugar content. It also has significant amounts of calcium, sodium, potassium, and vitamin C. **(Sheela et al., 2020)**. Research on elephant foot yam is crucial since it has several therapeutic benefits and is frequently used in Indian medicine, including Ayurveda **(Singh et al., 2020)**. It has been found to possess antibacterial, anticancer, antidiabetic, analgesic, anthelmintic and antifungal properties. Corm is used to treat a variety of conditions including bronchitis, asthma, abdominal pain, dysentery, splenomegaly, piles, elephantiasis, and rheumatic swellings **(A Hosseini et al., 2015)**. In the month of Bhadoh, farmers in Assam (India) consume a special dish consisting of EFY, which they think provides them strength **(Cui et al., 2020).** Some of the medicinal applications of EFY include the treatment of haemorrhoids, gulma (tumour conditions), asthila (prostate disorder), pliha (splenic disorders), kasa (cough), and svasa (breathing problems) (**R Mizanur et al., 2014**).

Various tuber extracts have been found to have analgesic, cytotoxic, immune-modulatory, anthelmintic, anti-inflammatory, hepatoprotective, and anxiolytic effects (**R A Harshvardhan et al., 2012**). Recent studies have shown that tuber extracts have cytotoxic and apoptotic effects against the human colon cancer cell line HCT-15 (**Anindita et al., 2013**). Elephant foot yam has several nutritional benefits, but it also contains anti-nutritional components as oxalate, phytate, and saponins, which can be diminished through different preparations before consumption (**O Sarada et al., 2012).** The major problem with consuming EFY is its acridity and/or oxalate level. Due to these problems, elephant foot yam cannot be grown for food (**Barua et al., 2022**). Inflammation can result from the itchy, stinging, and burning feelings that acridity generates in the mouth and throat. Itching may result from its friction with the skin, indicating how irritated it is **(Kumar et al., 2020**). Raphides, which are oxalate crystals that resemble needles, are what cause the acridity. In addition to being irritating, oxalate is thought to be toxic and anti-nutritional **(A Hosseini**

et al., 2015). If a person consumes more than 2 grammes of oxalate, they risk dying. Oxalates can render certain minerals, such as magnesium, iron, zinc, and calcium, unavailable for body by chelating them **(Singh et al., 2021)**. Therefore, eating foods high in oxalate may cause a mineral deficiency in the body. Additionally, oxalates crystals may accumulate in the kidney, causing renal stones and renal failure. Oxalates make up around 75% of kidney stones, and consuming oxalate-containing foods increases urine oxalate levels to varying degrees **(J H Pramod et al., 2012).**

Ultrasound is a nonthermal food processing method that is used to achieve good results in food processing techniques, such as improved mass transfer, food preservation, thermal treatment assistance, texture modification, and food analysis **(Wang et al., 2020)**. Ultrasonic waves (also known as supersonic waves) are sound waves with frequencies between 20 and 100 kHz **(Rahaman et al., 2021)**. Ultrasound causes 'cavitation' in liquids, pressure changes in gaseous media, and liquid movement in solid media **(Manzoor et al., 2020)**. These waves thought of as a type of vibration of high-frequency that produces microscale shear forces and fluid mixing **(R. Cui et al., 2020)**. Ultrasound is used to perform practically all of the unit processes that food goes through, including preservation, sorting and grading, as well as storage and processing. Ultrasonic cavitation has now gained appeal in a variety of applications, including the amplification of the emulsification of oils, the inactivation of microorganisms , the destruction of chemical and biological contaminants, chemical reactions, and so on (**Ojediran et al., 2020**). Furthermore, there is a variety of processing procedures where ultrasound is now being employed to improve food processing (**Barua et al., 2021**).

The study has following objectives:

1. To reduce oxalate content in Elephant foot yam by Ultrasound Assisted Extraction.
2. To study about Various Bioactive Compounds and Phytochemicals of Elephant foot Yam.
3. To Compare conventional and Novel methods of extraction by analysing Elephant foot Yam properties.

CHAPTER 2

REVIEW OF LITERATURE

In this chapter, review of related works pertinent to the topic of research was made in order to know the present status of research in the area. The knowledge of these studies would help the researchers to proceed in an appropriate direction in the present study and draw meaningful conclusions.

2.1 Origin and Distribution of Elephant Foot Yam Species

One of the earliest angiosperm genera, the genus Dioscorea, is thought to have originated in the Indo-Malayan region and Southeast Asia **(Kumar et al., 2017)**. The main Elephant foot yam species can be found in the three remote parts of the world: Tropical America, Southeast Asia and West Africa **(Kumar et al., 2017)**. These areas are the world's largest yam growing regions and exhibit a great deal of diversity **(Kumar et al., 2017**). Among 600 yam species only seven species are mostly consumed in West Africa, nine variants and 93 species are present in China, while five varieties and 14 species are present in Taiwan (**Price et al., 2017**). Two of them - D. cayennensis Lam. subsp. rotundata and D. alata L., D. cayennensis Lam. subsp. cayennensis are of particular significance as a staple crop, primarily in Western Africa, for about 100 million people. Seven to ten of these are cultivars (**Price et al., 2018**).

Scientific Classification	
Kingdom	Plantae
Division	Angiosperms
Class	Monocots
Order	Alisma tales
Family	Araceae
Genus	Amorphophallus
Species	*Paeoniifolius*
Synonyms	A campanulatus

Table 2.1 Scientific Classification of *Amorphallus paeoniifolius*

About 50 species are used as famine food or as staples in the wild. The famous well-known species of yam is Dioscorea villosa L., which is endemic to North America and is commonly known as wild yam (**Avula et al., 2014**). It is well known that China is where the familiar species Dioscorea esculenta (Lour.) Burk first appeared. According to reasearch, D. alata L. is the most commercially significant species and is widely dispersed throughout Pacific and the tropical Asia. It is originally from Tropical Myanmar, more specifically Southeast Asia and Thailand. The most well-known wild Dioscorea species, D. bulbifera L., is indigenous to Asia, , and tropical Africa (**Kumar et al., 2017**). Another wild species, D. pubera Blume, is found in temperate northern Australia and tropical Americas, China, moist parts of the Himalayas, Bhutan , Western Malaysia, and Central Nepal. It is a native of the Indo-China region. Unlike D. pentaphylla L., which is endemic to Tropical Asia and Eastern Polynesia and is found throughout those regions as well as South-Eastern Asia and North America (**Kumar et al., 2017**). There have been reports of around 50 different Dioscorea species in India, including those from, Kerala, Tamilnadu, Assam, Bihar, Odisha, West Bengal, , Gujarat, Rajasthan and Maharashtra (**Kumar**

et al., 2017). Out of 50 species of yam recorded, Assam has the most yam species (19), followed by Tamilnadu (16), Darjeeling and Sikkim (15), and Tamilnadu. High heights are where the untamed species D. prazeri Prain & Burkill and D. deltoidea Wall. ex Griseb. can be found (**Saikia et al., 2010**).

Due to its high yield production potential and widespread use as a vegetable in a variety of delectable cuisines, elephant foot yam is a tropical tuber crop that presents significant opportunities for adoption in tropical countries as a cash crop. It is a plant that originated in south-east Asia and is found growing wild in the Philippines, Malaysia, Indonesia, and other nations in the region. Its tubers are also used in numerous local ayurveda and unani medicinal formulations. The tubers are used in medications to treat piles, asthma, dysentery, and other abdominal problems because it is thought that they contain blood-purifying properties. It is traditionally grown on a commercial scale in the Indian states of Andhra Pradesh, Tamil Nadu, West Bengal, and Kerala. It is also known as Suran or Jimmikand. Local cultivars produced in the wild are typically utilised in the northern and eastern parts of India to produce vegetables, pickles, and homegrown ayurvedic remedies for a variety of diseases. The enormous amounts of calcium oxalate contained in wild plant tubers make them extremely acidic and irritate the tongue and throat. Elephant foot yam cultivation is gradually extending to other Indian states, including Bihar and Uttar Pradesh.

"Gajendra," a native variety from the Andhra Pradesh region's Kovuur, is the most widely grown type in the country. Since it is rarely grown economically in other nations, this crop also has excellent export potential from India. Elephant foot yam production, area, and yield statistics are not currently available, and for some states where it is grown commercially, only unpublished data are available. There hasn't yet been any research on the economics of this crop in India in the literature. Therefore, research has been conducted to determine the crop's cost of cultivation, measures of farm revenue, and resource-use efficiency in the states where it is grown commercially.

2.2 Pharmacological Studies of Elephant Foot Yam

There have been reports of antibacterial, antifungal, antimutagenic, hypoglycaemic, and immunomodulatory properties in Dioscorea species **(Kumar et al., 2017)**. Botryodiploidia theobromae extracts of Dioscorea bulbiferous and

Dioscorea alata have been found to exhibit antifungal properties. By reporting the antifungal and antimicrobial activities of wild yam D. pentaphylla against both gramme positive and gramme negative bacteria, including, Vibrio cholera, Pseudomonas aeruginosa, Streptococcus mutans, Shigella flexneri, Streptococcus pyogenes, Staphylococcus aureus, , Salmonella enteric-typhi, , and Klebsi. In a similar vein, D. hamiltonii leaf extract has been shown to exhibit antibacterial and antifungal effects against gram-positive bacteria and fungi. The gram-negative and gram-positive bacteria that the silver nanoparticles made from D. bulbifera tuber extracts were reported to be effective against. The bulbils of D. bulbifera have been shown to have analgesic and anti-inflammatory activities against paw oedema. It also possesses anthelmintic action against Fasciola gigantica and Pheritima posthuman. D. alata extract's cytotoxicity against human cancer cell lines has demonstrated the presence of anticancerous components. Adult Wister rats tested with the yam species D. oppositifolia showed anti-ulcer efficacy. Castor oil-induced diarrhoea and intestinal transit in rats were reportedly delayed by the methanolic and ethanolic extract of D. oppositifolia. For the treatment of type II diabetes, the anti-diabetic properties of D. alata and D. bulbifera have been proven.

Rasa	Katu, Kashaya
Guna	Ruksha, Tikshna, Guru, Vishada, Laghu
Vipaka	Katu
Veerya	Ushna
Karma	External – Shothahara, Vedanasthapana Internal – Arshaghna, Vatahara, Kaphahara Yakrit - Uttejaka

Table 2.2 Ayurvedic properties of *Amorphophallus paeoniifolius*

2.3 Food Value of Elephant Foot Yam Species

The nutritional, antinutritional, and physico-functional components of yams are all inherent food quality traits that are heavily utilised in human nutrition. Nutritional factors are phytochemicals that promote health, whereas anti-nutritional

factors are substances that have the opposite effect. For consumers and researchers, figuring out the value of these chemicals and their effects on human health is one of the biggest obstacles to implementing them in yam improvement programmes. The importance of these phytochemicals should be emphasised in order to comprehend if they have a positive or negative impact on human health.

2.4 Nutritional Parameters

Comparing yams to other tropical tuber crops, it has been determined that they contain a significant number of different dietary components. According to reports, yam tubers are a good source of important nutrients such lipids, proteins, starch, minerals and vitamins. Table compares the nutritional value of yams with that of other tuber crops.

Nutrient	**Unit**	**Composition**
Calories	Calories	71-135
Moisture	%	65-81
Protein	Gm	1.4-3.5
Fat	Gm	0.2-0.4
Carbohydrate	Gm	16.4-31.8
Fibre	gm	0.1-0.4
Ash	gm	0.6-1.7
Calcium	mg	12-69
Phosphorus	mg	17-61
Iron	mg	0.7-5.2
Sodium	mg	8-12
Potassium	mg	294-397

Beta carotene	mg	0.0-0.1
Thiamine	mg	0.01-0.11
Riboflavin	mg	0.01-0.04
Niacin	mg	0.3-0.8
Ascorbic acid	Mg	4-18

Table 2.3 – Nutritional values of yam (nutrients in 100gm of edible yam)

2.5 Proximate Composition

Proximate composition, which comprises ash, moisture, carbohydrate, crude fat, crude fibre, and crude protein is crucial for highlighting the quality of the food. The food's moisture content serves as a gauge for its food stability and water activity. Food with a low moisture level can be processed and stored for a long period, whereas food with a high moisture content is more susceptible to microbial illness. Food dehydration increases other food nutrients and lengthens its shelf life when it is preserved.

The amount of ash in the food indicates whether it contains essential dietary minerals that are good for the body's development. Compared to other tuber crops like cassava and potato, yam has less ash. Dietary fats aid in taste retention and absorption during cooking treatment , which results in increasing food palatability. According to researchers, dietary fats provided 1-2% of the food's calorific value, which is very sufficient for the diet. Yam has been shown to have a higher dietary fat or lipid content than potatoes and sweet potatoes.

The dietary fibre in food safeguards the healthy flora in the bowel and lowers the risk of cardiovascular disease and colon cancer. The high fibre content of the diet enhances the large intestine's digestion and absorption process, preventing constipation. According to studies, yam species contain higher dietary fibre than other tuber crops including, sweet potatoes, cassava, and potatoes. Protein supports cellular structure and functional processes as well as the control

of metabolic processes in all living things. Humans must consume a protein-rich diet on a daily basis. A diet with enough protein is indicative of one that is nutritionally adequate since it increases the calorific content of the food. It was deemed a good source of protein if the food's protein content made up 12 percent of its overall calorie content. According to reports, the yam species have more protein than other significant tuber crops like sweet potatoes and cassava.

Carbohydrate is a crucial component of the immediate composition of food that gives the body energy and is crucial to the structure and operation of cellular mechanisms. Both the food's organoleptic qualities and nutritional worth are enhanced by it. The texture and flavour of the food are made better by sugar and starch, which affects how people like the dish. Because different plants have different enzymatic activity for its manufacturing process, different plants have different amounts of starch. According to reports, yams contain less sugar and starch than cassava and potatoes. Due to the high levels of dietary fibre present, which plays a significant role as a functional meal, the dish contains a high proportion of non-starchy carbs. The additional non starchy carbohydrates including cellulose, lignin and hemicelluloses have a major impact on the yam's texture. According to reports, yams make up 12% of the energy food consumed by individuals in tropical regions, after sweet potato (2%), cassava (20%), and taro (4%).

2.6 Physico-functional Properties

The fundamental biochemical characteristics known as "physico-functional properties" show how the structure and functional characteristics of food relate to one another **(Afoakwa et al., 2012)**. Information on the behaviour of food ingredients in a food system during processing is provided by the functional parameters **(Afoakwa et al., 2012)**. The significant characteristics in the food sectors for bioprospecting of food ingredients include bulk density and gelatinization temperature. Other physico-functional features include water solubility index, paste clarity and iodine affinity to starch (IAS) **(Kumar et al., 2014)**. Different elements, such as the ratio of amylose to amylopectin and starch, affect the physico-functional qualities **(Kumar et al., 2021).** Numerous researchers have examined different physico-functional properties in the Dioscorea tuber and concluded that yam flour can be used to make food products **(Kumar et al., 2013)**. The proportional volume of packaging material is reflected in the bulk

density of the flour. Less bulk density is preferred since it suggests the sample will pack more tightly during storage or distribution. For the creation of complementary foods, flour with a high bulk density is preferable **(Rahman et al., 2021)**. The amount of water that flour can absorb to generate the necessary consistency of dough is known as its "water absorption capacity" (WAC) **(Singh et al., 2022).** The way that water and oil interact with the flour reflects how they affect the flavour and consistency of meals. High water absorption may guarantee the product's cohesion, which is a functional quality that is mostly crucial for ready-to-eat foods but may also be crucial for dough preparation. Due to the presence of hydrophilic components including polar amino acids and polysaccharides, as well as an increase in the amylose leaching and loss of the structural integrity of the starch, the flour has a higher WAC. Where viscosity is required in the formulation of some food products and baked goods, flour with a high WAC is appropriate **(Sonal et al., 2022)**. The amylose leaching from starch granules is related to the water solubility index (WSI). Food products with foam have better texture, consistency, and look. The amount of interfacial area produced by the protein in the flour is measured by its foam capacity (FC). Foam stability and capacity are negatively related. High foaming capacity flours may create substantial air bubbles that are encased in a thinner, less elastic protein coating. Paste clarity (PC) is a desired characteristic that affects the food's brightness and turbidity. The gelatinization of the flour's starch increases the paste's transmittance, and the resulting paste is more transparent than the suspension. They claimed that these flours made from wild yam tubers had a good chance of being employed as food ingredients in the future. One of the physico-functional characteristics of flour is the gelatinization temperature, which relates to the temperature needed to gelatinize the starch. Higher starch content flour required a lower gelatinization temperature.

2.7 Bioactive Component Of Elephant Foot Yam Species

The secondary metabolites derived from plants called bioactive components are employed as a defensive mechanism against different insects and pests. Numerous pharmacological effects are carried out by these bioactive components, which include, polypeptides , polyphenols, phenols, alkaloids, steroids, essential oils and terpenoids. Numerous bioactive substances, including, glycoside steroids , alkaloids, tannins, flavonoids, saponins, phenols, anthraquinones, etc. are known

to be present in significant amounts in the Dioscorea species. lists the tubers of various Dioscorea species that have been reported to contain various beneficial chemicals. Diosgenin is a steroidal sapogenin (C27) that is typically found in the Dioscorea family and is a member of the triterpene family. Diosgenin is obtained from about 15 different species of Dioscorea, with a market worth of $500 million. Diosgenin, botogenin, and kryptogenin are the three sapogenins that have been isolated from yam species. Diosgenin, a compound found in the Dioscorea genus, is used as a precursor in the manufacturing of steroid medications like cortisone.

Various Dioscorea species have been shown to contain other beneficial substances, including dioscorin and water-soluble polysaccharides. Dioscorin is a yam species storage protein that inhibits trypsin, inhibits carbonic anhydrase, is an antioxidant, an immunomodulator, and promotes the invasion of hypertension. Over 90% of the proteins in yam that can be extracted are made up of dioscorin. Only a few Dioscorea species, including D. opposita, D. alata, D. japonica, D. esculenta, and D. batata, have so far been documented to contain dioscorin. Some of the yam's active ingredients are progressively receiving attention for their therapeutic as well as nutritional characteristics. Additionally well-known active components from the tubers of Dioscorea species are allantoin and dioscin. Different Dioscorea species' cultivated germplasm has been found to contain significant amounts of allantoin and dioscin in China. The a-amylase and a-glucosidase activity, as well as the antioxidant and anti-dyslipidemic capabilities, are due to the allantoin of the yam species. Due to the presence of glucomannan, another bioactive component of yellow and white-water yams called water-soluble polysaccharides (WSP) has been shown to lower blood sugar and cholesterol levels, particularly LDL cholesterol. According to reports, D. opposita's WSP has hyperglycemic characteristics. The WSP can also strengthen the immunological system of the body. After administering WSP extract of D. opposita, Researchers noted an increase in lymphocyte, macrophage, and natural killer cell numbers. Numerous investigations on Dioscorea species, including D. oppositifolia, D. bulbifera, and D. alata, have focused on their pharmacogenetic and phytochemical properties. These studies revealed the presence of, saponin , amino acids, alkaloids, flavonoids, triterpenoids, tannins, and steroids.

2.8 Anti – Nutritional Factors In Elephant Foot Yam Species

Anti-nutritional factors are naturally occurring chemical substances produced by normal metabolism that decrease the body's ability to utilise nutrients. The nutritive value of the meal is decreased by antinutritional factors, which also impair the bioavailability of dietary components, particularly protein, minerals, and vitamins. After intake, the acrid tubers of yam species can cause throat and buccal cavity inflammation as well as skin irritation due to a variety of anti-nutritional factors **(Kumar et al., 2017).**

The antinutritional components of yams - saponin,alkaloid, oxalate, phenol, phytate, tannin, trypsin inhibitors and amylase inhibitors—are thought to be poisonous and bitter. The breakdown of starch to maltose is slowed or prevented by alpha amylase inhibitors, which change the catalytic action of the alpha amylase enzyme on starch. These are the glycoproteins that range in molecular weight from 45,000 to 49,000 kDa . Reduced starch digestion results from amylase enzyme inactivation. The pancreatic amylase enzyme and the amylase inhibitor combine in an equal ratio (1:1), and the amylase inhibitor attaches to the enzyme at a location other than the active site, inactivating the enzyme's catalytic activity through conformational changes. Compared to other commercial tuber crops, yam tubers have a higher alpha amylase concentration. The protein known as a trypsin inhibitor is a member of a large class of proteins called protease inhibitors. Trypsin inhibitor is a protease inhibitor that prevents trypsin and chymotrypsin from performing their respective enzymatic functions in the digestive system, causing indigestible complexes to form with dietary protein and preventing proper protein digestion. According to reports, wild yam tubers contain more trypsin inhibitors than those from cultivated species.

The greatest class of secondary metabolites made up of nitrogen bases produced from amino acids are called alkaloids. Alkaloids and their derivatives are important pharmacologically because of their analgesic, antispasmodic, and antibacterial characteristics . It has been claimed that wild yam species have higher alkaloid contents than do cultivated ones. According to reports, flavonoids are the polyphenols that are most prevalent in human diets. The structural makeup of flavonoids includes several benzene rings. The bioavailability of dietary minerals such as iron, zinc, and calcium is decreased when flavonoids are combined with

positively charged amino acids. These are strong water soluble antioxidants that also have anti-inflammatory, anti-microbial, and anti-carcinogenic characteristics. According to reports, the flavonoid concentration in some yam species has the antioxidant ability to scavenge free radicals . Foods and beverages have an astringent flavour because of tannins. It interacts with the food's dietary protein to produce complexes that precipitate the protein and reduce its availability. A higher tannin concentration degrades the protein content of food and hinders the absorption of iron. The use of plants with high tannin content for the treatment of illnesses such leucorrhoea, rhinnorrhea, wound healing, and diarrhoea has been documented. The presence of tannins in the yam species is what gives them their bitter flavour.

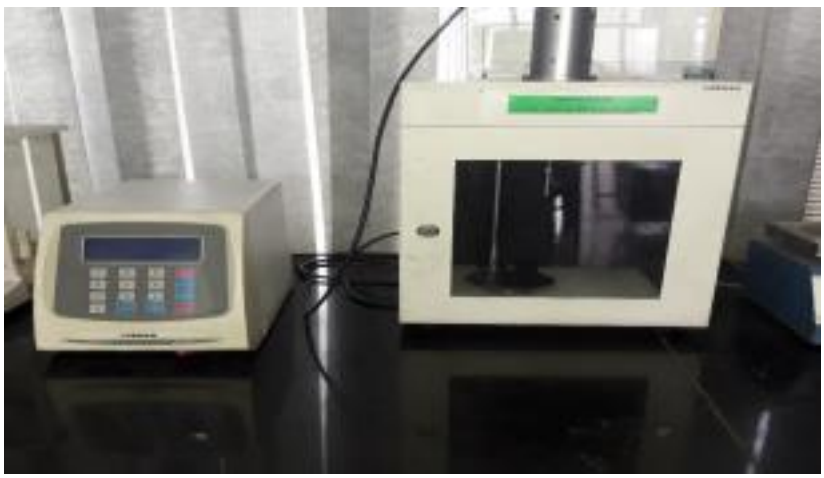
The naturally occurring substances known as saponins are composed of a sugar molecule combined with a triterpene or steroid glycone. There are two main categories of saponin: steroidal and triterpene. Blood hemolysis is caused by an increased concentration of saponins; however they also have therapeutic potentials including decreasing cholesterol and having anti-cancerous properties. According to reports, the main physiologically active component of yams is steroidal saponins. The methanolic extracts of D. villosa and D. cayenensis yielded a total of 21 steroidal saponins, six of which had aglycone skeletons. The D. bulbifera species of wild yam contains saponins that have hemolytic, antibacterial, and cholesterol-binding effects. The naturally occurring substances known as saponins are composed of a sugar molecule combined with a triterpene or steroid glycone. There are two main categories of saponin: steroidal and triterpene. Blood hemolysis is caused by an increased concentration of saponins; however, they also have therapeutic potentials including decreasing cholesterol and having anti-cancerous properties. According to reports, the main physiologically active component of yams is steroidal saponins. The methanolic extracts of D. villosa and D. cayenensis yielded a total of 21 steroidal saponins, six of which had aglycone skeletons. The D. bulbifera species of wild yam contains saponins that have hemolytic, antibacterial, and cholesterol-binding effects.

The growth of plants is inhibited by phenolic chemicals. Within plant tissue, they are typically associated with glucosyl residues. Phenols are classified as anti-nutrients because they impair the digestion of minerals, proteins, and carbohydrates, rendering them intractable. They also affect the mucosa of the

digestive system by inhibiting the function of digestive enzymes such amylase, trypsin, and chymotrypsin. The main factor in the tuber flesh turning brown when exposed to the air are the phenols from the species of yam. According to researchers, the yam species' phenol concentration increased their ability to act as antioxidants.

2.9 Ultrasonication

The application of ultrasound, a non-thermal food processing method, has been shown to have beneficial impacts in the processing of food, including food preservation, improved mass transfer, support for thermal treatments, texture modification, and food analysis. The frequency range of ultrasonic waves, sometimes known as supersonic waves, is 20 kHz to 100 kHz. Ultrasound induces "cavitation" in liquids, changes in gas pressure, and liquid movement in solid media, in that order. It can be thought of as a type of vibration of high frequency that produces microscale shear forces and fluid mixing. In order to use ultrasound processing as a cutting-edge food processing technology, to explore the, recent uses of high-intensity ultrasound in food processing, comprehend the cavitation mechanism and basic principles of ultrasound generation. Nearly all of the unit operations that are performed on food, such as, preservation, grading, and sorting as well as processing and storage, are covered by ultrasound. Degradation of the biological polymer was one of the first uses of ultrasound that was documented in the literature. Ultrasonic cavitation has gained popularity in recent years for a variety of uses, including enhancing chemical processes, emulsifying oils, degrading chemical and biological contaminants, inactivating germs, etc.



2.10 Research Surface Methodology

The theoretical model that connects some controllable variables (factors) to a response is frequently either unavailable or extremely complex. Information about the relationship between the causes and the response in this situation should be gathered empirically. The Box and Wilson-developed Response Surface Methodology (RSM) is a group of statistical and mathematical methods for studying situations like the one being posed using an empirical model. More specifically, its goals are as follows:

• To produce knowledge in the desired experimental field.

• To accurately calculate the experimental variance (pure error).

• To ensure that the suggested model and the experimental data are adequate (to make it easy to detect the lack of fit).

• To accurately and precisely forecast the observed reaction at locations inside the experimental domain where no tests were conducted.

• To offer step-by-step plans for carrying out experiments with various alternatives in response to the findings.

• To continue operating at a high level of efficiency within the constraints of time, money, and any other real-world constraints.

• To facilitate the easy detection of outlier data.

• To eliminate ambiguity and enable decision-making in uncertain situations.

RSM clearly encompasses much more than model fitting and model analysis. In fact, RSM, taken in its broadest sense, has taken centre stage in industrial experimentation.

CHAPTER 3

MATERIALS AND METHODS

Current study deals with the utilization of Elephant foot yam by employing Ultrasound assisted extraction for the extraction of bioactive compounds and reduction of oxalate content. The preliminary trials were done for the selection of extraction method and independent and dependent variables were selected for the final experiments on the basis of preliminary trials. Final experimental design was based on the Box Behnken Design (BBD) of Response Surface Methodology and experiments were performed accordingly. The study was carried in two phases. In the first phase, the dried yam powder was used for oxalate reduction through various extraction methods which involve conventional as well as novel technologies. The extraction was carried out by Ultrasound Assisted method. In the second phase, the optimized results obtained in the first phase served in the qualitative analysis in the experimentation of two major setups, viz. FT-IR as discussed later. This study was aim at reduction of oxalate, analysis of phytochemicals and utilizing the novel extraction methodologies by comparing it with traditional approaches. Among several traditional methods we have categorized a novel approach of ultrasonication for reduction of oxalate and extraction of various phytochemicals from elephant foot yam. The necessity of the given experiments lies in the fact that the conventional approaches tend to downgrade the quality and quantity.

Statistical Analysis of data obtained from different experimental runs was analyzed by using Design Expert 13 software. Multiple regression analysis was performed and second order models were fitted for each response. Data obtained from the statistical analysis was used to acquire optimized value of all the variables. Numerical optimization was conducted after setting the constraint values for the responses and independent variables. Graphical analysis using the contour plots was done to evaluate the effect of independent variables on the individual responses. Experiments were conducted to verify and validate the optimal results by comparing the experimental values with predicted values. This chapter provides the details of material and methodology used during the entire study of the investigation. All the experiments were performed in the Central instrumentation facility (CIF) Laboratory of department of Bioengineering, Integral University, Lucknow (India). Detail of the raw material collection and procurement, various

instruments and equipment's used for the experimentation, selection of independent and dependent variables, experimental design analysis has been discussed in this chapter.

3.1 Experimental Materials

3.1.1 Collection of raw materials

Elephant Foot yam was procured from local market of Lucknow. The yam was washed under running water and removal of outer covering was done with the help of knife. The yam was kept in Hot air oven for drying at 50º C for 24 hours. Then the dried yam was grinded in grinder to make it in powder form. Then the sieving is done for constant particle size 150µm. The yam powder was kept in Air tight container for further processing.

3.1.2 Chemical Glassware's And equipment

All chemical used during the experimentation were AR grade and purchased from the standard suppliers. The borosil grade glassware were used during the study. All Glassware cleaned, washed thoroughly with water and rinsed with distilled water and dried before use. Various equipment used during the study are in given table 3.1.

Equipment/Instruments	**Specifications/ Make**	**Purpose**
Electronic balance	MSW, 10A/VA Delhi Mettler AE 166, Capacity 100g, LC: 0.0001g	Weighing the sample
Grinder	Usha rapid mix 500-Watt copper motor mixer 500 watt	Size reduction
Hot air oven	IFTD.6.MS. Size 150mmL *900mm *600mm	Even Drying

Microwave oven	Model-MC-7148MS	Reduction of oxalate and Extraction of Phytochemicals
Ultrasonic probe Sonicator	Electronic industries Model EL-250	Reduction of oxalate and Extraction of Phytochemicals
Distillation unit	GLSI-SCU-25AQ	Distilled water
Spectrophotometer	Lasanay LI-2904	Spectrophotometric Analysis
Muffle furnace	GMP model Model no. KI-179	Ash estimation
Refrigerator	Model RS62K6227SL Samsung 220V-50Hz	Extract storage
Centrifuge machine	Model R8C Remi Frequency 50 Hz	Density separation

Table 3.1 List of equipment's/ instruments

3.2 Preliminary Experiments

Preliminary experiments were planned to adopt the suitable extraction technique, its parameters and their level and other factors for the final experiments. The different extraction techniques which were used in this study included Ultrasound Assisted Extraction, Boiling and NaCl treatments. The novel extraction methodologies conducted in the optimization experiments were compared to the results of traditional approaches as mentioned in the reviewed literature in the results and discussion section.

S. No	Parameter	Level	Value of Level	Response
1	Solvent	**1**	Distilled Water	Oxalate content Extract yield
2	Particle size	**2**	150,300 (µm)	
3	Solvent Volume	**6**	10,20,30,40,50,60	
4	Sample size	**1**	5 g	

Table 3.2 The various parameters considered for preliminary experiments

3.2.1 Boiling

In extra boiling water, the EFY cubes were cooked for 10, 20, 30, and 40 minutes. After heating, the EFY cubes and soak water were separated. The EFY cubes were placed on blotting paper and allowed to cool in the air before being dry in hot air oven for 24 hours at 50°C. Other features such as acridity, oxalate content (through titrimetric method) and sensory acridity, were investigated **(Kumar et al., 2017)**.

A. Effect of boiling on oxalate content of Elephant foot yam –

Sodium, potassium, and ammonium salts are soluble oxalate (water soluble) crystals found in plants **(Abiodun et al., 2014)**. Oxalic acid is a water-insoluble

calcium, iron, or magnesium salt that chelates metal ions. The production of calcium oxalate crystals in the kidneys (renal stones) and a reduction in mineral bioavailability are two of the most noteworthy effects of oxalates on the human body. In different Dioscorea species, total oxalate levels ranged from 67 to 104 mg/100g, while soluble oxalate levels ranged from 37 to 85 mg/100g **(Ansil et al., 2014)**. In different types of EFY, the amount of soluble oxalate found ranged from 2.91 to 18.50 mg/100g **(Kumar et al., 2014)**. Boiling is one of the most effective ways for humans to make meals more enjoyable and less hazardous. The amount of oxalate in the food was significantly reduced after it was cooked. The soluble oxalate concentration dropped 40.9 percent from 12.97 mg/ 100g (0 min boiling) to 7.66 mg/ 100g (40 min boiling), while the total oxalate content (soluble and insoluble combined) dropped 48.7% from 72.39 mg/ 100g (0 min boiling) to 37.14 mg/ 100g (40 min boiling) (40 min boiling).

It also shows that as boiling time increased, both oxalates decreased, with the greatest reduction occurring during the first 10 minutes of boiling; the oxalate content in yam cooked for longer periods (20, 30, and 40 minutes) did not differ significantly from that obtained after the first 10 minutes of boiling. In a range of root crops, including colocasia, wild yam, Japanese taro, and trifoliate yam tuber, boiling has been demonstrated to reduce oxalates. Thermal degradation/breakdown at higher temperatures, as well as oxalates leaching in the cook water, could have caused the decline **(Kumar et al., 2014)**. Oxalate leaching is aided by the skin's weakening during the boiling process. The influence of boiling, a common cooking method, on oxalate and acridity issues in Amorphophallus paeoniifolius was examined. Boiling lowered soluble and total oxalate levels, as well as sensory acridity **(Sonal et al., 2022).** A decrease in total phenolic content, as well as DPPH activity, and an increase in solids loss in cook water occurred at the same time as per the studies (**Xu et al., 2014).**

Boiling Time (minutes)	**Reduced Oxalate %**
10	21.5
20	22.9
30	23.8

40	24.7
50	25.6
60	26.9

Table 3.3 – Reduction of oxalate through boiling

3.2.2 NaCl Treatment

Yam slices were immersed for 60 minutes in six different NaCl solutions (5 - 30%). For the control (P0), yam that has not been soaked, either soaking NaCl and water, P1 – P6 are being soaked at different concentrations.

Ca oxalate reduction is significantly aided by NaCl solution. The results of soaking yam in various concentrations of NaCl solution revealed substantial differences. The research content of Ca oxalate (ppm) shows that the different treatments have distinct Ca oxalate content, as well as different percentage decreases in Ca oxalate content. The sample without NaCl solution soaking or control sample (P0) had the greatest average Ca oxalate level of 102.44 ppm, whereas the sample with 10 percent NaCl solution soaking had the lowest average Ca oxalate content of 78.92 ppm. The average decrease in the highest Ca oxalate level was 22.89 percent from P2, whereas the average drop in the lowest Ca oxalate content was 13.61 percent from P1. The results showed that soaking in a 10% NaCl (P2) solution is the best way to lower the Ca oxalate level in yam. This solution can reduce the highest oxalate concentration of 22.89 percent. Because the outcome of an average reduction of its content is lower than P2, which is 20,96%,

adding NaCl concentrations over 10%, i.e., 15% (P3), has no meaningful influence on the drop percentage of oxalate content. Researchers found that flouring reduced oxalate concentration in Bogor taro by soaking in a 5 percent NaCl solution for 30 minutes and soaking in a 7.5 percent and 10 percent NaCl solution for 60 minutes. The best results came from soaking taro in 10 percent NaCl for 60 minutes, which reduced the oxalate concentration by 26.83 percent. The addition of a salt concentration greater than 5%, i.e. 7,5%, for 30 minutes resulted in a 5% drop in oxalate level, which was not substantially different. While soaking in 7.5 percent NaCl can reduce oxalate by 12.73 percent, the reduction was less when compared to soaking in 5 percent NaCl, which can reduce oxalate by 22.47 percent **(Singh et al., 2021**).

NaCl Concentration %	**Treatment time (minutes)**	**Reduced oxalate %**
5	60	12.2
10	60	15.4
15	60	19.8
20	60	21.5
25	60	22.1
30	60	22.4

Table 3.4 – Reduction of oxalate through Salinity treatment

The preliminary experiments were conducted with the aim that the extraction technique and the variables having greater influence on the response i.e. Oxalate reduction, would be selected as operating parameters for final experiments. Preliminary experiments showed that the Ultrasound Assisted Extraction gives the more yield and less oxalate % among all the techniques used for extraction. In solvents, distilled water will be selected for the final experiments

as it provided the best results and as per review of literature it was found suitable because of its improved food grade quality. Thus on the basis of preliminary experiments and on the basis of review of literature Solvent (Water) was set as constant parameter for final experiments. For Ultrasound Assisted Extraction the Ultrasound Temperature, Treatment Time, Solid-Solvent ratio were set as independent variable with three levels each.

3.3 Selection of constant parameters

Those parameters which do not affect the process directly but are needed somewhere in the process and need to be identified to fix the values are called constant parameters. As per the preliminary trials and literature reviewed, three parameters were fixed as constant parameters which included, sample weight (15 g) and solvent (water), Particle size (150µm).

3.3.1 Sample weight

Selection of sample quantity was based on the fact that enough extract should be recovered so that the analysis could be done easily. Too small the amount of sample taken during experiments the magnification of results would be quite poor. At the same time, it also depended on the capacity of the flasks used in the experimental procedures. After preliminary trials, 15 gram yam powder was found adequate to conduct all the experiments for efficient extraction analysis of responses.

3.3.2 Solvent

Selection of solvent was based on the principle of percolation between pores opened during the extraction procedures. During the treatment, the solvent molecule penetrates/percolates within the substrate to extract the essential compound. Thus the overall quality and quantity of solvent utilized in the extraction phase is essential in every regard. The chemical behavior viz. ionic behavior and covalent nature of the different characterized solvents is also essential in extraction process. The results of selection in this regard are presented in the results and discussion section. Thus on the basis of preliminary experiments and on the basis of review of literature water was selected as the solvent and the sample size 150µm and sample weight 15 gram for final experiments. **Table 3.3** gives the list of constant parameters for the final experiments.

S. No	Parameter	Constant
1	Solvent	Water
2	Sample Size	15 g
3	Particle size	150 µm

Table 3.5 Constant Parameters for final experiments

3.4 Selection of independent variables

The variables which could be varied within a certain range during the study to see the effect on dependent variables are called independent variables. For the current research,
independent variables considered were Ultrasound temperature, treatment time and Solid-Solvent ratio for ultrasound assisted extraction as these factors affect the extract yield and oxalate reduction of yam powder. Range and values of these variables were decided based on review of literature and preliminary trials.

3.4.1 Particle size for UAE

During Preliminary experiments, in all extraction techniques, 2 levels of Particle size of samples (150µm and 300µm) were tested for getting the best extract yield. As per the review of literature, particle size of the raw material for the extraction varied in the range of 150 and 300 µm. It was observed that extract yield increased significantly with decreasing particle size and higher yield was observed in case of powdered raw material having smaller particle size because smaller size materials have less penetration depth that leads to uniform ultrasound exposure. Therefore, for the final extraction, the particle size of yam powder was selected around the preliminary size of ranging with 1 level i.e., 150 µm.

3.4.2 Volume of solvent for UAE

Solvent Volume for extraction is a parameter which leads to the diffusion of maximum extract during extraction. Solvent Volume doesn't affect the quality of extract but may have an impact on the quantity of extract achieved through

extraction. In the preliminary experiments, Solvent Volume (10, 20, 30, 40, 50, 60) was taken for experiments. In order to attain the best possible ratio of sample to solvent for the extraction of bioactive compounds, Solid-solvent was taken as variable in the final experiment. Thus, based on the literature review and preliminary experiments, for the reduction of oxalate and extraction of bioactive compounds, three levels of Solvent Volume (10, 20 and 30) were selected for the final experiments.

3.4.3. Ultrasound temperature

In this study, the ultrasound assisted extraction processes the temperature categorized was also same as per the aforementioned criterion. The Ultrasound temperature was standardized as per the literature cited trials were performed at the central value of 20℃, which ascertains the given levels viz. 10,20,30 ℃ in the Ultrasound assisted extraction. In order to analyze the effect of Ultrasound temperature on the extraction yield, along with the central level of 20 ℃, the lower and higher levels around the central levels were also considered for the final experiments.

3.4.4 Sonication time

Based on the literature review, sonication time of 20 min as central point was selected for the actual experiments in the range of 10-30mins. The central point was on trial basis for analyzing the yield range and feasibility in the actual experiments. During the preliminary experiments, it was observed that the yield was significant enough to proceed for further experiments when samples were subjected to the sonication time of 20 mins. Using critical considerations of the literature review and the preliminary trials, Sonication time for the extraction for the final experiments were chosen in vicinity of 20 min, viz. 10, 20, 30mins. Thus, a total of three levels of sonication time were selected for the final experiments of Ultrasound assisted extraction.

3.5 Dependent variables (responses)

Numbers of responses were selected to study the effect of independent variables on extraction of phytochemicals and reduction of oxalate from Elephant foot yam. Yam extract was analyzed for six responses which comprised of Extract yield, Oxalate content, Total phenolic content (TPC), DPPH Antioxidant activity,

Total Flavonoid content, Total tannin content. Besides these six responses, the optimized extract sample was screened for FTIR analysis for the presence and identification of the bioactive compounds.

3.6.Experimental Design

Box Behnken Design (BBD) of response surface methodology is used for optimization of Ultrasound assisted extraction for extraction of bioactive compounds and reduction of oxalate. The design consisted of 17 randomized runs with five replicates at the central point. For the designed experiments, three variables having (Ultrasound temperature, treatment time, Solid-Solvent ratio) for Ultrasound assisted extraction were selected for the experiments. **Table 3.4** and **Table 3.6** represent the actual and coded independent variables for extraction. **Table 3.6** and **Table 3.7** represent the BBD matrix of experiments for Yam extract.

Table 3.6 - Coded levels for independent variables in UAE

Independent Variables	**Coded Levels**			
Name	**Code**	**-1**	**0**	**1**
		Actual Levels		
Ultrasound temperature (℃)	A	20	30	40
Treatment Time (min)	B	10	15	20
Solvent Volume(ml/g)	C	10	20	30

Table 3.7 - Experimental design BBD for Ultrasound Assisted Extraction

Std.	Run	Ultrasonication Time (minutes)	Ultrasound temperature (°C)	Solid solvent ratio (w/v)
8	1	20	30	30
4	2	20	40	20
5	3	10	30	10
16	4	15	30	20
9	5	15	20	10
7	6	10	30	30
2	7	20	20	20
3	8	10	40	20
10	9	15	40	10
17	10	15	30	20
15	11	15	30	20
6	12	20	30	10
12	13	15	40	30
14	14	15	30	20
1	15	10	20	20
13	16	15	30	20
11	17	15	20	30

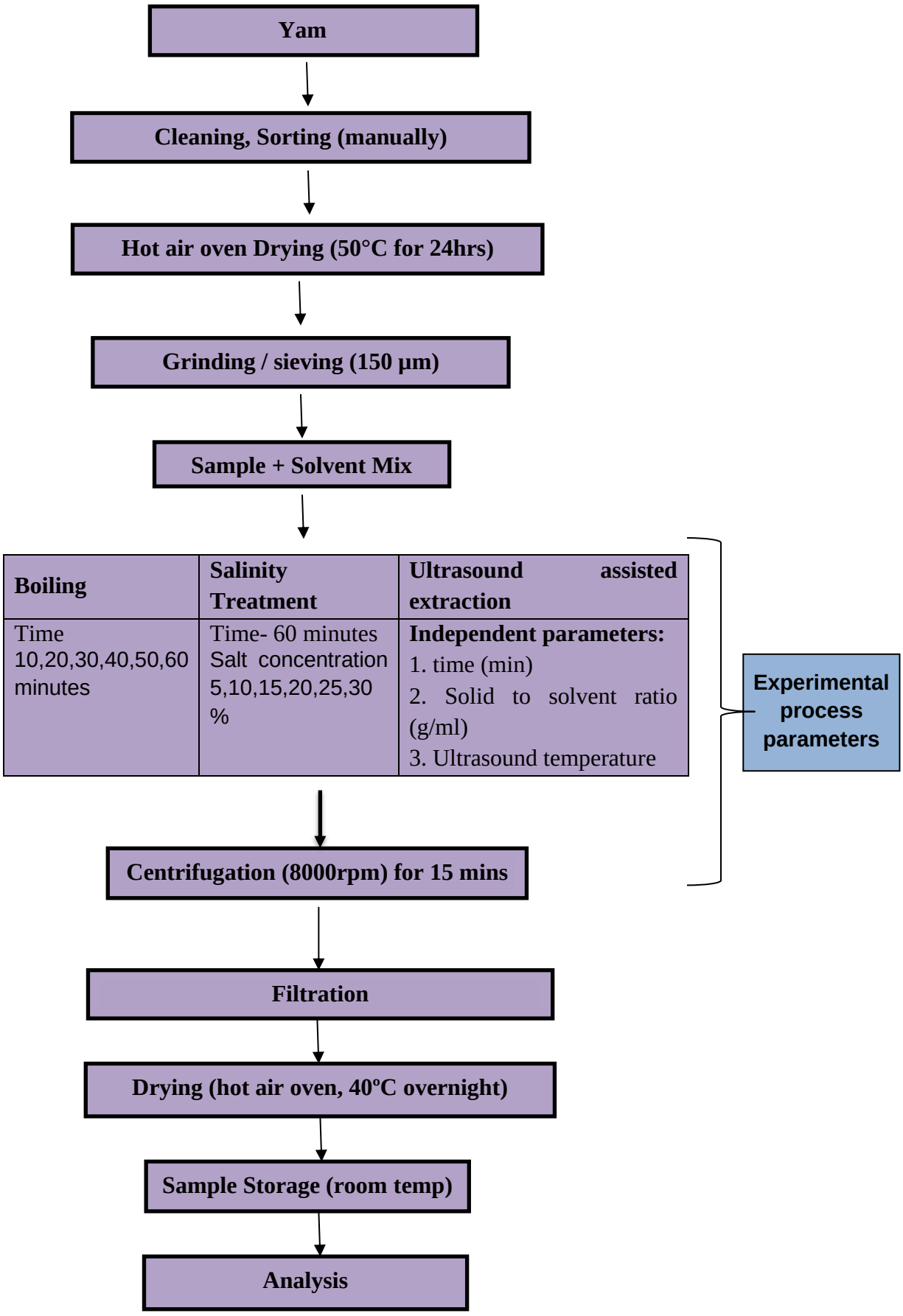


Fig. 3.1 Experimental plan

Table 3.8 Dependent Variables

S. No.	Responses
1	Oxalate content
2	Total Phenolic Content
3	Antioxidant Activity
4	Total Flavonoid content
5	Total Tannin content
6	Extract yield

3.7 Experimental Procedure

The present research deals with the Optimization of the process for extraction of phytoconstituents and reduction of oxalate from yam by employing ultrasound techniques and for their application in food. Experiments were conducted according to the experimental matrix given in **Table 3.6** and **Table 3.7** generated by Design expert (version 13). The entire study was carried out in three phases.

In first phase the dried Yam powder was used for the extraction of yam extract containing bioactive compounds and reduction in oxalate. Thus, UAE ultrasound temperature (20, 30, 40°C), treatment time (10,15, 20 min), solvent volume (10, 20, 30) were being selected. In second phase a total of six responses were evaluated in this phase in order to determine the reduction in oxalate content and bioactive compounds present in the yam extract. These responses included Oxalate content, Extract yield, Total phenolic content (TPC), Total Flavonoid content (TFC), Total Tannin content (TTC) and DPPH Antioxidant activity.

In the third phase, the optimized results from the 1 extraction methodologies were compared. In this section, the Fourier Transform Infrared Spectroscopy (FTIR) analysis was also done for the extract after ultrasound treatment.

3.7.1 Ultrasound assisted extraction of yam extract

Several combinations at designed levels were analyzed for individual responses. The yam powder and solvent mix was kept for sonication at different ultrasound temperature and time combinations as discussed above.

3.7.2 Centrifugation

Centrifugation is a technique which involves the application of centrifugal force to separate particles from a solution according to their size, shape, density, viscosity of the medium and rotor speed. This process is used to separate two miscible substances, but also to analyse the hydrodynamic properties of macromolecules. More-dense components of the mixture migrate away from the axis of the centrifuge (move to the outside), while less-dense components of the mixture migrate towards the axis, i. e., move to the centre.

The need for the utilization of this technique was easier accessibility of extract and overall easier filtration. The thin brown coloured filtrate collected after extraction was filled in the centrifuge tubes for centrifugation for further clarification. The filtrate was then centrifuged for 15 min at 8000 rpm. After centrifugation, the supernatant was collected in a beaker for further processing.

3.7.3 Filtration

Filtration is any of various mechanical, physical or biological operations that separate solids from fluids (liquids or gases) by adding a medium through which only the fluid can pass. The fluid that passes through is called the filtrate. Extraction vessel, followed by treatment, was kept under ambient conditions for settling of the slurry. This slurry (sample + solvent mix) was filtered using Whatman No. 1 filter paper to separate the solid residue and liquid extract. After complete filtration, the solid residue is retained on the filter paper

3.7.4 Drying

After centrifugation the supernatant was kept in hot air oven for drying. It was kept for overnight at 40ºC till all water get evaporated. After that dried extract was stored at room temperature in powdered form for further analysis.

3.8 Chemical Analysis of Responses

3.8.1 Characterization of yam extract

The yam extract extracted from yam powder was evaluated for the

following responses:

3.8.1.1 Extract Yield:

The extract yield (%) is a measure of the solvent's efficiency to extract specific components from the original material and it is defined as the amount of extract recovered in mass compared with the initial amount of whole plant **(JH Pramod et al., 2012).** It was determined for each techniques tested. The dry extract obtained after drying was weighed to obtain the extraction yield. Yield of the extract obtained was calculated as follows:

$$\text{Yield (\%)} = \frac{\text{Weight of extract recovered}}{\text{Weight of dry powder}} \text{ X } 100$$

3.8.1.2 Total Phenolic Content (TPC)

Approximately, 200µL sample was added to 1.5 mL of diluted Folin-Ciocalteu reagent (1:10, v/v) and was incubated for 5 minutes at room temperature. The mixture was then added with 1.5 mL of 0.566 M Na2CO3. The absorbance of the mixture was measured at 725 nm using a spectrophotometer (Genesys 20, United State) after 90 minutes of incubation. The standard gallic acid range 0-125 mg/ml was constructed and the same analysis procedure as samples was conducted. The result was expressed as mg of GAE per amount of sample in g.

3.8.1.3 Antioxidant Activity

Reagents:

0.2 mM DPPH: 0.0078 g DPPH was dissolved in methanol to make the volume 100 ml.

Procedure

An aliquot of 1.5 ml of sample solution was mixed with 1.5 ml Methanolic solution of DPPH (0.2 mM). The reaction mixture was incubated for 30 minutes in dark at room temperature. The absorbance of resulting solution was measured at 517 nm. For the control, the assay was conducted in same manner but methanol was used instead of sample solution. Analysis was done in triplicate for each extracts**.** DPPH scavenging capacity of tested sample was measure as a decrease in the absorbance and was calculated as:

$$\text{Antioxidant Activity \%} = \frac{\text{Control absorbance} - \text{extract absorbance}}{\text{Control absorbance}} \text{ X } 100$$

3.8.1.4 Total Tannin Content (TPC)

The percentage composition of tannin in the extract was determined using Swain's method [19] with some modifications. One ml of sample extract was pipetted into a 50 ml volumetric flask consist of 20 ml distilled water, 2.5 ml Folin-Denis reagent and 10 ml of 17% Na2CO3. The mixture was made up to mark to 50 ml with distilled water, mixed well and allowed to stand for 20 minutes until bluish-green coloration developed. Standard tannic acid solutions of range 0-500 ppm were treated similarly as the sample above. The absorbance of the tannic acid standard solutions as well as the samples was read using a spectrophotometer (Genesys 20, United State) at a wavelength of 760 nm after the bluish-green color was fully developed. Tannin content was determined by a tannic acid standard curve and expressed as mg of tannic acid equivalence (TAE) per 100 g of dried sample.

3.8.1.5 Total Flavonoid Content (TFC)

Aluminium chloride colorimetric method was used with some modifications to determine flavonoid content. One millilitre of the extract was mixed with 3 ml of methanol,
0.2 ml of 10% aluminium chloride, 0.2 ml of 1M potassium acetate and 5.6 ml of distilled water and remains at room temperature for 30 minutes. Sample blank was prepared in a similar way by replacing aluminium chloride with distilled water. The absorbance was measured at 420 nm. Quercetin was used as standard (1 mg/ml). Flavonoid contents were determined from the standard curve and were expressed as quercetin equivalent (mg/g of the extracted compound).

3.8.1.6 Oxalate Content

The classical method used in the determination of total oxalate is the titrimetric method (Adeniyi et al., 2009). The titration method is described as follows: 2 g of the flour was weighed and digested with 10 ml 6 M HCl for one hour and cooled. It was made up to mark in the 250 ml volumetric flask and filtered. Two 125 ml of the filtrate was measured into the beakers and 3-4 drops of methyl red was added. Concentrated NH4OH solution was added drop wise to the test solution until the color changes from salmon pink to faint yellow color and the pH of the solution was determined. Each portion was heated to 90°C, cooled and filtered to remove the precipitate. Again the filtrate was heated to 90°C and 10 ml of 5% CaCl2 solution was added with continuous stirring. The solution was

decanted and the precipitate completely dissolved in 10ml of 20% (v/v) H_2SO_4 solution. The filtrate was made to 300 ml mark and aliquot of 125 ml of the filtrate was heated until near boiling, which was then titrated against 0.05 M standardized potassium tetraoxomanganate (VII) to give a pink color (which persisted for 30 s) at end point.

$$\text{Oxalate content} = \frac{T \times Vme \times Df \times 105}{ME \times MF}$$

Where: T = Titre value of $KMNO_4$,

Vme= volume- mass equivalent (that is, 1 ml of 0.05 m $KMNO_4$, = 0.00228 g of anhydrous oxalic acid),

Df= dilute factor (Vt/A that is, total volume of titrate/ Aliquot used),

Mf= mass of sample used,

ME= molar equivalence of $KMNO_4$ in oxalate concentration in g/dm3

3.9 Utilization of yam Extract

After the successful extraction of yam extract (Reduction of oxalate & Bioactive compounds) from yam powder, the extract can be utilized in the foods. Yam extract can be utilized as a nutraceutical in food material.

3.10 Data Analysis

3.10.1 Statistical analysis and optimization of variables

The model development was done using response surface methodology through use of Design expert 13 version. Complete second order model as given in Equation was fitted to the data and the model adequacy was tested using R^2(coefficient of multiple determination) and Fisher's F-test. The parametric effect on various responses was done through the interpretation of developed models. Regarding four independent variables, a second order response function has the following general formula

$$Y = \beta_0 + [\beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4] + [\beta_{11}X_{12} + \beta_{22}X_{22} + \beta_{33}X_{32} + \beta_{44}X_{42}] + [\beta_{12}X_1X_2 + \beta_{23}X_2X_3 + \beta_{34}X_3X_4 + \beta_{41}X_4X_1] \quad (3.17)$$

Where,

β_0 is the intercept, β_1, β_2, β_3 and β_4 are coefficients of the linear terms, β_{11}, β_{22}, β_{33} and β_{44} are quadratic coefficients and β_{12}, β_{23}, β_{34} and β_{41}

Multiple regression analysis was used to analyse the experimental data in order to develop response functions and obtain variable parameters optimized corresponding to best outputs. The values of model coefficients and related statistics in terms of lack of fit and p value were obtained through the program. The value of p represents the probability of significance. A model with lower values of p was considered better. The models having p-value lower than 0.1 were accepted.

The sign and magnitude of the coefficient explain the nature of the effect. Negative sign at linear level means decrease in the response when the level of the predictor is increased while positive sign indicates increase in the response. Significant negative interaction suggests that the level of one of the predictors can be increased while that of other decreased for constant value of the response. Positive interaction means the response is minimum at center point and it increases with increase or decrease or both the variables from center point. Positive coefficient of a quadratic term indicates the minimum response at center value of the parameter and it increases with increase or decrease in parameter level. Negative coefficient of the quadratic term shows the maximum response at center value and it decreases with increase or decrease in parameter level.

3.10.2 Adequacy of the model

The second order polynomial equation was solved using a statistical approach called the method of least square (MLS) which is a multiple regression technique used to fit a mathematical model to a set of experimental data generating the lowest residual possible. The results of regression analysis were obtained in terms of ANOVA, regression coefficient and associated statistics, standard deviation, coefficient of determination (R^2), lack of fit, etc. These terms describe adequacy of predictive model and effect of independent parameters on the responses.

3.10.3 Test for significance of the regression model

This test was performed as an analysis of variance (ANOVA) by calculating the F-ratio, which is the ratio between the regression mean square and the mean square error. The F-ratio, also called the variance ratio, is the ratio of variance due to the effect of a factor (in this case the model) and variance due to the error term. This F-ratio was used to calculate the p-value of the model which was finally used

to measure the significance of the model under investigation. $p<0.01$ corresponds to the fact that the variable is significant at 1% level of significance. Similarly $p<0.05$ and $p<0.10$ corresponds to the fact that the variable is significant at 5% and 10% level of significance, respectively.

Additionally, checks were carried out in order to determine whether the model actually describes the experimental data. The checks performed here included determining the various coefficient of determination (R^2). In addition to the above, the adequacy of the model was also investigated by the examination of residuals. The residuals are the difference between the respective, observed responses and the predicted responses examined using the normal probability plots of the residuals and the plots of the residuals versus the predicted response. If the model is adequate, the points on the normal probability plots of the residuals should form a straight line.

3.10.4 Numerical optimization of independent variables

The term optimization has been commonly used in analytical chemistry as a means of discovering conditions at which to apply a procedure that produces the best possible response. Optimizing refers to improving the performance of a system, a process, or a product in order to obtain the maximum benefit from it**.** Among the most relevant multivariate techniques used in analytical optimization is response surface methodology (RSM). It can be well applied when a response or a set of responses of interest are influenced by several variables. The objective is to simultaneously optimize the levels of these variables to attain the best system performance. Thus in the light of above mentioned facts, independent variables with respect to the measured responses were optimized using response surface methodology. For the first phase reduction of oxalate was taken into consideration. After that, the optimized condition was predicted by the model. The optimized points could be a single point or a range of points in which all the possible combinations would yield good results. The goal was set for significant dependent variables and independent variables as per the required criteria of variables. Based on the goals, software gave the multiple solutions of optimized independent variables. Among all the optimized solutions, the best optimized solution for optimum values of independent variables was selected on the basis of the criteria that the optimum values should be close to the variable values and higher desirability.

3.10.5 Graphical Analysis

Graphical analysis was done with the help of contour plots showing the effect of independent variables on the responses. The combination of two independent variables was selected by keeping other two at optimum values obtained during numerical optimization. Besides contour graphs, perturbation graphs are also drawn to see the deviation of the independent variables at the optimized point.

3.10.6 Validation of optimal points

Experiments were conducted to validate the optimal results as given by Design Expert 13 software. The optimized values were verified and predicted and actual values were compared to determine the validity of the model and optimal results.

CHAPTER 4

RESULTS AND DISCUSSION

An intensive study was conducted for process optimization for reduction of oxalate and extraction of various phytochemicals from yam by employing ultrasound extraction technique with application of phytochemicals as nutraceuticals in food. The study included the preliminary trials for the selection of solvent and its volumetric ratio (compared to substrate) for efficient extraction and selection of independent variables for getting optimized yield and maximum oxalate reduction of the yam extract.

In the preliminary trials conducted for the selection of independent variables for conducting final experiments. The final experimental plan was based on the Box–Behnken Design (BBD) of Response Surface Methodology (RSM) to evaluate optimized values and the experiments were performed accordingly.

The entire study was conducted in two phases. In first phase, the dried yam powder was used for reduction of oxalate and phytochemical extraction which contains bioactive compounds and in second phase the extract was analyzed quantitatively and qualitatively using various responses such as Reduced oxalate, Extract yield, Total phenolic content (TPC), Total Flavanoid content (TFC), Total Tannin content (TTC), DPPH Antioxidant activity. Besides these six responses, in third phase the optimized extract sample was qualitatively characterized for FTIR analysis for the presence and identification of the bioactive compounds.

Experimental data of the extraction study was analyzed statistically as well as graphically followed by optimization of independent variables for getting the best results. Regression analysis of variables was obtained employing Response Surface Methodology (RSM) with Box-Behnken Design (BBD) for all variables. ANOVA was employed to critically investigate/examine the models. A full second order model was fitted into each response and was further utilized to interpret the significance of variables on the response. If the model was found adequate, the best fit equation was generated as a means to draw contour plots for indicating the effect of independent variables on the linear and interactive responses graphically. Finally, conclude by the optimization of different process conditions using design expert software 13.

Optimization was done to generate the optimum points of the independent variables for the best possible combinations of independent variables. Further,

actual experiments were performed at optimal points, were compared with optimized results to verify the model.

4.1 Preliminary Experiments

In the preliminary phase of experimental plan, the aim was observation and selection of several parameters for the decision of selection of independent variable levels in actual experiment. They include:

Solvent: Water

Temperature: 20,30,40,50℃

Solvent volume: 10,20,30, 40, 50,60

Time: 10, 15,20, 25, 30 minutes

Sample size: 5gm

With respect to the observable response in terms of extract yield. The preliminary experiments were conducted for Boiling, NaCl treatment and Ultrasound processing method. The observable response i.e. extract yield and oxalate reduction provided following results.

1) All three methods responded by showing maximum extract yield with water as extraction solvent. Conventional method shows less oxalate reduction as compared to UAE.
2) The minimum level of particle size i.e. 150 shows the high yield whereas, maximum particle size i.e. 300 µm showed least yield results during Boiling, NaCl treatment and UAE processing.
3) In higher levels of solvent volume i.e. 50 and 60 ml the yield results deteriorated. The similar observation was also found in the lowest level i.e. 10 ml as well.

4.2 Analysis for yam extract

During the first phase of study, the dried yam powder was used for the reduction of oxalate and extraction of phytochemicals. Ultrasound assisted extraction was conducted for the whole study with three independent variables having 3 levels of each respectively. The independent variables with their predefined levels for UAE viz. Ultrasound temperature (20,30,40 ℃), Sonication time (10,15,20 mins), Solvent (Water) volume (10,20,30 ml) and particle size (150,300 µm). Box Behnken Design (BBD) approach of Response Surface

Methodology (RSM) was used for the design of experiments. A total of 17 experiments were conducted and were analysed for six responses which comprised of Reduced oxalate, Extract yield, Total phenolic content (TPC), Total Flavonoid Content (TFC), Total Tannin Content (TTC), DPPH Antioxidant activity. Results of the Experimental data showing the effect of all independent variables on the responses has been presented in table 4.1 and 4.2. Beside these six responses of the optimized extract sample with the best results were screened and Fourier Transform Infrared Spectroscopy (FTIR) was also conducted for the optimized results of UAE and for control.

As per results of **Table 4.1** the extract yield ranged from (30.5 – 49.2 %), Oxalate ranged from (29.3 – 43.2), TPC ranged from (19.1 – 38.8 %), TFC ranged from (39.5 – 59.8), TTC ranged from (3.5 – 7.8), Antioxidant ranged from (59.1 – 78.8 %), for UAE.

The results of **Table 4.1, 4.2,** for responses were analyzed numerically, statistically and graphically. The values of the process parameters in the model were analyzed statistically to check significant effects on the particular responses. The graphical analysis of all the responses and optimization of the process parameters was carried out. Finally, the optimized data was compared with the actual experiments check the statistical validation/significance of the model.

Table 4.1 Experimental data on UAE of yam extract

Std	Run	A:time	B:temperature	C:solid solvent ratio	yield	Reduced oxalate content	Total phenol	total flavonoid	total tannin	Antioxidant content
		minutes	℃	w/v	%	%	GAE/g	QE/g	TE/g	%
8	1	20	30	30	49.2**	43.2	38.8	59.8	7.8	78.8
4	2	20	40	20	48.1	40.5	36.9	58.6	7.4	76.9
5	3	10	30	10	30.5*	29.3	19.1	39.5	3.5	59.1
16	4	15	30	20	38.5	34.3	26.2	47.1	4.7	66.2
9	5	15	20	10	34.1	32.8	23.8	42.3	4.1	63.8
7	6	10	30	30	32.3	30.9	22.9	41.2	3.8	62.9
2	7	20	20	20	45.6	39.6	35.6	55.8	7.1	75.6
3	8	10	40	20	32.3	29.6	19.5	39.8	3.8	59.5
10	9	15	40	10	39.1	33.6	25.7	44.5	4.4	65.7
17	10	15	30	20	39.8	34.4	26.3	47.3	4.9	66.3
15	11	15	30	20	39.7	34.7	26.8	47.8	5.8	66.8
6	12	20	30	10	44.3	37.8	35.2	55.6	6.8	75.2
12	13	15	40	30	40.4	36.9	28.8	48.9	6.3	68.8
14	14	15	30	20	39.6	34.5	26.6	47.2	5.2	66.6
1	15	10	20	20	30.9	28.5	18.7	38.9	3.3	58.7
13	16	15	30	20	39.7	34.6	25.9	48.5	5.5	65.9
11	17	15	20	30	40.6	35.2	27.2	44.7	6.1	67.2

***Minimum ** maximum**

Response	Intercept	A	B	C	AB	AC	BC	A^2	B^2	C^2
Oxalate	34.50	5.35	0.5625	1.59	-0.0500	0.9500	0.2250	0.3625	-0.3125	0.4375
P=		<0.0001	0.0006	<0.0001	0.7252	0.0002	0.1435	0.0297	0.0513	0.0134
Yield	39.10	7.65	1.09	1.81	0.2750	0.7750	-1.30	0.2234	-0.3422	0.4321
P=		<0.0001	0.0003	<0.0001	0.3646	0.0232	0.0012	0.0032	0.0234	0.3241
Total phenol	26.36	8.29	0.7000	1.74	0.1250	-0.0500	-0.0750	1.97	-0.6550	0.6700
P=		<0.0001	0.0037	<0.0001	0.6058	0.8351	0.7553	<0.0001	0.0229	0.0208
Total flavonoid	47.58	8.80	1.26	1.59	0.4750	0.6250	0.5000	2.31	-1.62	-0.8650
P=		<0.0001	0.0005	0.0001	0.1549	0.0741	0.1372	<0.0001	0.0008	0.0206
Total Tannin	5.32	1.84	0.1625	0.6500	0.3422	0.5432	-1.32	0.4223	0.3221	0.4231
P=		<0.0001	0.2609	0.0004	0.0431	0.0034	0.3233	0.4123	0.0032	-0.4224
Antioxidant	66.36	8.29	0.7000	1.74	0.1250	-0.0500	-0.0750	1.97	-0.6550	0.6700
P=		<0.0001	0.0037	<0.0001	0.6058	0.8351	0.7553	<0.0001	0.0229	0.1782

Table 4.2 Coefficients table for UAE

4.2.1 Statistical analysis of yam extract for UAE

Statistical Analysis of the responses viz. Reduced oxalate, Extract yield, Total phenolic content (TPC), Total Flavonoid Content (TFC), Total Tannin Content (TTC), DPPH Antioxidant activity in terms of the effect of independent variables (Ultrasound temperature, Sonication time, solid - solvent ratio) on responses was conducted by Design Expert 13. The output data for all the responses was examined and based on the results, independent variables were optimized for getting the best outputs. The regression Analysis and other statistical data of the variables was obtained employing response surface methodology With BBD. A full second- order model was fitted into each response. The data from all the experiments were analyzed and the predicted regression equations were developed utilizing the multiple regression. ANOVA was applied to investigate the models. The model was analyzed to translate the impact of variables on the individual responses. Experimental results of extraction are tabulated in **Table 4.1 and 4.2.** In the following sections all the responses analyzed statistically have been discussed in detail.

4.2.1.1 Oxalate Content

Yam oxalate content is the most important response as being the treasure trove for all the compounds. The oxalate obtained, as shown in **Table 4.1**, reduction of oxalate is from 29.3 – 43.2%. Maximum reduction of oxalate is 43.2% for the yam sample was obtained at the experiment run 1 having experimental conditions of Ultrasound temperature at 30 ℃, Sonication time at 20 min, Solvent volume 1:30 and particles size was 150µm. On other hand, the minimum oxalate reduction is 29.3% was obtained at the experiment run no. 3 having independent variable conditions of Ultrasound temperature at 30 ℃, Sonication time at 10 min, Solid solvent ratio 1:10. Results of the present study in terms of oxalate, when compared with the past studies of **(Rahaman et al., 2021),** was found 5.65% higher than their result of 24.2% oxalate. Increased reduction of oxalate in the present study could be credited to the Ultrasound assisted extraction because it is effective for extraction as its mechanical effects on the process by increasing the penetration of solvent into the product due to disruption of the cell walls produced by acoustical cavitation **(Yadu et al., 2017).**

Response surface quadratic model was used and ANOVA was performed using partial sum of squares methods to check the adequacy of the model for the oxalate . Model significance was checked for both model and model factors. The linear model

factors viz. Ultrasound temperature(A), Sonication Time (B) and Solvent volume (C) and, quadratic model factors; pure quadratic terms (A^2, B^2, C^2) and interaction quadratic terms (AB, AC, BC) were analyzed depending on P values. The statistical significance of individual independent variables and their quadratic interaction values of the oxalate can be explained by the p-value given in **Table 4.2.** It can be concluded from the **Table 4.2** that the model was found significant at 1% level of significance. The results revealed that for oxalate of all linear and pure quadratic terms of Ultrasound temperature (A), solvent volume (B) and sonication time (C) were significant at 1% and5% level of significance respectively.

A second order polynomial equation was used to fit the coded variables (A, B, C) for oxalate using multiple regression analysis. **Table 4.2** shows the coefficient of determination (R^2) for the independent variable, their interaction in coded form and their corresponding p value. The value of the R^2 for the oxalate was found as 0.9980 which implies that the model

could account for 95.24% data. The lack of fit value for regression model was not significant, which indicates that the model equation was adequate to describe the oxalate. For better suitability of the model, the difference between the predicted and adjusted should be less than 0.2, the adequate precision should be greater than 4 and whereas C.V. should not exceed 10%. In this case, the "Pred R^2" of 0.9732 was in reasonable agreement with the "Adj R^2" of 0.9954, the adequate precision was found to be 69.6760 and the C.V was 0.7867% thereby verifying the accuracy and suitability of model. The coefficient of determination (R^2) and adjusted determination coefficient (Adj R^2) were reasonably close to 1, indicating a high degree of correlation between the observed and predicted values.

Response 1: Reduced oxalate content

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	257.23	9	28.58	382.91	< 0.0001	significant
A-time	228.98	1	228.98	3067.67	< 0.0001	
B-temperature	2.53	1	2.53	33.91	0.0006	
C-solid solvent ratio	20.16	1	20.16	270.10	< 0.0001	
AB	0.0100	1	0.0100	0.1340	0.7252	
AC	3.61	1	3.61	48.36	0.0002	
BC	0.2025	1	0.2025	2.71	0.1435	
A^2	0.5533	1	0.5533	7.41	0.0297	
B^2	0.4112	1	0.4112	5.51	0.0513	
C^2	0.8059	1	0.8059	10.80	0.0134	
Residual	0.5225	7	0.0746			
Lack of Fit	0.4225	3	0.1408	5.63	0.0641	not significant
Pure Error	0.1000	4	0.0250			
Cor Total	257.76	16				

Std. Dev.	0.2732	**R^2**	0.9980
Mean	34.73	**Adjusted R^2**	0.9954
C.V. %	0.7867	**Predicted R^2**	0.9732
		Adeq Precision	69.6760

Table 4.3 – Regression analysis for oxalate reduction

A second order polynomial equation **(Eq. 4.1)** was developed representing an empirical relationship between the response (oxalate reduction) and the independent variables, Ultrasound temperature (A), Sonication Time (B) and solid - solvent volume

(C). The equation for oxalate is given below:

Oxalate (%) = 34.50 + 5.35A + 0.56B + 1.59C-0.05AB+0.22BC+0.36A^2-0.31B^2+0.43 C^2 (4.1)

The equation included both significant and non-significant terms. Non-significant terms were removed from the model and then the equation **(Eq. 4.1a)** was regenerated, that describes only the effect of significant process variables on Oxalate from yam. The equation is as follows: Oxalate (%) = 34.50 + 5.35A + 0.56B + 1.59C + 0.36A^2-0.31B^2+0.43 C^2 (4.1a)

The importance of the model developed equation was evaluated from their coefficients of correlation. As shown in the final equation 4.1a, the response oxalate was affected by all the linear terms of Ultrasound temperature (A), Ultrasound Time (B), Solid-solvent ratio (C) and their pure quadratic terms (A^2, B^2, C^2).Coefficients of linear terms (B, C) were positive and the oxalate was positively affected by linear terms barring the linear term (A, B) which was had negative coefficient, thus showing negative effect. The coefficients of quadratic terms were negative and the response oxalate was negatively affected by quadratic terms. From the positive effects, Ultrasound temperature (B) had highest effect on response oxalate reduction. Similarly, pure quadratic term of Ultrasound temperature (B^2) had highest effect on response oxalate reduction from negative coefficients.

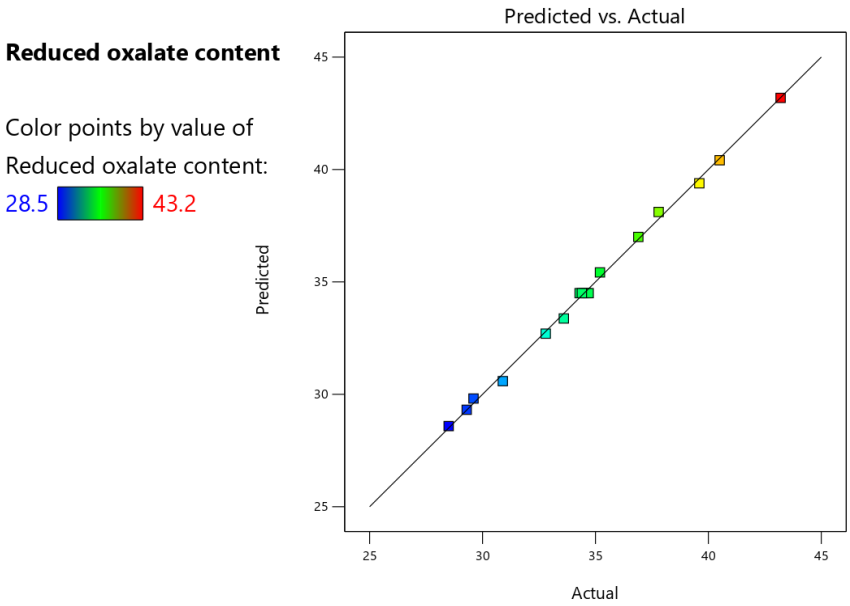


Figure 4.1 - Experimental v/s predicted graph of oxalate reduction

Model adequacy and validation was evaluated using the Experimental vs Predicted graph **(Fig. 4.1)** and the full length data of actual, predicted and error for oxalate was obtained from **(Appendix A1)**. It was observed that the predicted values (28.2-58.3%) from the model are significantly close to the actual experimental values (30.5-49.2%) with a negligible error (0.94to 4.67%). This close proximity of the model prediction with the experimental data assures the adequacy and validation of the second order model for the oxalate.

4.2.1.2 Extract Yield

Yam extract yield is the most important response as being the treasure trove for all the bioactive compounds. The extract yield obtained, as shown in **Table 4.1**, ranged from 30.5 to 49.2%. Maximum extract yield of 49.2% for the yam sample was obtained at the experiment run 1 having experimental conditions of Ultrasound temperature at 30 ℃, Sonication time at 20 min, Solvent volume 1:30 and particles size was 150µm. On other hand, the minimum extraction yield of 30.5% was obtained at the experiment run no. 3 having independent variable conditions of Ultrasound temperature at 30 ℃, Sonication time at 10 min, Solid solvent ratio 1:10. Results of the present study in terms of extract yield, when compared with the past studies of **(Kumar et al., 2017),** was found 5.65% higher than their result of 44.11% extract yield. Increased extraction yield in the present study could be credited to the Ultrasound assisted extraction because it is effective for extraction as its mechanical effects on the process by increasing the penetration of solvent into the product due to disruption of the cell walls produced by acoustical cavitation **(Singh et al., 2021).**

Response surface quadratic model was used and ANOVA was performed using partial sum of squares methods to check the adequacy of the model for the extract yield. Model significance was checked for both model and model factors. The linear model factors viz., Ultrasound temperature(A), Sonication Time (B) and Solvent volume (C) and, quadratic model factors; pure quadratic terms (A^2, B^2, C^2) and interaction quadratic terms (AB, AC, BC) were analyzed depending on P values. The statistical significance of individual independent variables and their quadratic interaction values of the extract yield can be explained by the p-value given in **Table 4.2.** It can be concluded from the **Table 4.2** that the model was found significant at 1% level of significance. The results revealed that for Extract yield of all linear and pure quadratic

terms of Ultrasound temperature (A), solvent volume (B) and sonication time (C) were significant at 1% and5% level of significance respectively.

A second order polynomial equation was used to fit the coded variables (A, B, C) for extract yield using multiple regression analysis. **Table 4.2** shows the coefficient of determination (R^2) for the independent variable, their interaction in coded form and their corresponding p value. The value of the R^2 for the extract yield was found as 0.9935 which implies that the model could account for 95.24% data. The lack of fit value for regression model was not significant, which indicates that the model equation was adequate to describe the extract yield. For better suitability of the model, the difference between the predicted and adjusted should be less than 0.2, the adequate precision should be greater than 4 and whereas C.V. should not exceed 10%. In this case, the "Pred R^2" of 0.9808 was in reasonable agreement with the "Adj R^2" of 0.9896 , the adequate precision was found to be 50.9362 and the C.V was 1.48% thereby verifying the accuracy and suitability of model. The coefficient of determination (R^2) and adjusted determination coefficient (Adj R^2) were reasonably close to 1, indicating a high degree of correlation between the observed and predicted values.

Response 2: Extract yield

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	513.39	6	85.56	255.23	< 0.0001	significant
A-time	468.18	1	468.18	1396.51	< 0.0001	
B-temperature	9.46	1	9.46	28.22	0.0003	
C-solid solvent ratio	26.28	1	26.28	78.39	< 0.0001	
AB	0.3025	1	0.3025	0.9023	0.3646	
AC	2.40	1	2.40	7.17	0.0232	
BC	6.76	1	6.76	20.16	0.0012	
Residual	3.35	10	0.3353			

Lack of Fit	2.18	6	0.3634	1.24	0.4363	not significant
Pure Error	1.17	4	0.2930			
Cor Total	516.74	16				

Std. Dev.	0.5790	**R^2**	0.9935
Mean	39.10	**Adjusted R^2**	0.9896
C.V. %	1.48	**Predicted R^2**	0.9808
		Adeq Precision	50.9362

Table 4.4 Regression analysis for extract yield

A second order polynomial equation **(Eq. 4.1)** was developed representing an empirical relationship between the response (Extract Yield) and the independent variables viz. Ultrasound Temperature (A), Sonication Time (B) and solvent volume (C). The equation for extract yield is given below:

Extract Yield (%) = $39.10+7.65A+1.09B+1.81C+0.27AB+0.77AC-1.30BC+0.22A^2-0.34B^2+0.43C^2$ (4.1)

The equation included both significant and non-significant terms. Non-significant terms were removed from the model and then the equation **(Eq. 4.1a)** was regenerated, that describes only the effect of significant process variables on extract yield from yam. The equation is as follows:

Extract Yield (%) = $39.10+7.65A+1.09B+1.81C+0.22A^2-0.34B^2+0.43C^2$ (4.1a)

The importance of the model developed equation was evaluated from their coefficients of correlation. As shown in the final equation 4.1a, the response yield was affected by all the linear terms of, Ultrasound Power (A), Sonication Time (B) and solvent volume (C) and their pure quadratic terms (A^2, B^2, C^2).Coefficients of linear terms (B, C) were positive and the response yield was positively affected by linear terms barring the linear term (A, B) which was had negative coefficient, thus showing negative effect. The coefficients of quadratic terms were negative and the response yield was negatively affected by quadratic terms. From the positive effects, Ultrasound temperature (B) had highest effect on response yield. Similarly, pure quadratic term of

Ultrasound temperature (B^2) had highest effect on response yield from negative coefficients.

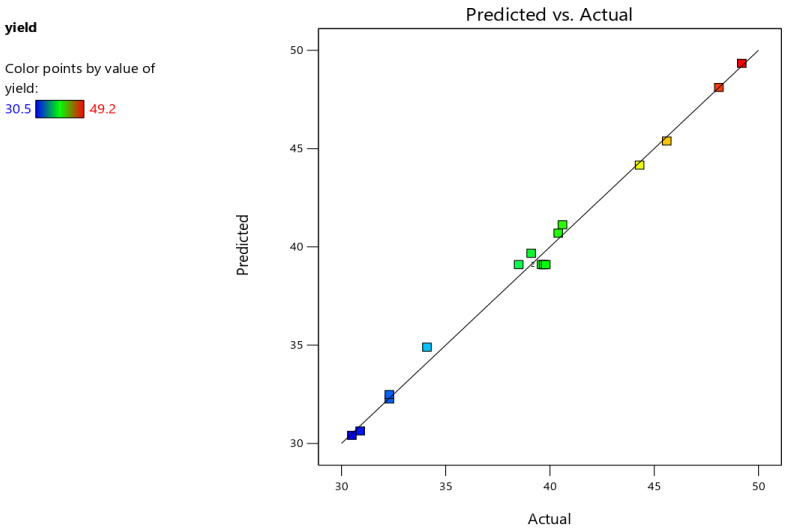


Figure 4.2 – Experimental v/s predicted graph of extract yield

Model adequacy and validation was evaluated using the Experimental vs Predicted graph **(Fig. 4.2)** and the full length data of actual, predicted and error for extract yield was obtained from **(Appendix A1)**. It was observed that the predicted values (29.3-48.1%) from the model are significantly close to the actual experimental values (30.5-49.2%) with a negligible error (0.94to 4.67%). This close proximity of the model prediction with the experimental data assures the adequacy and validation of the second order model for the extract yield.

4.2.1.3 Total Phenolic Content (TPC)

Total phenolic content (TPC) is an important response for the assessment of bioactive compounds present in yam extract. **Table 4.1** shows the results of the total of 17 experimental runs which were performed as per the combinations given by the software (Design Expert 13) and their corresponding values for total phenolic content. Total phenol content was expressed in Gallic acid equivalent (mg of Gallic acid/g extract). The TPC obtained, as shown in **Table 4.1**, ranged from 19.1-38.8mg GAE/g. Maximum TPC of 38.8% for the yam sample was obtained at the experiment run 1 having experimental conditions of Ultrasound temperature at 20, Sonication time at 20 min, solvent volume 1:20. On other hand, the minimum TPC of 19.1% was obtained at the experiment run no. 3 having independent variable conditions of Ultrasound temperature 30, Sonication time at 20 min, Solvent volume 1:30. Extraction of phenolic compounds from plant material is influenced by the chemical nature of the compound, the extraction method employed, sample particle size, the solvent used, extraction conditions (time, temperature), as well as the presence of interfering substances **(Kumar et al., 2017).**

Response surface quadratic model was used and ANOVA was performed using partial sum of squares methods to check the adequacy of the model for the TPC. Model significance was checked for both model and model factors, linear model factors Ultrasound temperature (A), Sonication Time (B) and Solvent volume (C) and, quadratic model factors; pure quadratic terms (A^2, B^2, C^2) and interaction quadratic terms (AB, AC, BC) depending on P values. The significance of each independent variables and their interaction on the phenol can be explained by the p-value given in **Table 4.2.** It can be observed from the **Table 4.2** that the model was found significant at 1% level of significance. The results revealed that for TPC of all linear and pure quadratic terms of Ultrasound temperature (A), Solvent volume (B) and Sonication Time (C) were significant at 1% and5% level of significance respectively.

A second order polynomial equation was used to fit the coded variables (A, B, C) for TPC using multiple regression analysis. **Table 4.2** shows the coefficient of determination (R^2) for the independent variable, their interaction in coded form and their corresponding p value. The value of the R^2 for the phenol was found as 0.9975 which implies that the model could account for 96.68% data. The lack of fit value for regression model was not significant, which indicates that the model equation was adequate to describe the phenol. For better suitability of the model, the difference between the predicted and adjusted should be less than 0.2, the adequate precision should be greater than 4 and whereas C.V. should not exceed 10%. In this case, the "Pred R^2" of 0.8219 was in reasonable agreement with the "Adj R^2" of 0.9943, the adequate precision was found to be 56.7995 and the C.V was 1.70% thereby verifying the accuracy and suitability of model. The coefficient of determination (R^2) and adjusted determination coefficient (Adj R^2) were reasonably close to 1, indicating a high degree of correlation between the observed and predicted values.

Response 3: Total phenol

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	597.59	9	66.40	309.96	< 0.0001	significant
A-time	549.46	1	549.46	2565.01	< 0.0001	
B-temperature	3.92	1	3.92	18.30	0.0037	
C-solid solvent ratio	24.15	1	24.15	112.74	< 0.0001	
AB	0.0625	1	0.0625	0.2918	0.6058	
AC	0.0100	1	0.0100	0.0467	0.8351	
BC	0.0225	1	0.0225	0.1050	0.7553	
A^2	16.34	1	16.34	76.28	< 0.0001	
B^2	1.81	1	1.81	8.43	0.0229	

C^2	1.89	1	1.89	8.82	0.0208	
Residual	1.50	7	0.2142			
Lack of Fit	1.01	3	0.3358	2.73	0.1782	not significant
Pure Error	0.4920	4	0.1230			
Cor Total	599.09	16				

Std. Dev.	0.4628		**R^2**	0.9975
Mean	27.29		**Adjusted R^2**	0.9943
C.V. %	1.70		**Predicted R^2**	0.9718
			Adeq Precision	56.7995

Table 4.5 Regression analysis for Total phenol content

A second order polynomial equation **(Eq. 4.2)** was developed representing an empirical relationship between the response (TPC) and the independent variables Ultrasound temperature (A), Sonication Time (B) and Solvent volume (C). The equation for TPC is given below:

$$\text{TPC (mg GAE/g)} = 26.36+8.29A+0.70B+1.74C+0.12AB-0.05AC-0.07BC+1.97A^2-0.65B^2+0.67C^2 \quad (4.2)$$

The equation included both significant and non-significant terms. Non-significant terms were removed from the model and then the equation **(Eq. 4.2a)** was regenerated, that describes only the effect of significant process variables on TPC. The equation is as follows:

$$\text{TPC (mg GAE/g)} = 26.36+8.29A+0.70B+1.74C+1.97A^2-0.65B^2+0.67C^2 \quad (4.2a)$$

The importance of the model developed equation was evaluated from their coefficients of correlation. As shown in the final equation 4.2a, the response TPC was affected by all the linear terms of, Ultrasound temperature (A), Sonication Time (B) and Solvent volume (C)and pure quadratic terms (B^2, C^2).Coefficients of linear terms (B, C) were positive and the response phenol was positively affected by linear terms barring the linear term (A, B) which was had negative coefficient, thus showing negative effect. The coefficients of quadratic terms were negative and the response phenol was negatively affected by quadratic terms. From the positive effects, Ultrasound temperature (A) had highest effect on response phenol . Similarly, their interactive term BC had highest effect on response from positive effect and pure quadratic term of Ultrasound temperature (B^2) had highest effect on response phenol from negative coefficients.

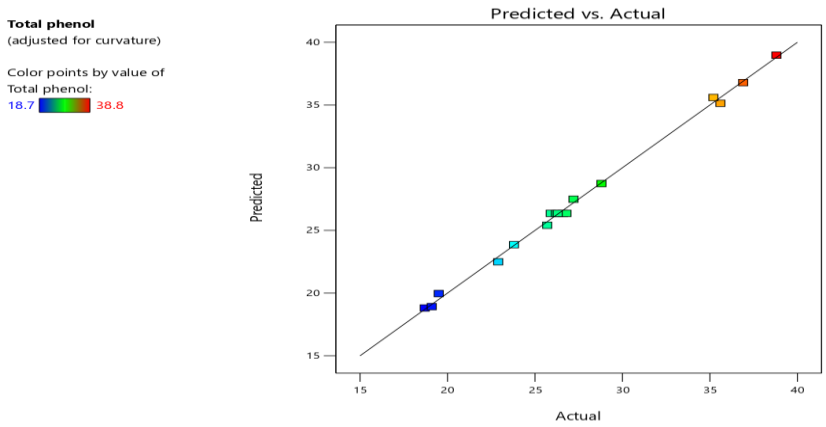


Figure 4.3 Experimental v/s predicted graph for Total Phenolic Content

Model adequacy and validation was evaluated using the Experimental vs Predicted graph **(Fig. 4.2)** and the full length data of actual, predicted and error for TPC was obtained from **(Appendix A1)**. It was observed that the predicted values (18.3-37.1%) from the model are significantly close to the actual experimental values (19.1-38.8%) with a negligible error (0.43 to 11.47%). This close proximity of the model prediction with the experimental data assures the adequacy and validation of the second order model for the total phenolic content.

4.2.1.4 Total Flavonoid content (TFC)

Yam extract containing bioactive compounds was evaluated for flavonoid content. **Table 4.1** shows the values for flavonoid expressed in %QE/g. The flavonoid obtained, as shown in **Table 4.1**, ranged from 39.5-59.8%. Maximum flavonoid content is 59.8% for the yam sample was obtained at the experiment run 1 having experimental conditions of Ultrasound temperature at 30, Sonication time at 20 min, Solvent volume 1:30. On other hand, the minimum flavonoid is 39.5% was obtained at the experiment run no. 3 having independent variable conditions of Ultrasound temperature at 40°C , Sonication time at 10 min, Solvent volume 1:20. Results of the present study in terms of flavonoid, when compared with the past studies of **(Singh et al., 2021)**, was found higher than their result of 47.56% total flavonoid content. As in the case of polyphenols, the flavonoid is influenced by the distribution of the analyzed segment in vegetable material.

Response surface quadratic model was used and ANOVA was performed using partial sum of squares methods to check the adequacy of the model for the flavonoid. Model significance was checked for both model and model factors, linear model factors Ultrasound

temperature (A), Sonication Time (B) and Solvent volume (C) and, quadratic model factors; pure quadratic terms (A^2, B^2, C^2) and interaction quadratic terms (AB, AC, BC) depending on P values. The significance of each independent variables and their interaction on the extract Flavonoid can be explained by the p-value given in **Table 4.2.** It can be observed from the **Table 4.2** that the model was found significant at 1% level of significance. The results revealed that for flavonoid of all linear and pure quadratic terms of Ultrasound temperature (A), Solvent volume (B) and Sonication Time (C) were significant at 1% and5% level of significance respectively.

A second order polynomial equation was used to fit the coded variables (A, B, C) for extract yield using multiple regression analysis. **Table 4.2** shows the coefficient of determination (R^2) for the independent variable, their interaction in coded form and their corresponding p value. The value of the R^2 for the flavonoid was found as 0.9964 which implies that the model could account for 96.48% data. The lack of fit value for regression model was not significant, which indicates that the model equation was adequate to describe the flavonoid content. For better suitability of the model, the difference between the predicted and adjusted should be less than 0.2, the adequate precision should be greater than 4 and whereas C.V. should not exceed 10%. In this case, the "Pred R^2" of 0.9707 was in reasonable agreement with the "Adj R^2" of 0.9918, the adequate precision was found to be 46.7159 and the C.V was 1.25% thereby verifying the accuracy and suitability of model. The coefficient of determination (R^2) and adjusted determination coefficient (Adj R^2) were reasonably close to 1, indicating a high degree of correlation between the observed and predicted values.

Response 4: Total flavonoid

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	690.67	9	76.74	216.13	< 0.0001	significant
A-time	619.52	1	619.52	1744.78	< 0.0001	
B-temperature	12.75	1	12.75	35.91	0.0005	
C-solid solvent ratio	20.16	1	20.16	56.78	0.0001	
AB	0.9025	1	0.9025	2.54	0.1549	
AC	1.56	1	1.56	4.40	0.0741	
BC	1.0000	1	1.0000	2.82	0.1372	
A^2	22.47	1	22.47	63.28	< 0.0001	
B^2	10.98	1	10.98	30.93	0.0008	
C^2	3.15	1	3.15	8.87	0.0206	
Residual	2.49	7	0.3551			

Lack of Fit	1.14	3	0.3792	1.13	0.4385	not significant
Pure Error	1.35	4	0.3370			
Cor Total	693.16	16				

Std. Dev.	0.5959		**R^2**	0.9964
Mean	47.50		**Adjusted R^2**	0.9918
C.V. %	1.25		**Predicted R^2**	0.9707
			Adeq Precision	46.7159

Table 4.6 Regression analysis for total flavonoid content

A second order polynomial equation **(Eq. 4.3)** was developed representing an empirical relationship between the response (flavonoid) and the independent variables Ultrasound temperature (A), Sonication Time (B) and Solvent volume (C). The equation for flavonoid activity is given below:

Flavonoid content (%) = $47.58+8.80A+1.26B+1.59C+0.47AB+0.62AC+0.50BC+2.31A^2-1.62B^2-0.8C^2$

(4.3)

The equation included both significant and non-significant terms. Non-significant terms were removed from the model and then the equation **(Eq. 4.3a)** was regenerated, that describes only the effect of significant process variables on flavonoid from yam extract. The equation is as follows:

Flavonoid content (%) = $47.58+8.80A+1.26B+1.59C+2.31A^2-1.62B^2-0.8C^2$

(4.3a)

The importance of the model developed equation was evaluated from their coefficients of correlation. As shown in the final equation 4.3a, the response flavonoid content was affected by all the linear terms of Ultrasound temperature (A), Sonication Time (B) and Solvent volume (C)and pure quadratic terms (B^2, C^2).Coefficients of linear terms (B, C) were positive and the response flavonoid was positively affected by linear terms barring the linear term (A, B) which was had negative coefficient, thus showing negative effect. The coefficients of quadratic terms were negative and the response flavonoid was negatively affected by quadratic terms. From the positive effects, Ultrasound temperature (B) had highest effect on response flavonoid. Pure

quadratic term of Ultrasound temperature (B^2) had highest effect on response flavonoid content from negative coefficients.

Model adequacy and validation was evaluated using the Experimental vs Predicted graph **(Fig. 4.3)** and the full length data of actual, predicted and error for flavonoid was obtained from **(Appendix A2)**. It was observed that the predicted values (38.6 – 58.4) from the model are significantly close to the actual experimental values (39.5 – 59.8) with a negligible error (0.004 to 2.04%). This close proximity of the model prediction with the experimental data assures the adequacy and validation of the second order model for the flavonoid.

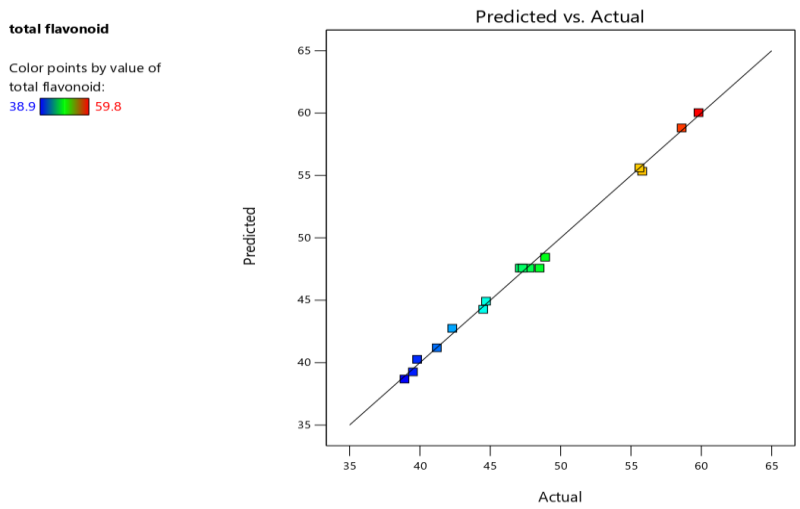


Figure 4.4- Experimental v/s predicted graph for Total flavonoid content

4.2.1.5 Total Tannin content (TTC)

Yam extract containing bioactive compounds was evaluated for Tannin content. **Table 4.1** shows the values for tannins are expressed in %. The tannins obtained, as shown in **Table 4.1**, ranged from 3.5 – 7.8%. Maximum tannin content of 7.8% for the yam sample was obtained at the experiment run 1 having experimental conditions of Ultrasound temperature at 30, Sonication time at 20 min, Solvent volume 1:30. On other hand, the minimum tannin of 3.5% was obtained at the experiment run no. 3 having independent variable conditions of Ultrasound temperature at 40 , Sonication time at 10 min, Solvent volume 1:20. Results of the present study in terms of tannin content, when compared with the past studies of **(Singh et al., 2021)**, was found 2.5% higher than their result of 5.8% tannin content. As in the case of polyphenols, the tannin is influenced by the distribution of the analyzed segment in vegetable material.

Response surface quadratic model was used and ANOVA was performed using partial sum of squares methods to check the adequacy of the model for the tannin content. Model

significance was checked for both model and model factors, linear model factors Ultrasound temperature (A), Sonication Time (B) and Solvent volume (C) and, quadratic model factors; pure quadratic terms (A^2, B^2, C^2) and interaction quadratic terms (AB, AC,BC) depending on P values. The significance of each independent variables and their interaction on the tannin can be explained by the p-value given in **Table 4.2.** It can be observed from the **Table 4.2** that the model was found significant at 1% level of significance. The results revealed that for tannin content of all linear and pure quadratic terms of Ultrasound temperature (A), Solvent volume (B) and Sonication Time (C) were significant at 1% and5% level of significance respectively.

A second order polynomial equation was used to fit the coded variables (A, B, C) for Tannin using multiple regression analysis. **Table 4.2** shows the coefficient of determination (R^2) for the independent variable, their interaction in coded form and their corresponding p value. The value of the R^2 for the tannin was found as 0.9390 which implies that the model could account for 96.48% data. The lack of fit value for regression model was not significant, which indicates that the model equation was adequate to describe the tannin content. For better suitability of the model, the difference between the predicted and adjusted should be less than 0.2, the adequate precision should be greater than 4 and whereas C.V. should not exceed 10%. In this case, the "Pred R^2" of 0.8972 was in reasonable agreement with the "Adj R^2" of 0.9249, the adequate precision was found to be 26.2266 and the C.V was 7.35% thereby verifying the accuracy and suitability of model. The coefficient of determination (R^2) and adjusted determination coefficient (Adj R^2) were reasonably close to 1, indicating a high degree of correlation between the observed and predicted values.

Response 5: Total tannin

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	30.60	3	10.20	66.70	< 0.0001	significant
A-time	27.01	1	27.01	176.63	< 0.0001	
B-temperature	0.2112	1	0.2112	1.38	0.2609	
C-solid solvent ratio	3.38	1	3.38	22.10	0.0004	
Residual	1.99	13	0.1529			
Lack of Fit	1.20	9	0.1333	0.6769	0.7128	not significant
Pure Error	0.7880	4	0.1970			
Cor Total	32.59	16				

Std. Dev.	0.3911		**R^2**	0.9390
Mean	5.32		**Adjusted R^2**	0.9249
C.V. %	7.35		**Predicted R^2**	0.8972
			Adeq Precision	26.2266

Table 4.7- Regression analysis for total tannin content

A second order polynomial equation **(Eq. 4.3)** was developed representing an empirical relationship between the response (tannin content) and the independent variables, Ultrasound temperature (A), Sonication Time (B) and Solvent volume (C). The equation for tannin is given below:

Tannin content (%) = $5.32+1.84A+0.16B+0.65C+0.34AB+0.54AC-1.32BC+0.42A^2+0.32B^2+0.42C^2$

(4.3)

The equation included both significant and non-significant terms. Non-significant terms were removed from the model and then the equation **(Eq. 4.3a)** was regenerated, that describes only the effect of significant process variables on tannin from yam extract. The equation is as follows:

Tannin content (%) = $5.32+1.84A+0.16B+0.65C+0.42A^2+0.32B^2+0.42C^2$

(4.3a)

The importance of the model developed equation was evaluated from their coefficients of correlation. As shown in the final equation 4.3a, the response tannin was affected by all the linear terms of, Ultrasound temperature (A), Sonication Time (B) and Solvent volume (C)and

pure quadratic terms (B^2, C^2).Coefficients of linear terms (B, C) were positive and the response Tannin was positively affected by linear terms barring the linear term (A, B) which was had negative coefficient, thus showing negative effect. The coefficients of quadratic terms were negative and the response tannin was negatively affected by quadratic terms. From the positive effects, Ultrasound temperature (B) had highest effect on response tannin . Pure quadratic term of Ultrasound temperature (B^2) had highest effect on response tannin from negative coefficients.

Model adequacy and validation was evaluated using the Experimental vs Predicted graph **(Fig. 4.3)** and the full length data of actual, predicted and error for tannin content was obtained from **(Appendix A2)**. It was observed that the predicted values (2.4 - 6.5) from the model are significantly close to the actual experimental values (3.5 – 7.8) with a negligible error (0.004 to 2.04%). This close proximity of the model prediction with the experimental data assures the adequacy and validation of the second order model for the tannin .

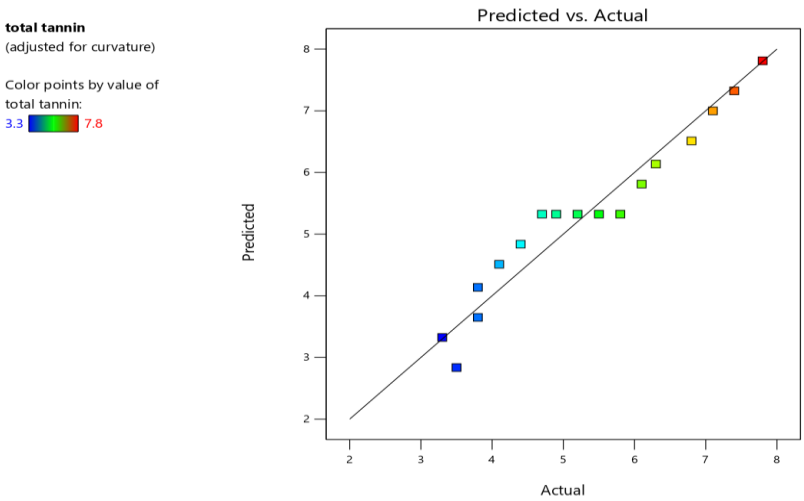


Figure 4.5- Experimental v/s predicted graph for Total tannin content

4.2.1.6 Antioxidant Activity (DPPH)

Yam extract containing bioactive compounds was evaluated for antioxidant activity using DPPH assay. **Table 4.1** shows the values for DPPH Antioxidant Activity expressed in % antioxidant activity. The Antioxidant activity obtained, as shown in **Table 4.1**, ranged from 59.1 – 78.8%. Maximum antioxidant activity of 78.8% for the yam sample was obtained at the experiment run 1 having experimental conditions of Ultrasound temperature at 30, Sonication time at 20 min, Solvent volume 1:30. On other hand, the minimum Antioxidant activity of 59.1% was obtained at the experiment run no. 3 having independent variable conditions of Ultrasound temperature at 40, Sonication time at 10 min, Solvent volume 1:20. Results of the present study in terms of

Antioxidant activity, when compared with the past studies of **(Kumar et al., 2017)**, was found 22.882% higher than their result of 56.31%antioxidant activity. As in the case of polyphenols, the antioxidant activity is influenced by the distribution of the analyzed segment in vegetable material.

Response surface quadratic model was used and ANOVA was performed using partial sum of squares methods to check the adequacy of the model for the Antioxidant activity. Model significance was checked for both model and model factors, linear model factors Ultrasound temperature (A), Sonication Time (B) and Solvent volume (C) and, quadratic model factors; pure quadratic terms (A^2, B^2, C^2) and interaction quadratic terms (AB, AC,BC) depending on P values. The significance of each independent variables and their interaction on the Antioxidant can be explained by the p-value given in **Table 4.2.** It can be observed from the **Table 4.2** that the model was found significant at 1% level of significance. The results revealed that for Antioxidant activity of all linear and pure quadratic terms of Ultrasound temperature (A), Solvent volume (B) and Sonication Time (C) were significant at 1% and5% level of significance respectively.

A second order polynomial equation was used to fit the coded variables (A, B, C) for Antioxidant using multiple regression analysis. **Table 4.2** shows the coefficient of determination (R^2) for the independent variable, their interaction in coded form and their corresponding p value. The value of the R^2 for the Antioxidant was found as 0.9975 which implies that the model could account for 96.48% data. The lack of fit value for regression model was not significant, which indicates that the model equation was adequate to describe the antioxidant activity. For better suitability of the model, the difference between the predicted and adjusted should be less than 0.2, the adequate precision should be greater than 4 and whereas C.V. should not exceed 10%. In this case, the "Pred R^2" of 0.9718 was in reasonable agreement with the "Adj R^2" of 0.9943, the adequate precision was found to be 56.7995 and the C.V was 0.6878% thereby verifying the accuracy and suitability of model. The coefficient of determination (R^2) and adjusted determination coefficient (Adj R^2) were reasonably close to 1, indicating a high degree of correlation between the observed and predicted values.

Response 6: Antioxidant content

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	597.59	9	66.40	309.96	< 0.0001	significant
A-time	549.46	1	549.46	2565.01	< 0.0001	
B-temperature	3.92	1	3.92	18.30	0.0037	
C-solid solvent ratio	24.15	1	24.15	112.74	< 0.0001	
AB	0.0625	1	0.0625	0.2918	0.6058	
AC	0.0100	1	0.0100	0.0467	0.8351	
BC	0.0225	1	0.0225	0.1050	0.7553	
A^2	16.34	1	16.34	76.28	< 0.0001	
B^2	1.81	1	1.81	8.43	0.0229	
C^2	1.89	1	1.89	8.82	0.0208	
Residual	1.50	7	0.2142			
Lack of Fit	1.01	3	0.3358	2.73	0.1782	not significant
Pure Error	0.4920	4	0.1230			
Cor Total	599.09	16				

Std. Dev.	0.4628		**R^2**	0.9975
Mean	67.29		**Adjusted R^2**	0.9943
C.V. %	0.6878		**Predicted R^2**	0.9718
			Adeq Precision	56.7995

Table 4.8- Regression analysis for antioxidant

A second order polynomial equation **(Eq. 4.3)** was developed representing an empirical relationship between the response (Antioxidant Activity) and the independent variables Ultrasound temperature (A), Sonication Time (B) and Solvent volume (C). The equation for antioxidant activity is given below:

$$\text{Antioxidant Activity}(\%) = 66.36+8.29A+0.70B+1.74C+0.12AB-0.05AC-0.07BC+1.97A^2-0.65B^2+0.67C^2 \quad (4.3)$$

The equation included both significant and non-significant terms. Non-significant terms were removed from the model and then the equation **(Eq. 4.3a)** was regenerated, that describes only the effect of significant process variables on antioxidant activity from Yam extract. The equation is as follows:

Antioxidant Activity (%) = $66.36+8.29A+0.70B+1.74C+1.97A^2-0.65B^2+0.67C^2$

(4.3a)

The importance of the model developed equation was evaluated from their coefficients of correlation. As shown in the final equation 4.3a, the response antioxidant activity was affected by all the linear terms of Ultrasound temperature (A), Sonication Time (B) and Solvent volume (C) and pure quadratic terms (B^2, C^2).Coefficients of linear terms (B, C) were positive and the response Antioxidant was positively affected by linear terms barring the linear term (A, B) which was had negative coefficient, thus showing negative effect. The coefficients of quadratic terms were negative and the response antioxidant activity was negatively affected by quadratic terms. From the positive effects, Ultrasound temperature (B) had highest effect on response antioxidant . Pure quadratic term of Ultrasound temperature (B^2) had highest effect on response antioxidant activity from negative coefficients.

Model adequacy and validation was evaluated using the Experimental vs Predicted graph **(Fig. 4.3)** and the full length data of actual, predicted and error for Antioxidant Activity was obtained from **(Appendix A2)**. It was observed that the predicted values (58.5 – 77.6) from the model are significantly close to the actual experimental values (59.1 – 78.8) with a negligible error (0.004 to 2.04%). This close proximity of the model prediction with the experimental data assures the adequacy and validation of the second order model for the antioxidant activity.

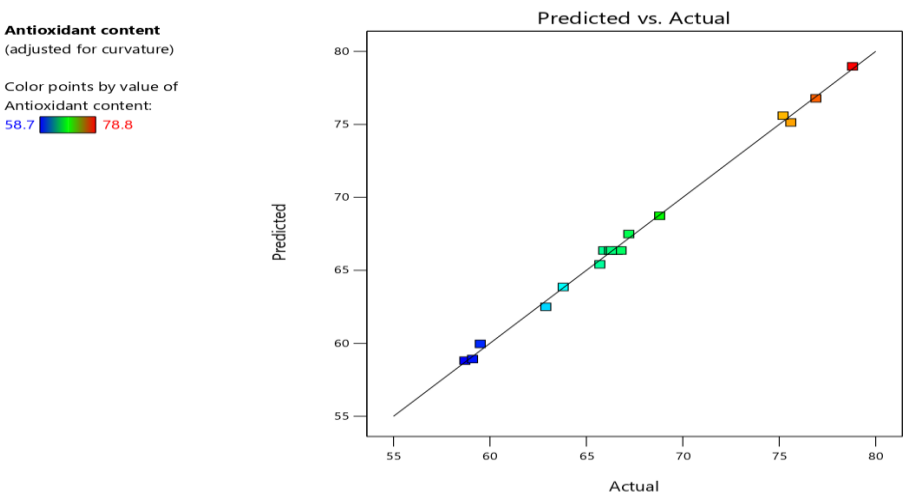


Figure 4.6- Experimental v/s predicted graph for antioxidant

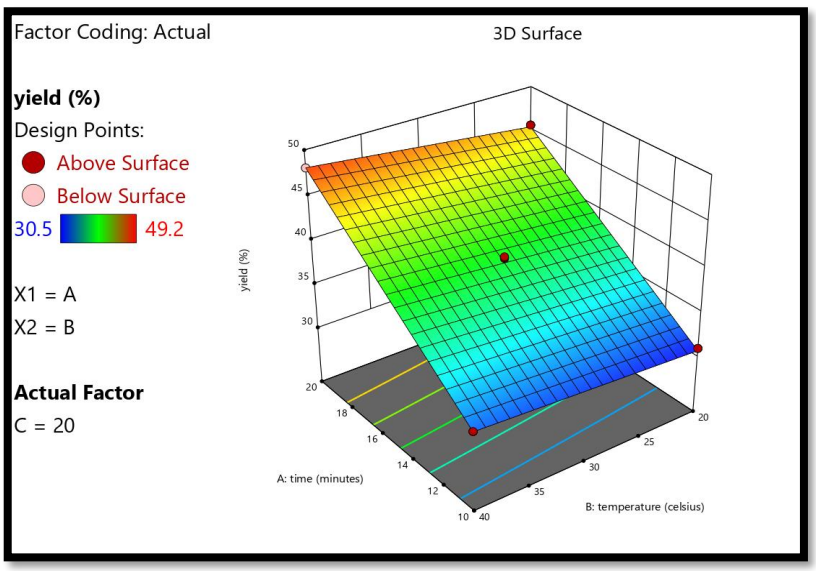
4.3 Graphical analysis of independent variable of UAE

After the optimization of process parameters, graphical analysis for all the responses was done considering the optimized conditions. Graphical analysis was done for understanding the trend of various responses with respect to levels of significant process variables. To determine the operating range for the best result, graphs were drawn using software Design expert 13.

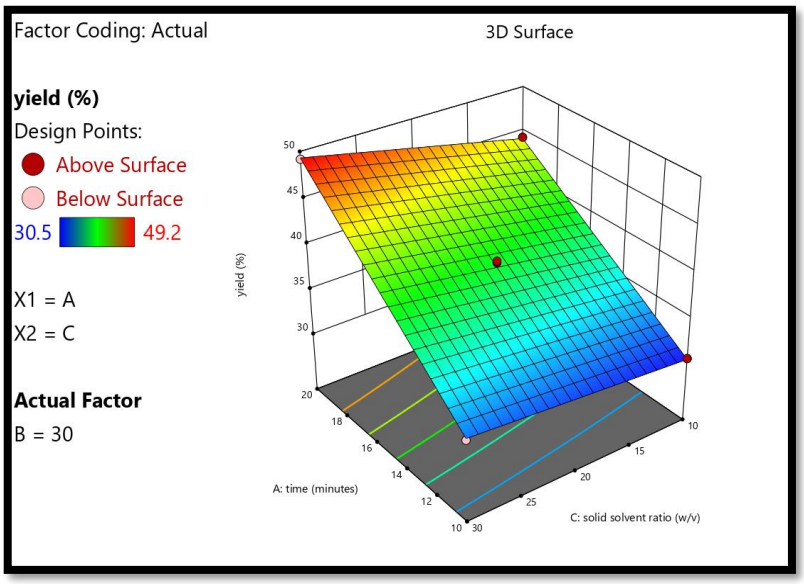
4.3.1 Extract yield

In Fig. **4.1,** at linear level the extract yield varies with particle size at optimum conditions of ultrasound temperature 39.93℃, Time 20 min, Solvent volume 29.08 for achieving maximum extract yield. The graph shows extract yield increases with the decrease in particle size 150 µm. Smaller the particle size greater the surface and hence the mass transfer efficiency increases. Grinding breaks the plant cell wall, thus facilating the active compound to release to the extraction solvent and enhance the yield. The readings are supported by **(Kumar et al, 2017)**

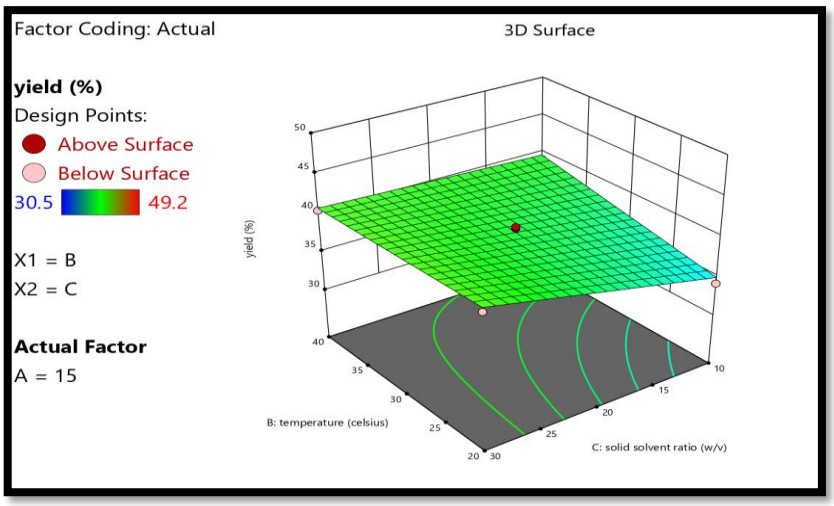
The graph shows extract yield increases with the power and decrease after further increase in power. This is due ultrasound wave can facilitate the cell walls of target sample to disrupt, also can accelerate the diffusing and dissolving of target components in the liquid medium. However, higher ultrasonic power can weaken the cavitation effect because the cavitation bubbles in this case more likely grow too big to collapse Moreover, excessive cavitation bubbles production can hinder the mass transfer and lead the ultrasound waves to scatter, which weaken the effect of ultrasonic temperature. The readings are supported by **(Singh et al., 2021)**



(a)



(b)



(c)

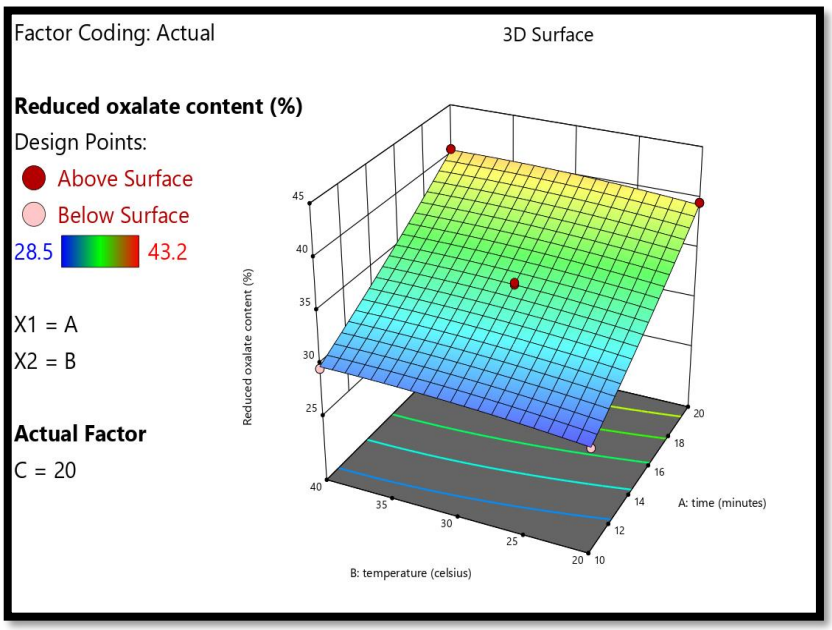
Figure 4.7 - Graphical analysis of yield (a, b and c) (a) interaction between time and temperature; (b) interaction between time and solid-solvent ratio; (c) interaction between temperature and solid-solvent ratio

4.3.2 OXALATE

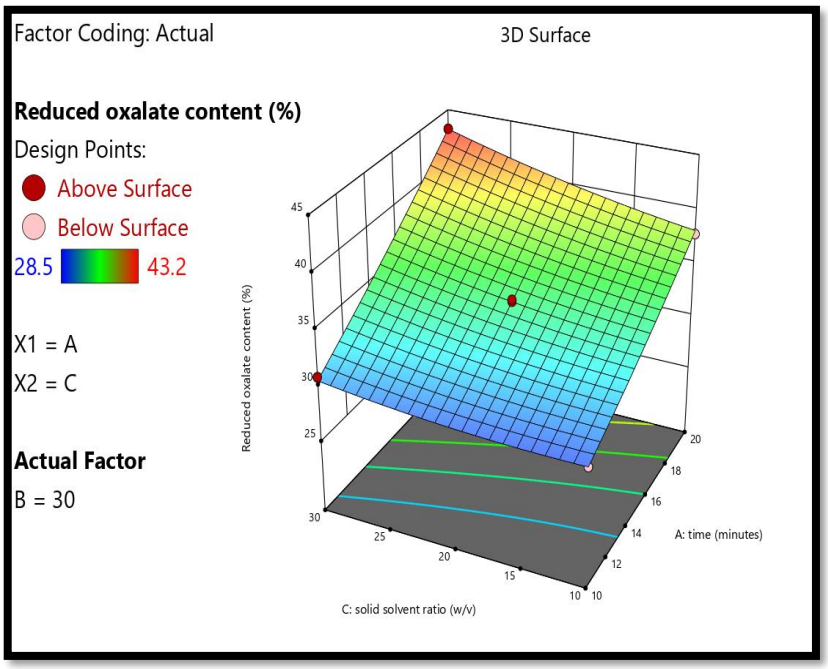
In Fig. **4.8a,** at linear level the oxalate varies with particle size at optimum conditions of ultrasound temperature 39.93°C, Time 20 min, Solvent volume 29.08 for achieving maximum reduction in oxalate. The graph shows oxalate decreases with the decrease in particle size 150 µm. Smaller the particle size greater the surface and hence the mass transfer efficiency increases. Grinding breaks the plant cell wall, thus facilating the active compound to release to the

extraction solvent and enhance the oxalate reduction. The readings are supported by **(Kumar et al, 2017)**

The graph shows oxalate decreases with the temperature. This is due ultrasound wave can facilitate the cell walls of target sample to disrupt, also can accelerate the diffusing and dissolving of target components in the liquid medium. However, higher ultrasonic power can weaken the cavitation effect because the cavitation bubbles in this case more likely grow too big to collapse Moreover, excessive cavitation bubbles production can hinder the mass transfer and lead the ultrasound waves to scatter, which weaken the effect of ultrasonic temperature. The readings are supported by **(Singh et al., 2021)**



(a)



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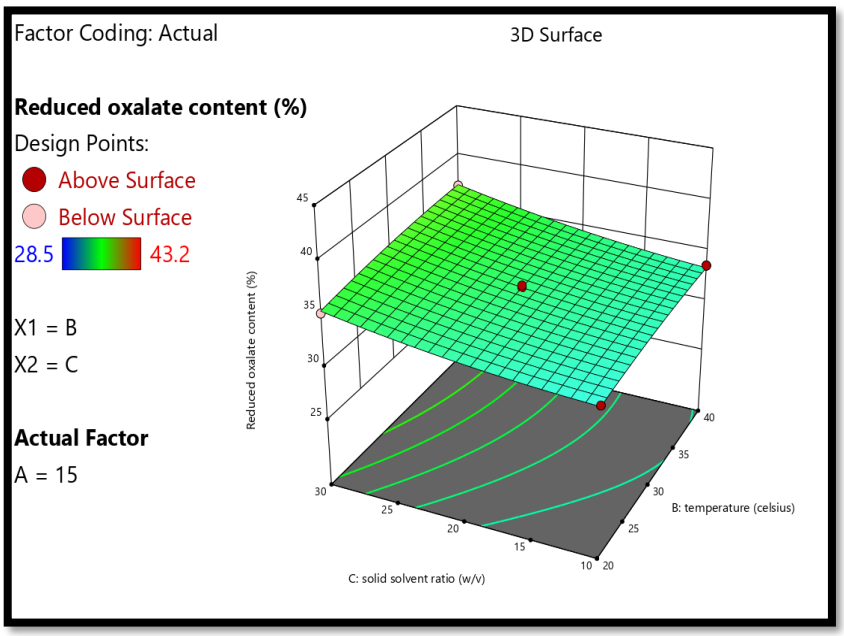


(c)

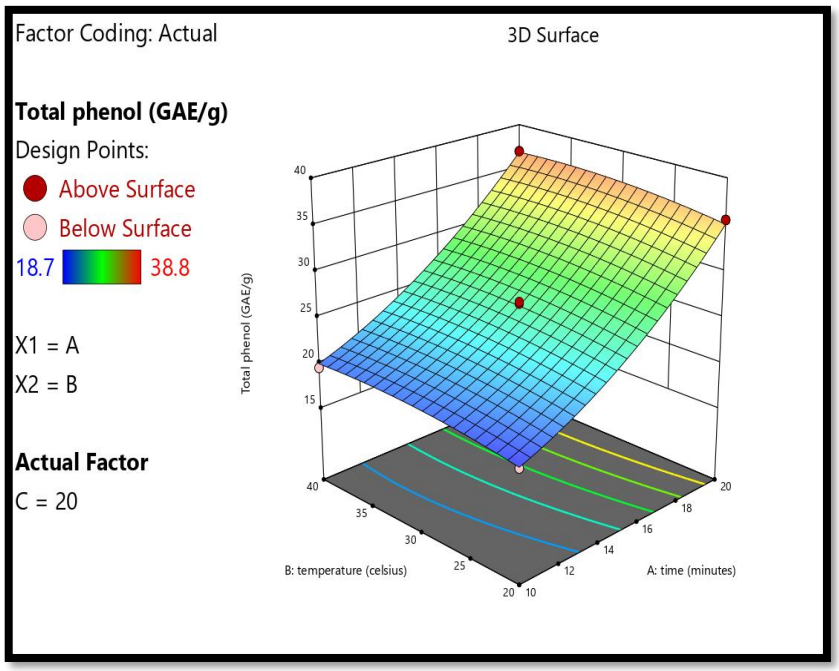
Figure 4.8- Graphical analysis of oxalate (a, b and c) (a) interaction between time and temperature; (b) interaction between time and solid-solvent ratio; (c) interaction between temperature and solid-solvent ratio

4.3.3 Total Phenol Content (TPC)

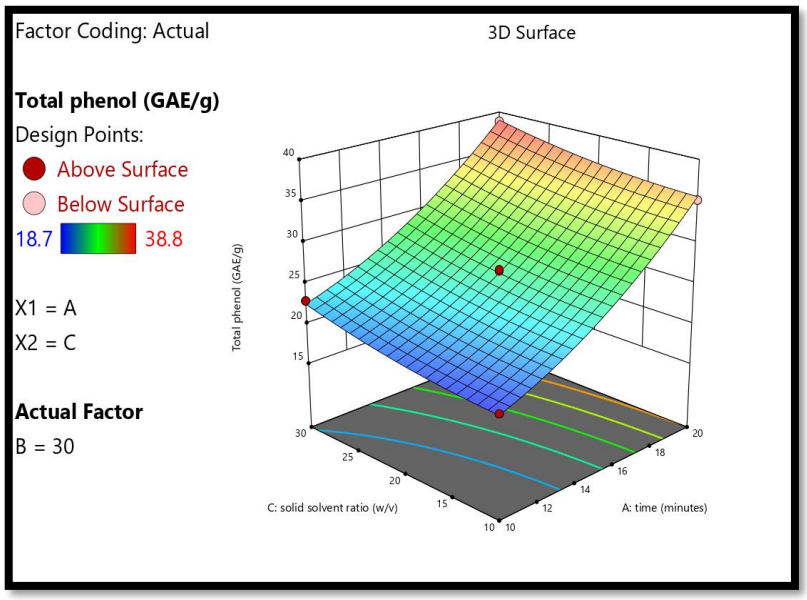
In Fig. **4.8a,** at linear level the phenol varies with particle size at optimum conditions of ultrasound temperature 39.93°C, Time 20 min, Solvent volume 29.08 for achieving maximum

phenol. The graph shows phenol increases with the decrease in particle size 150 µm. Smaller the particle size greater the surface and hence the mass transfer efficiency increases. Grinding breaks the plant cell wall, thus facilating the active compound to release to the extraction solvent and enhance the phenol. The readings are supported by **(Xu et al., 2014)**

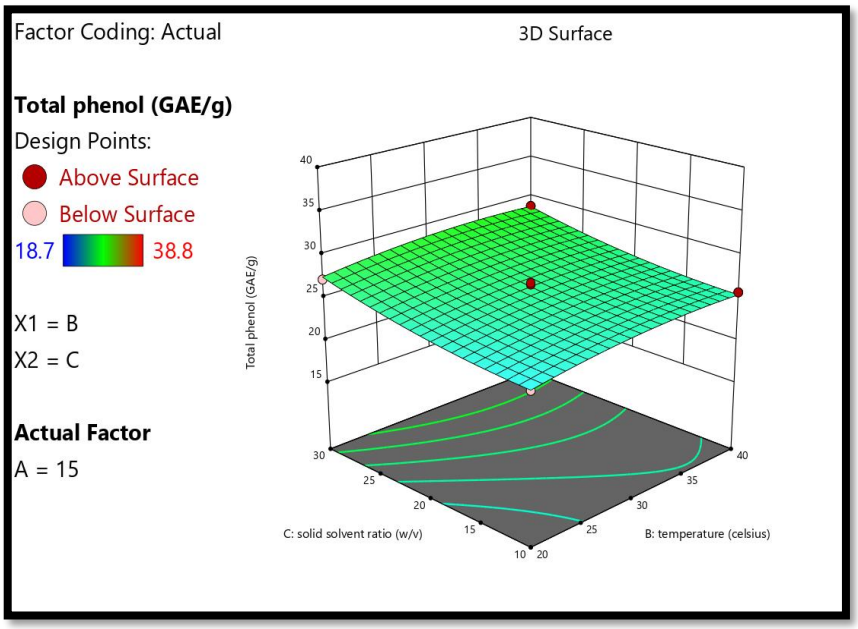
The graph shows phenol increases with the temperature. This is due ultrasound wave can facilitate the cell walls of target sample to disrupt, also can accelerate the diffusing and dissolving of target components in the liquid medium. However, higher ultrasonic power can weaken the cavitation effect because the cavitation bubbles in this case more likely grow too big to collapse Moreover, excessive cavitation bubbles production can hinder the mass transfer and lead the ultrasound waves to scatter, which weaken the effect of ultrasonic temperature. The readings are supported by **(Sheikh et al., 2013)**



(a)



(b)



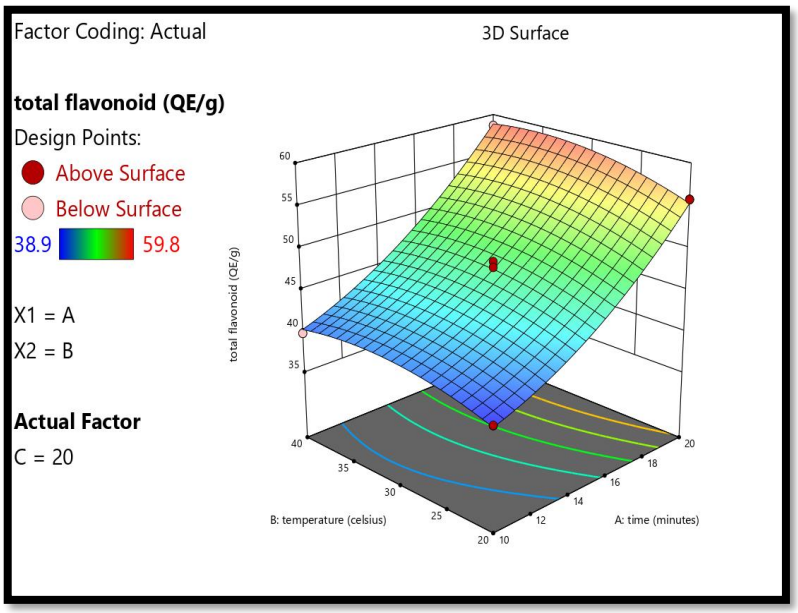
(c)

Figure 4.9 Graphical analysis of phenol (a, b and c) (a) interaction between time and temperature; (b) interaction between time and solid-solvent ratio; (c) interaction between temperature and solid-solvent ratio

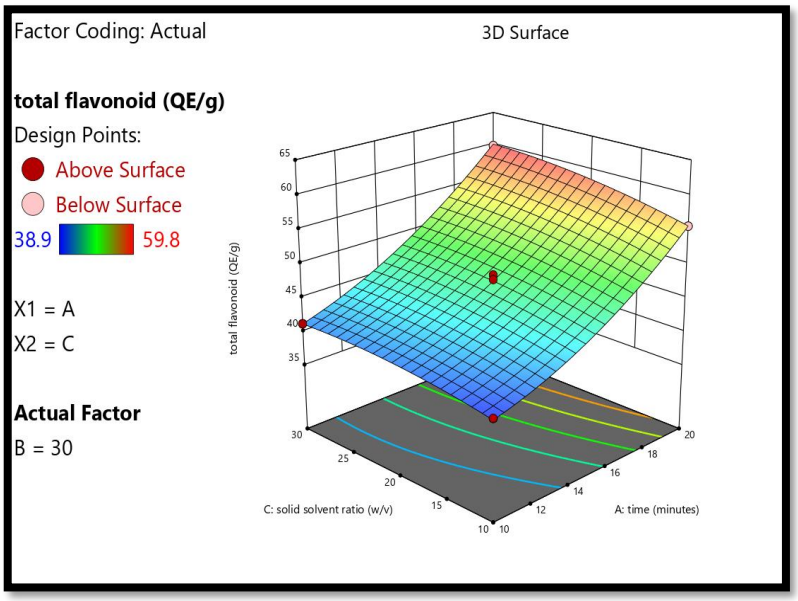
4.3.4 Total Flavonoid Content (TFC)

In Fig. **4.8a,** at linear level the flavonoid varies with particle size at optimum conditions of ultrasound temperature 39.93°C, Time 20 min, Solvent volume 29.08 for achieving maximum flavonoid content. The graph shows flavonoid increases with the decrease in particle size 150

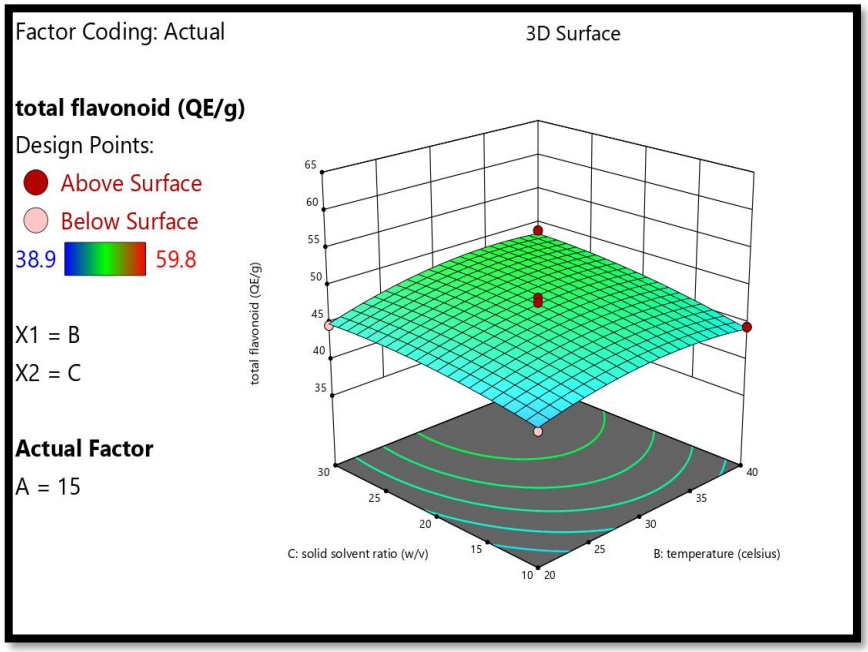
µm. Smaller the particle size greater the surface and hence the mass transfer efficiency increases. Grinding breaks the plant cell wall, thus facilating the active compound to release to the extraction solvent and enhance the flavonoids. The readings are supported by **(Xu et al.,2014)** The graph shows flavonoid increases with the temperature. This is due ultrasound wave can facilitate the cell walls of target sample to disrupt, also can accelerate the diffusing and dissolving of target components in the liquid medium. However, higher ultrasonic power can weaken the cavitation effect because the cavitation bubbles in this case more likely grow too big to collapse Moreover, excessive cavitation bubbles production can hinder the mass transfer and lead the ultrasound waves to scatter, which weaken the effect of ultrasonic temperature. The readings are supported by **(Sheikh et al., 2013)**



(a)



(b)



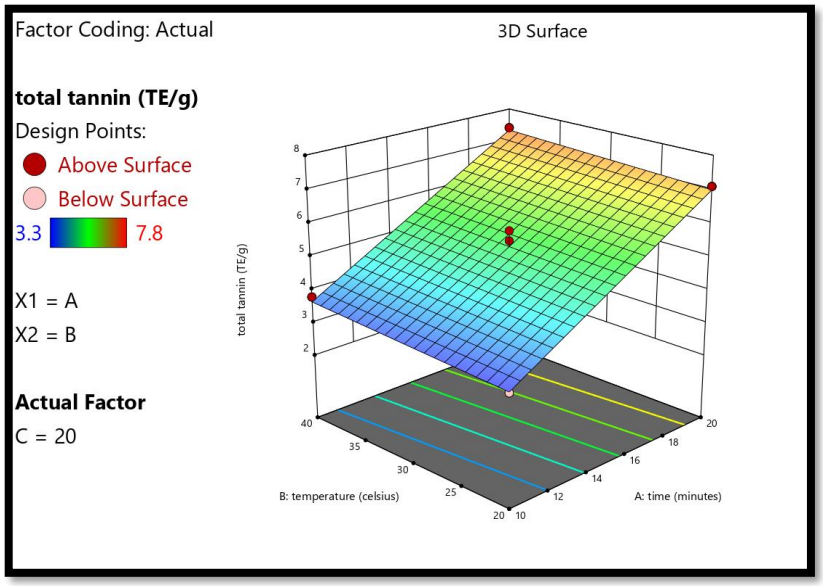
(c)

Figure 4.10 - Graphical analysis of flavonoid (a, b and c) (a) interaction between time and temperature; (b) interaction between time and solid-solvent ratio; (c) interaction between temperature and solid-solvent ratio

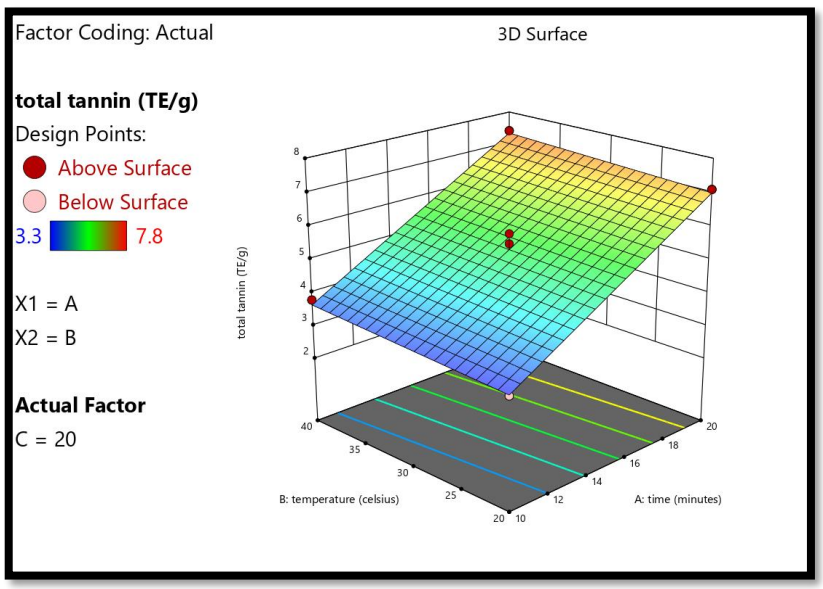
4.3.5 Total Tannin content (TTC)

In Fig. **4.8a,** at linear level the tannin varies with particle size at optimum conditions of ultrasound temperature 39.93℃, Time 20 min, Solvent volume 29.08 for achieving maximum tannin . The graph shows tannin increases with the decrease in particle size 150 µm. Smaller the particle size greater the surface and hence the mass transfer efficiency increases. Grinding breaks the plant cell wall, thus facilating the active compound to release to the extraction solvent and enhance the tannin . The readings are supported by **(Xu et al, 2014)**

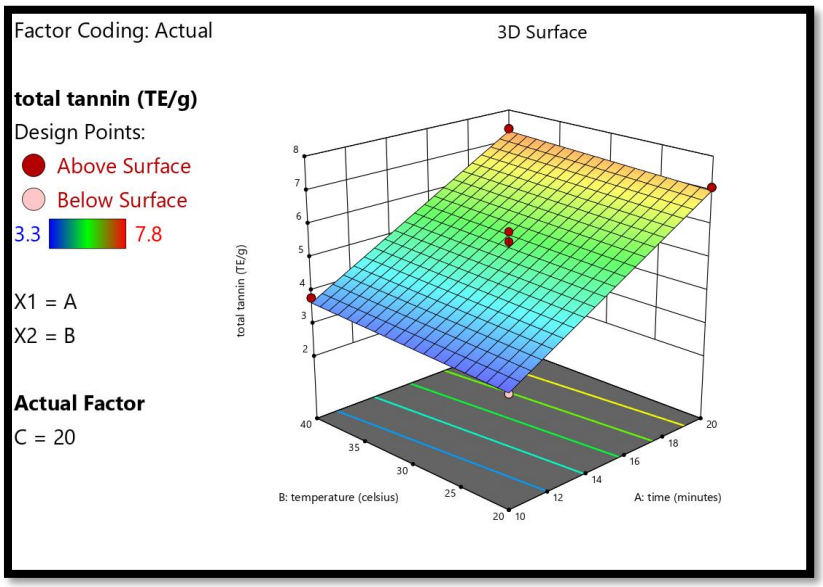
The graph shows tannin increases with the Temperature. This is due ultrasound wave can facilitate the cell walls of target sample to disrupt, also can accelerate the diffusing and dissolving of target components in the liquid medium. However, higher ultrasonic temperature can weaken the cavitation effect because the cavitation bubbles in this case more likely grow too big to collapse Moreover, excessive cavitation bubbles production can hinder the mass transfer and lead the ultrasound waves to scatter, which weaken the effect of ultrasonic temperature. The readings are supported by **(Sheikh et al., 2013)**


Factor Coding: Actual
3D Surface
total tannin (TE/g)
Design Points:
Above Surface
Below Surface
3.3
7.8
X1 = A
X2 = B
Actual Factor
C = 20
total tannin (TE/g)
B: temperature (celsius)
A: time (minutes)

(a)


Factor Coding: Actual
3D Surface
total tannin (TE/g)
Design Points:
Above Surface
Below Surface
3.3
7.8
X1 = A
X2 = B
Actual Factor
C = 20
total tannin (TE/g)
B: temperature (celsius)
A: time (minutes)

(b)



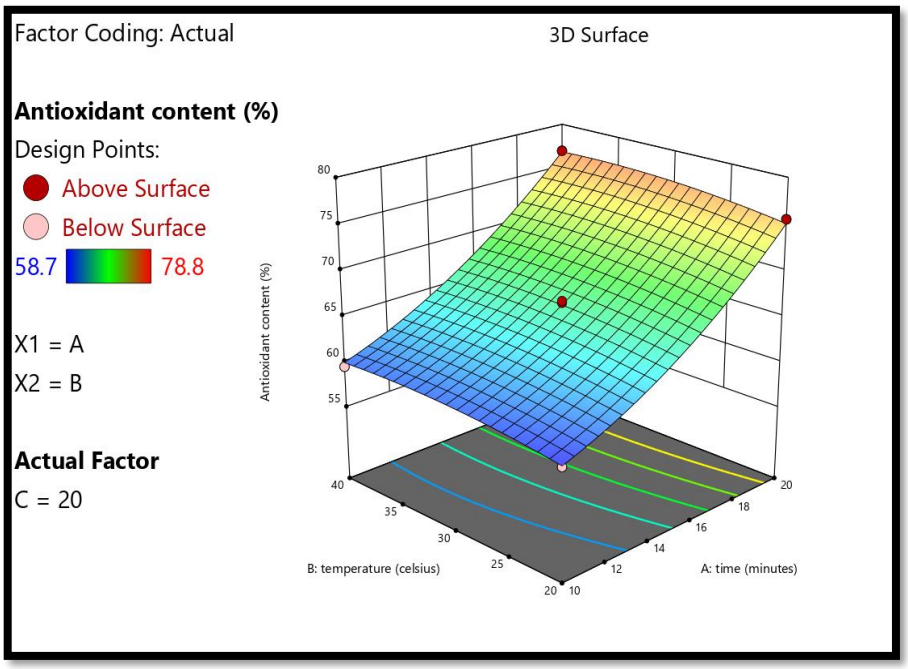
(c)

Figure 4.11 Graphical analysis of TTC (a, b and c) (a) interaction between time and temperature; (b) interaction between time and solid-solvent ratio; (c) interaction between temperature and solid-solvent ratio

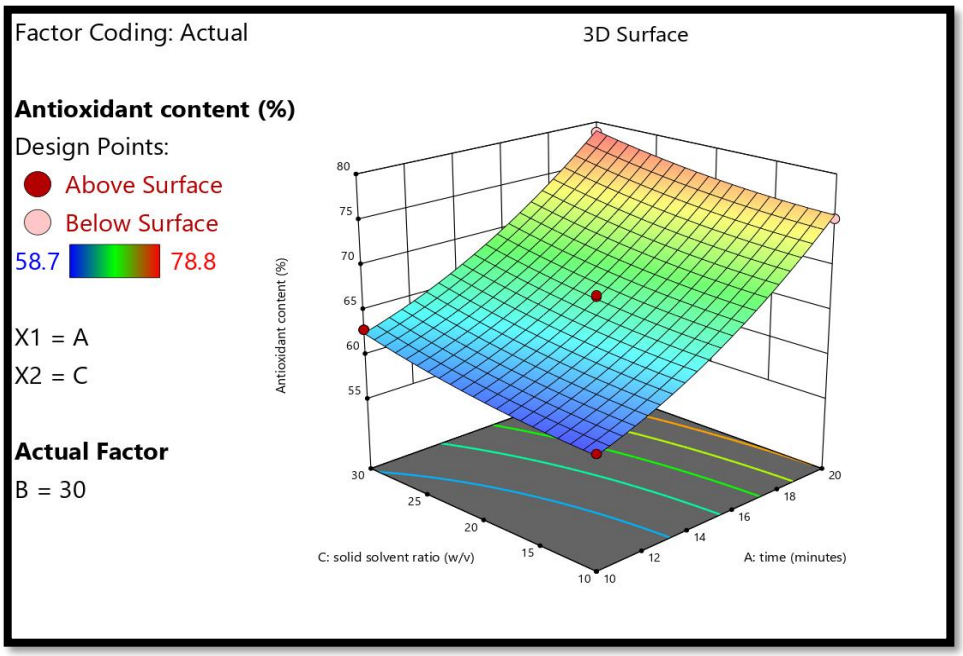
4.3.6 Antioxidant Activity

In Fig. **4.8a,** at linear level the antioxidant varies with particle size at optimum conditions of ultrasound temperature 39.93℃, Time 20 min, Solvent volume 29.08 for achieving maximum antioxidant. The graph shows antioxidant increases with the decrease in particle size 150 µm. Smaller the particle size greater the surface and hence the mass transfer efficiency increases. Grinding breaks the plant cell wall, thus facilating the active compound to release to the extraction solvent and enhance the antioxidant . The readings are supported by **(Kumar et al, 2017)**

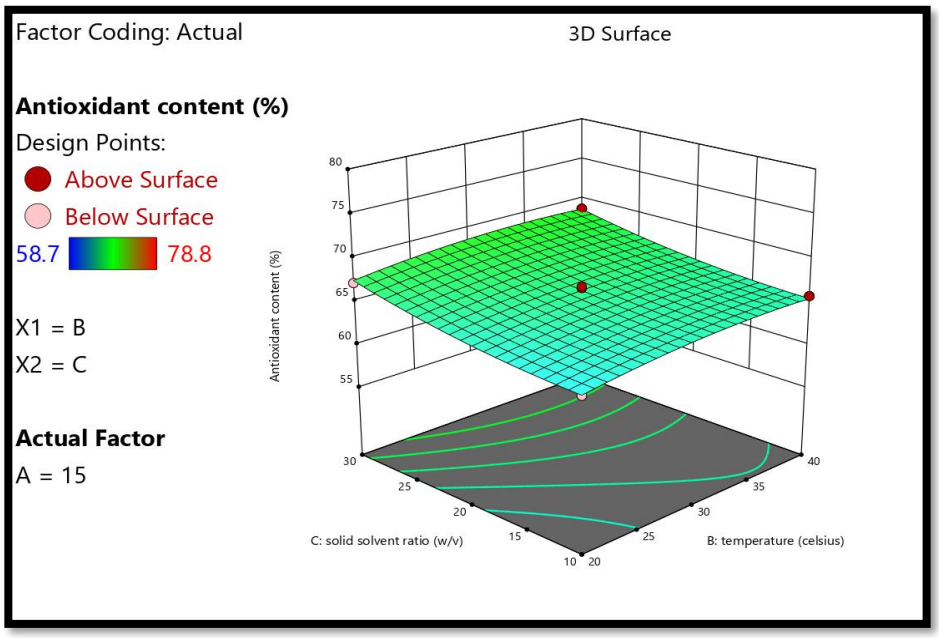
The graph shows antioxidant increases with the temperature. This is due ultrasound wave can facilitate the cell walls of target sample to disrupt, also can accelerate the diffusing and dissolving of target components in the liquid medium. However, higher ultrasonic power can weaken the cavitation effect because the cavitation bubbles in this case more likely grow too big to collapse Moreover, excessive cavitation bubbles production can hinder the mass transfer and lead the ultrasound waves to scatter, which weaken the effect of ultrasonic temperature. The readings are supported by **(Xu et al., 2014)**


Factor Coding: Actual
3D Surface
Antioxidant content (%)
Design Points:
Above Surface
Below Surface
58.7
78.8
X1 = A
X2 = B
Actual Factor
C = 20
Antioxidant content (%)
B: temperature (celsius)
A: time (minutes)

(a)


Factor Coding: Actual
3D Surface
Antioxidant content (%)
Design Points:
Above Surface
Below Surface
58.7
78.8
X1 = A
X2 = C
Actual Factor
B = 30
Antioxidant content (%)
C: solid solvent ratio (w/v)
A: time (minutes)

(b)



(c)

Figure 4.12 - Graphical analysis of antioxidant (a, b and c) (a) interaction between time and temperature; (b) interaction between time and solid-solvent ratio; (c) interaction between temperature and solid-solvent ratio

4.4 Optimization of parameters for yam extract

Optimization is a procedure of getting best output of various possible values of input parameters for any process. In this study, numerical optimization was carried using design expert 13 version statistical software. The goal was to reduce the oxalate content and to fixed to maximize the extracted bioactive compounds quantitatively as well as qualitatively using the optimized parameters (Ultrasound temperature, Sonication Time, Solvent Volume) among the number of experiments performed. All the responses of Reduce oxalate, extract yield, Total Phenolic Content, Total Flavonoid content, Total Tannin content, DPPH Antioxidant Activity were considered for the optimization. Among all the optimized solutions, the best optimized solution for optimum values of independent variables was selected on the basis of the criteria that the optimum values would fit the experimental setup and should be of higher desirability.

To optimize the process parameters, the goal was set in range for all the independent variables from lower and upper limits of their values. In case of responses, the goal was fixed to maximize and minimize. All the responses and independent variables were given similar importance (+++). The criterion for goal set up for optimization of reduce oxalate and extraction of bioactive compounds from yam extract is given in **Table. 4.9.** Based on mentioned criteria, the optimization was carried out. During optimization, 54 solutions were generated, out of which the one that suited the criteria most was selected.

Table. 4.9 Criteria for optimization of UAE of yam extract					
Parameter	**Goal**	**Lower Limit**	**Upper Limit**	**Unit**	**Importance**
Ultrasound temperature	In Range	20	40	W/V	+++
Sonication Time	In Range	10	20	min	+++
Solid Solvent Ratio	In Range	10	30	ml	+++
Oxalate content	Minimize	29.3	43.2	%	+++
Extract yield	Maximize	30.5	49.2	%	+++
Total Phenolic Content	Maximize	19.1	38.8	GAE/g	+++
DPPH Antioxidant Activity	Maximize	59.1	78.8	%	+++
Total Flavonoid content	Maximize	39.5	59.8	QE/g	+++
Total Tannin Content	Minimize	3.5	7.8	TE/g	+++

Based on mentioned criteria, the optimization was carried out. During optimization, 65 solutions were obtained, out of which the one that suited the experimental setup the most and having highest desirability of 1 was selected. The most suitable optimum values are given in the **Table.4.10**. The optimum result of Extraction of bioactive compounds was obtained when the Ultrasound temperature was 39.93℃, Sonication Time 20 min, Solid - Solvent volume 29.08 w/v .

Table. 4.10 Optimum values of independent variables and responses		
Parameter	**Unit**	**Optimum Value**
Ultrasound temperature	℃	39.93
Sonication Time	min	20
Solid-Solvent Volume	ml	29.08
Oxalate content	%	42.37
Extract yield	%	49.21
Total Phenolic Content	GAE/g	37.95
DPPH Antioxidant Activity	%	77.95
Total Flavonoid content	QE/g	57.39
Total Tannin content	TE/g	7.67

4.5 Verification of optimized results

Optimized set of experimental conditions was obtained from Design Expert Software 13 by setting the goals as represented on the Table 4.12 and Table 4.13. The predicted optimized results of the response variable were verified by conducting the experiments and evaluating their attributes at optimized combinations of independent variables. The experiments were conducted and actual values of all the responses were compared with predicted values. The actual values of the responses have been presented in Table 4.14.and were found closer to the predicted values. Thus it was concluded that the optimum set of conditions as predicted by the model are correct and validate the model.

Table. 4.11 Verification of actual and predicted values					
Parameter	**Unit**	**Predicted values**	**Experimental values**	**Residual Error**	**% Error**
Ultrasound temperature	℃	39.93	30.56	-0.00092	0.091668
Sonication Time	min	19.87	20.78	0.000111	0.01111
Solid-Solvent Volume	ml	29.08	30.89	0.000195	0.019508
Oxalate content	%	42.37	43.26	0.074654	2.771026
Extract yield	%	49.21	49.25	0.335343	0.103293
Total Phenolic Content	mg GAE/g	37.95	38.8	0.214232	0.375967
Total Flavonoid content	QE/g	57.39	59.8	0.355123	0.342321
Total Tannin content	TE/g	7.67	7.85	0.152976	0.654321
DPPH Antioxidant Activity	%	77.95	78.8	0.214223	0.398478

4.6 Comparison of overall optimized trends

The optimized values obtained using BBD of RSM were explained in detail in previous sections. The individual values showed optimized values of all the dependent variables by standardizing trends in the available data. The UAE processing optimized results were compared with conventional methodology in the following **Table 4.25.**

Table 4.12 Comparison of optimized trends in processing

S. No.	Parameters	UAE	Boiling	Nacl
1	Extract yield	49.2	23.3	12.2
2	Total Phenolic Content	38.8	12.4	10.8
3	DPPH Antioxidant Activity	78.8	34.5	23.4
4	Total Flavonoid content	59.8	26.7	12.7
5	Total Tannin Content	7.8	4.52	6.77
6	Oxalate content	43.2	55.6	65.7

The comparisons of optimized processing values reflect the excellence of UAE methodology over conventional technique. An objective look over different values reveal following results.

1) Maximum extract yield and minimum oxalate can be seen in UAE methodology. This is due to its inherent nature of non-thermal processing technology. Minimum extract yield

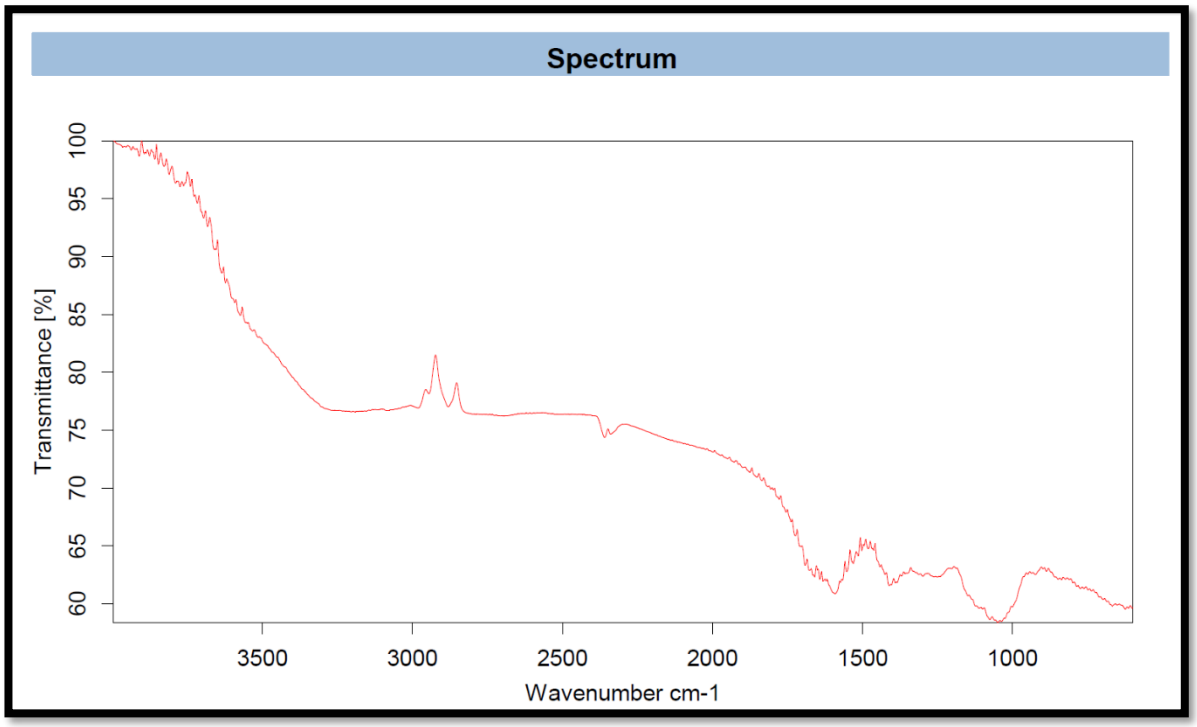
values in conventional methodology indicate the destruction of chemical structures essential in dye extraction.

2) Moreover, similar observation can be seen in TPA values due to inherent advantage of UAE over conventional methodology.
3) Antioxidant activity values show UAE superiority over other methods.
4) In terms of powder properties, the comparisons of different values conclude the overall effectiveness of UAE over conventional technology.
5) The optimization value of individual treatment when compared on the basis of reduced oxalate shows the best and most acceptable in terms of phytochemical quality.

4.7 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSIS-

Fourier transform infrared spectroscopy (FTIR) The functional groups of ultrasonic-based modified yam molecules were measured by Bruker Vertex 70 FTIR spectrometer (Bruker Optics, Germany). In brief, the yam was treated under ultrasonication with various influencing parameters like temperature, time and solid-solvent ratio. Further, to remove the moisture completely, the ultrasound modified yam was dried in a hot air oven at 50 ◦C for 24 h. The spectrums of samples were recorded at wavelength of 1000-3500 cm^{-1}.

Impact of ultrasonication on functional groups of molecules by FTIR analysis - Sample 233

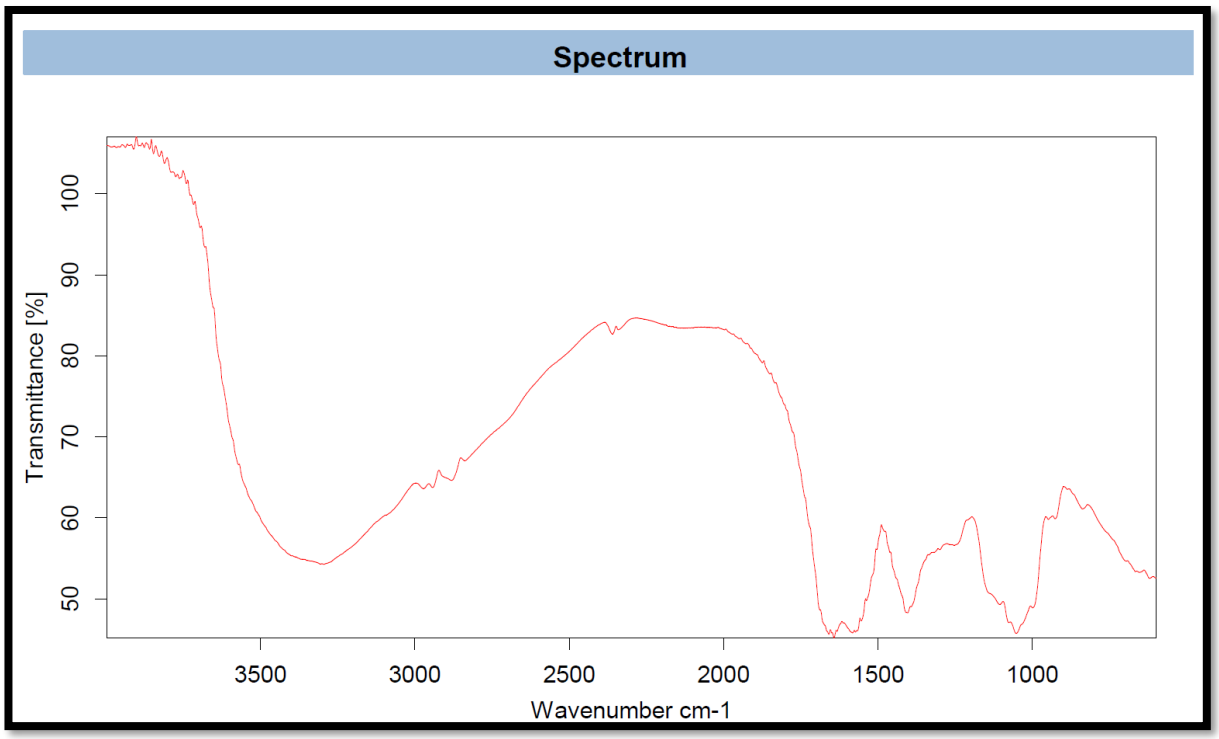


- A strong broad peak at around 3204.40 cm^{-1} shows C-H stretching
- A sharp peak at 1591.25 cm^{-1} representing C=O stretching.
- A broad peak at 1048.86 cm^{-1} shows elongation of C–O group

FT-IR spectra presented bands associated to stretching, flexion and deformation corresponding to the main functional groups characteristic of the elephant foot yam. The spectra of four yam samples were shown, indicating that ultrasound treatment caused minute changes in the type of chemical groups, but no newly produced chemical groups were found in yam.

For treated sample with Ultrasound technique for 20 minutes at 30℃ with 1:30 solid solvent ratio, FTIR spectra have revealed that positions of the characteristic absorption peaks have changed after the ultrasound treatment, and minor reductions was noticed in their intensities. Similar findings were reported by Monroy et al., 2018 during the ultrasound treatment of elephant foot yam starch analysis. As shown in figure the broadband appeared at around 3204.40 cm^{-1} resembles the C-H stretching and Ultrasound treatment reduced the intensity of this band. A sharp peak was observed at 1591.25 cm^{-1} represents C=O stretching. Another broad peak at 1048.86 cm^{-1} shows elongation of C-O group. FTIR spectra of elephant foot yam caused structural changes at a molecular level. In previously studied, conformational changes in the yam was observed in the spectra associated with the band range of 1000-1500 $cm^{-1.}$

Sample 242



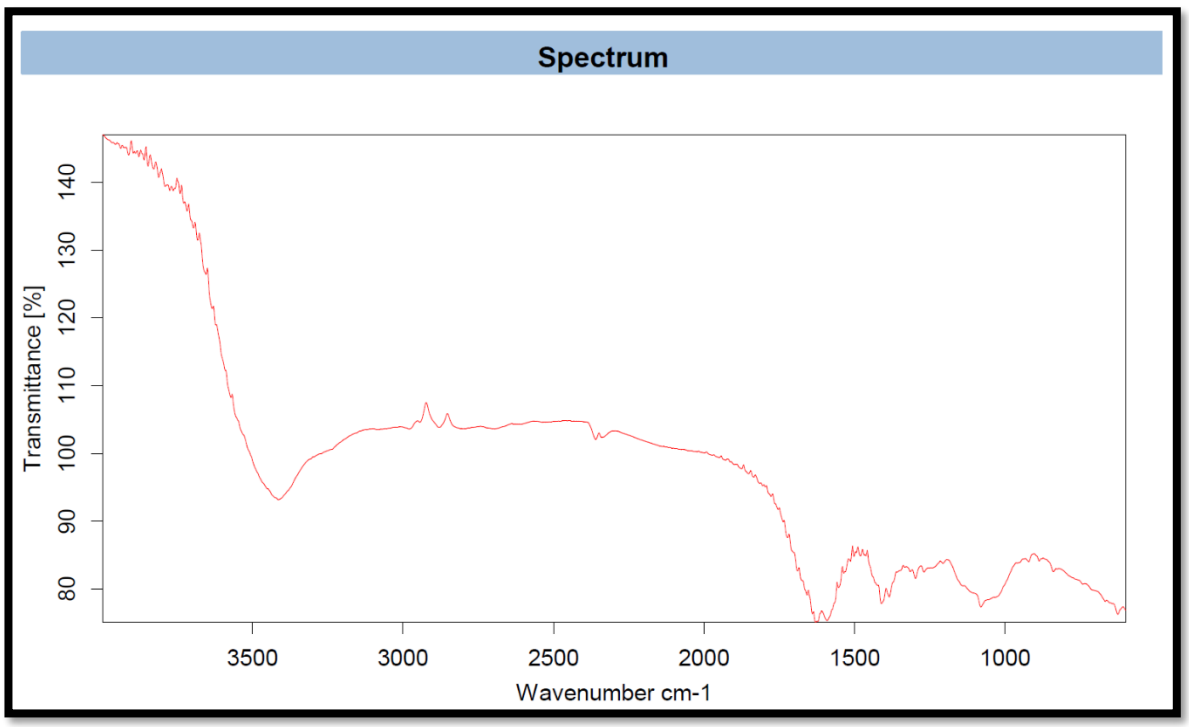
- A broad peak at wavenumber 3294.6 cm^{-1} shows C-H bonding with cumulative O-H stretching.
- A small peak at wavenumber 1642.17 cm^{-1} represents better C=O bonding.
- A small peak at wavenumber 1403.49 cm^{-1} was noticeable that shows weak C=O stretching.
- A sharp peak was observed at wavenumber 1052.08 cm^{-1} C-O bonding.

FT-IR spectra presented bands associated to stretching, flexion and deformation corresponding to the main functional groups characteristic of the elephant foot yam. The spectra of four yam

samples were shown, indicating that ultrasound treatment caused minute changes in the type of chemical groups, but no newly produced chemical groups were found in yam.

For treated sample with Ultrasound technique for 20 minutes at 40℃ with 1:20 solid solvent ratio, FTIR spectra have revealed that positions of the characteristic absorption peaks have changed after the ultrasound treatment, and minor reductions was noticed in their intensities. Similar findings were reported by Monroy et al., 2018 during the ultrasound treatment of elephant foot yam starch analysis. As shown in figure the broadband appeared at around 3294.6 cm^{-1} resembles the C-H bonding with cumulative O-H stretching and Ultrasound treatment increased the intensity of this band. A small peak was observed at wavenumber 1403.49 cm^{-1} was noticeable represents weak C=O stretching. Another sharp peak was observed at wavenumber at 1052.08 cm^{-1} shows elongation of C-O group. FTIR spectra of elephant foot yam caused structural changes at a molecular level. In previously studied, conformational changes in the yam was observed in the spectra associated with the band range of 1000-1500 $cm^{-1.}$

Sample 222



- A broad peak at wavenumber 3414.25 cm^{-1} shows strong O-H bonding with cumulative C-H stretching.
- A small peak at wavenumber 1629.53 cm^{-1} represents better C=O bonding.
- A small peak at wavenumber 1080 cm^{-1} was noticeable that shows presence of weak C-O bonds in the sample.

FT-IR spectra presented bands associated to stretching, flexion and deformation corresponding to the main functional groups characteristic of the elephant foot yam. The spectra of four yam samples were shown, indicating that ultrasound treatment caused minute changes in the type of chemical groups, but no newly produced chemical groups were found in yam.

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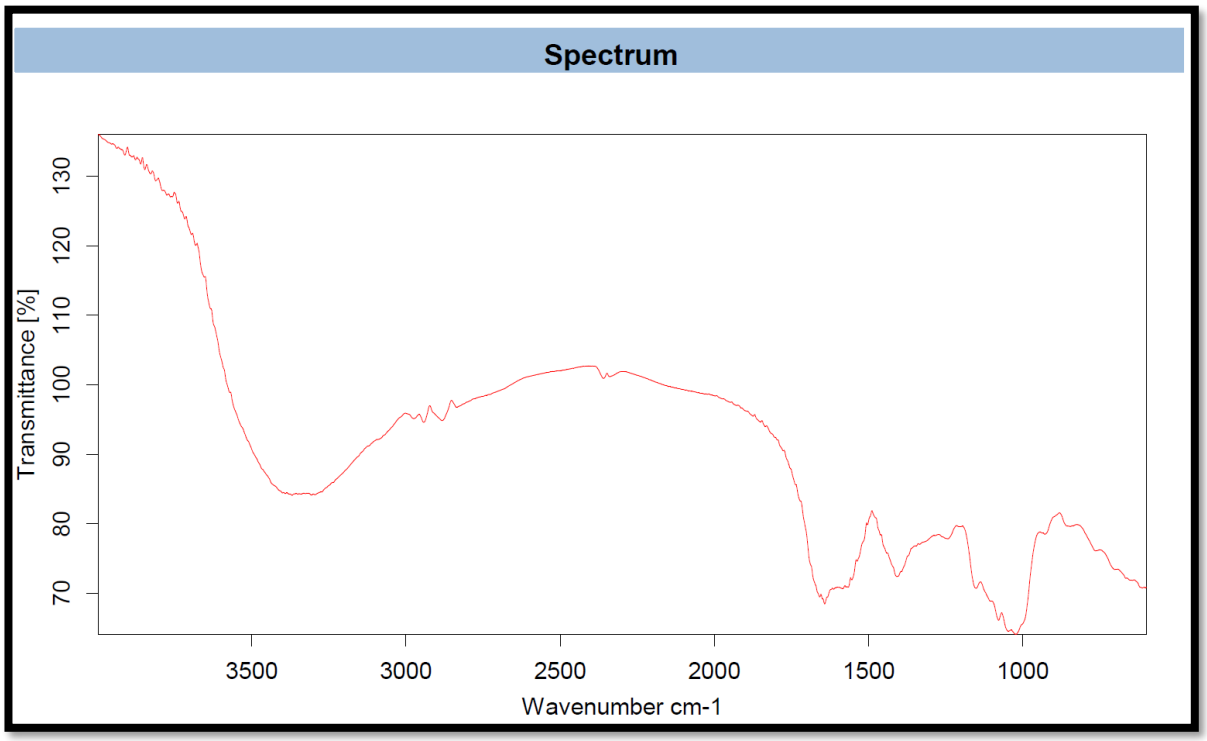

- A broad peak at wavenumber 3369.1 cm^{-1} shows strong presence of O-H bonding.
- A small peak at wavenumber 1642.21 cm^{-1} represents better C=O bonding.
- A small sharp peak at wavenumber 1408.11 cm^{-1} was present that shows same C=O stretching like previous sample.
- A small peak was observed at wavenumber 1022.36 cm^{-1} C-O stretching.

Sample 231

FT-IR spectra presented bands associated to stretching, flexion and deformation corresponding to the main functional groups characteristic of the elephant foot yam. The spectra of four yam

samples were shown, indicating that ultrasound treatment caused minute changes in the type of chemical groups, but no newly produced chemical groups were found in yam.

For treated sample with Ultrasound technique for 20 minutes at 30℃ with 1:10 solid solvent ratio, FTIR spectra have revealed that positions of the characteristic absorption peaks have changed after the ultrasound treatment, and minor reductions was noticed in their intensities. Similar findings were reported by Monroy et al., 2018 during the ultrasound treatment of elephant foot yam starch analysis. As shown in figure the broadband broad peak appeared at around 3369.1 cm^{-1} resembles the strong presence of O-H bonding and Ultrasound treatment reduced the intensity of this band. A small sharp peak was observed at 1642.21 cm^{-1} represents C=O stretching. Another small sharp peak at wavenumber 1408.11 cm^{-1} shows same C=O stretching like the previous sample. Another small peak was observed at wavenumber 1022.36 cm^{-1} shows C-O stretching. FTIR spectra of elephant foot yam caused structural changes at a molecular level. In previously studied, conformational changes in the yam was observed in the spectra associated with the band range of 1000-1500 $cm^{-1.}$

CHAPTER 5

SUMMARY & CONCLUSION

This chapter deals with summary and conclusions drawn on the basis of experimental work done for the project work entitled " Ultrasound assisted reduction in oxalate of elephant foot yam– an efficient and novel approach for mankind". As an alternative to traditional procedures, several unique techniques have been developed that offer advantages in terms of extraction time, solvent usage, extraction yields, and reproducibility. Because oxalate and acridity are common problems in Amorphallus paeoniifolius, an effort has been made to reduce calcium oxalate from yam using boiling, NaCl treatment and Ultrasound. While traditional methods such as boiling and Nacl treatment are effective for oxalate reduction, they also cause phytochemical loss, making them ineffective. Ultrasound, a revolutionary food technology technique, is successful in preserving numerous bioactive components while significantly lowering oxalate levels. The diversity of elephant foot yam in India must be investigated in terms of its potential for food and health benefits. The antioxidant activity of the yam can help to reduce the detrimental effects of free radical reactions, which is good for the consumers. The existence of promising levels of phenolic compounds as well as a reasonable total flavonoid content percentage back up the prior assertion. The elephant foot yam is high in caloric content and a good source of nutrients. Thus, the elephant foot yam can be used as feed or food, but it must be treated before eating due to its antinutritional value. When compared to other physical and chemical food processing activities, ultrasonication is regarded a clean technique with a high potential for customer acceptability. It is a physical alteration procedure that may or may not result in significant chemical changes. If its potential for the development of new goods is fully realised, ultrasound might have a significant presence in the food industry. While this technology has immense potential, it must be properly developed and scaled up for each and every culinary application.

Based on test results, following conclusions were drawn:

Ultrasonication, a novel technique has its immense and positive effects in the reduction of oxalate in Elephant foot yam as well as having a stable nature on keeping the bioactive compounds in yam , because of this treatment various phytochemicals like phenol, flavonoid, antioxidants show increment in the yam extraction. Ultrasonication is a very promising technique which helps in the extraction of various phytochemicals but in this research work our main focus is on the reduction of oxalate content in yam which acts as an antinutritional component thus not acceptable in the food world for consumption. Oxalate mainly causes acridity and kidney stones which are very harmful for humans. To reduce this oxalate content many conventional methods like boiling, NaCl treatment were given but reduction of oxalate is not much as well as many bioactive compounds also got denatured thus, Ultrasonication assisted extraction is done for the reduction of oxalate and phytochemicals analysis by increasing the phytochemicals content.

Various analysis were carried out during the experiment these include Oxalate content, Extract yield, Total phenol content, Total flavonoid content, Total tannin content and Antioxidant content.

1. Extract yield

   The yield was found more after the ultrasonication treatment in elephant foot yam. The highest yield was found with the sample having time 20 minutes temperature 30℃ and 1:30 solid-solvent ratio whereas the lowest yield was found in the sample having time 10 minutes temperature 30℃ and solid-solvent ratio is 1:10.

   Thus, yield obtained was depend on the amount of solid solvent ratio used as well as the ultrasonication treatment given.

2. Oxalate content-

   The reduction of oxalate was our main parameter for the whole work and it was successfully accomplished with the ultrasonication treatment as it works on the principle of acoustic cavitation in which the molecules ruptures as ultrasonic waves gets into them and the whole matter oozes out which maintain uniformity in the sample. The best reduction of oxalate was found in the sample having time 20 minutes temperature 30℃ and 1:30 solid-solvent ratio whereas the lowest oxalate reduction was found in the sample having time 10 minutes temperature 30℃ and solid-solvent ratio is 1:10.

3. Total phenol content-

   Phenolic compounds are the important analysis parameter as it helps in maintaining the nutritional background of a substance. In elephant foot yam the highest phenolic compounds were found with the sample having time 20 minutes temperature 30℃ and 1:30 solid-solvent ratio whereas the lowest phenolic content was found in the sample having time 10 minutes temperature 30℃ and solid-solvent ratio is 1:10.

4. Total flavonoid content-

   Flavonoids are found in abundance in the elephant foot yam, the same properties were seen after the ultrasonication treatment which conclude that the flavonoids contents gets increased after the treatment and also ultrasonication keeps these compounds stable. In elephant foot yam the highest flavonoid compounds were found with the sample having time 20 minutes temperature 30℃ and 1:30 solid-solvent ratio whereas the lowest flavonoid content was found in the sample having time 10 minutes temperature 30℃ and solid-solvent ratio is 1:10.

5. Total tannin content-

   Tannins are the antinutritional factor mainly found in elephant foot yam, they tend to cause acridity and other effects which are not acceptable in the food world thus as the reduction of oxalate was targeted at the same time tannins were also reduced to a very safe level that will not cause any harmful effects and this come possible because of the ultrasonication. In elephant foot yam the highest tannins compounds were found with the sample having time 20 minutes temperature 30℃ and 1:30 solid-solvent ratio whereas the lowest tannins content was found in the sample having time 10 minutes temperature 30℃ and solid-solvent ratio is 1:10.

6. Antioxidant content-

Antioxidants plays a major role in the nutritional background of any substance it is also regarded as the backbone of bioactive components, because of which various diseases, allergies can be treated. After the ultrasonication treatment in elephant foot yam the antioxidants level boost up to a new height which makes the yam very beneficial. Because of this parameter incorporation of yam can be used in the food world as a nutraceutical. In elephant foot yam the highest antioxidants were found with the sample having time 20 minutes temperature 30℃ and 1:30 solid-solvent ratio whereas the lowest antioxidants was found in the sample having time 10 minutes temperature 30℃ and solid-solvent ratio is 1:10.

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