JIMMA UNIVERSITY

JIMMA INSTITUTE OF TECHNOLOGY

SCHOOL OF CHEMICAL ENGINEERING

EXTRACTION AND OPTIMIZATION OF ANCHOTE (*COCCINIA ABYSSINICA*) TUBER STARCH AND ITS APPLICATION AS STABILIZER FOR YOGURT PRODUCTION

BY

GUDETA SHUMA

OCTOBER 2019

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN PROCESS ENGINEERING

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DECLARATION

I declare that the thesis hereby submitted by me for the Masters of Science Degree in Chemical Engineering (Process Engineering stream) at Jimma Institute of Technology, Jimma University is my own independent work and has not previously been submitted by me or another person to another University/Faculty.

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ABSTRACT

Anchote is an indigenous tuber crop found in the Western and South Western part of Ethiopia. It belongs to the family Cucurbitaceae and the Afan Oromo name for Coccinia abyssinica. Particularly, Anchote is known in Kellem Wollega, Western Wollega, Eastern Wollega, Illu Abba Boora, and Jimma. Starch can be extracted from anchote tuber by chemical method. This study investigates the production of starch from anchote tuber which is underutilized crop and used as indigenous food. The Production of anchote starch from Anchote tuber was done by different chemical solutions such as sodium chloride, sodium metabisulphite, ammonia with different concentrations (0.5, 1 and 1.5%) and control with water. The starch was isolated by cleaning, peeling, cutting into small pieces, wet grinding, filtration, sedimentation, decantation of the supernatant, drying, and the starch yield was recovered. The maximum yield of the starch produced was 20.86% fresh weight basis with a 1.5% sodium chloride solution. The optimum yield of starch was characterized by physicochemical and functional properties. Bulk density, true density, tapped density, Carr's index, and Hausner ratio of anchote starch were 0.46,1.5,0.65, 23.97, and 1.32 respectively. The X-ray Diffraction result of the starch had the strongest sharp peak diffraction pattern at 17.12° of 2θ angle which indicates the B-type starch. The moisture, ash, fat, fiber, protein, total carbohydrate, amylose content, and starch purity were 12.14%, 0.30%, 0.0%4, 1.64%, 1.66%, 83.82%, 17.42%, and 95.6% respectively. The pH of the starch was 7.32 which was almost neutral. The functional properties of anchote starch such as water absorption capacity and oil absorption capacity were 0.17ml/g for both. swelling power and solubility index of anchote starch with increasing temperatures (30, 50, 70 and 90℃) were respectively (1.90, 2.53, 8.10, and 11.65) and (0.31, 0.9, 4.8, and 15.43%). The extracted starch was used as a stabilizer in yogurt manufacturing with different proportions (0.5, 1 and 1.5%). The yogurt produced with 1.5% anchote starch shows good results of pH and syneresis. The titratable acidity and sensory result showed the best result for yogurt prepared with 1% and 1.5% anchote starch.

Keywords: Anchote, Starch, Stabilizer, Yogurt, Optimization

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ABBREVIATIONS

AOAC – Association of Official Analytical Chemist

CI – Carr's Index

CMC – carboxyl methyl cellulose

dwb – dry weight basis

FAO – Food and Agriculture Organization

fwb – fresh weight basis

HR – Hausner Ratio

M.C – Moisture Content

OAC – Oil Absorption Capacity

SI – Solubility Index

SNNP – Southern Nation Nationalities and Peoples

WAC – Water Absorption Capacity

XRD – X-Ray Diffraction

CHAPTER 1

INTRODUCTION

1.1 Background

Starch is complex carbohydrate (polysaccharides) which is only extracted from a plant. In our daily consumption starch is incorporated as the main ingredient of the food to gives energy to our body. It also used as a food additive to improve nutritional, functional, and aesthetic value. Starch is an important ingredient in food industries because of its ease of production in high amounts, ease of availability, and low cost (Jobling, 2004; Ammar, 2018).

Food industries such as the confectionery processing industry, bakery industry (bread, biscuits, snacks, etc.), beverage industry (concentrated juice, soft drinks), dairy industry (in yogurt manufacturing) and other complemented food processing industry are some examples. Non-food manufacturing industries such as pharmaceuticals, plastic, textiles, can also use starches as their raw material (Jobling, 2004).

Starch is produced from different crops such as cereals, roots, tubers, and legumes. Cereals contain (40 to 90%) of starch followed by tubers (65 to 85%). Root crops also contain (30 to 70%) starch. Legumes contain a lower amount of starch (25 to 50%)(Santana and Meireles, 2014). The extraction method depends on the type and final product applications. Wheat, maize, rice, and sorghum are vital sources of starch. Tuber crop is the second source of starch. Potato sweet potato, cassava, and yam are an important starch source tuber crops dominantly used as a human being's food in Africa (Chandrasekara, 2016).

Some tubers such as potatoes, cassava, and sweet potato were exploited in food industries for their starch products. But anchote is the underutilized tuber crop that was not promoted for starch production industrial use. Development of an appropriate processing method for anchote starch extraction can increase the use of anchote starch with desirable properties(Jangchud *et al.*, 2003). In general, starch is extracted from tubers by wet method through rasping, sieving and decantation. Functional, chemical, and pasting characteristics of starch extracted from sweet potato by different chemical solution such as sodium metabisulphite, sodium chloride and distilled water was studied by(A.Surendra and Parimalavalli, 2014).

Anchote is an indigenous tuber crop and cultural food product in the western part of Ethiopia and is Afan Oromo's name for *Coccinia abyssinica* pronounced as "Anchootee". It belongs to the family *Cucurbitaceae* particularly known in Kellem Wollega, western Wollega, eastern Wollega, Illu Abba Boora, and Jimma. It is a drought resistance tuber crop that is stored underground pit, available when another crop is not adequate. Therefore, it can involve in food security during environmental change(Yosef and Tileye, 2013). It is used as cultural food in these areas especially during ceremonies and holidays such as New Year celebration, ‘meskel’ festival, and wedding. It is also consumed as fasting food when animal product is not allowed. The producer and local society also use Anchote as herbal medicine. Some recent studies report that Anchote (*Coccinia abyssinica*) contains good amounts of minerals such as calcium, iron, phosphorus, and zinc(Habtamu, 2014). Anchote is a good source of edible starch next to potato(Habtamu and Kelbessa, 1997). It is an important source of starch and protein among the tuber crops. The high content of calcium is evident for the healing ability of Anchote for bone fracture and strengthens the lactating women. Adding of anchote flour to wheat flour in the production of bread can improve the nutritional quality of the bread. It can also represent the dependence of wheat flour which imported and will save the foreign currency for the country. Anchote starch and flour address the nutritional shortage challenges in wheat breads such as dietary fiber, protein, and minerals. (Demelash, 2016). Although, it is used locally for different food purposes without mixed or mixed with other food materials such as spices. For examples anchote paste, anchote soup, Anchote slice, anchote juice, etc. Anchote contains a high amount of dietary fibres which has benefit of physiological effects to the human body, as they stimulate and accelerate intestinal contraction and transit(Girma and Dereje, 2015).

In dairy industries, starches are used as stabilizer and thickener. Starches can be used in yogurt production as a stabilizer to improve the viscosity and sensory properties by decreasing the whey separation during storage and as well as enhance the ratio of total solids. For example, potato starch, corn starch, and cassava starch are the most known(Ammar, 2018; Malik *et al*, 2012). There are also some synthetic or inorganic stabilizers commonly known such as Carboxyl Methyl Cellulose (CMC) and gelatin. Gelatin is a synthetic stabilizer produced by partial hydrolysis of collagen from the connective tissue of animals(Alakali *et al*, 2007).

However, the demand for gelatin is decreasing in recent years because of the increasing concern of the organic stabilizer. Also, synthetic stabilizer is expensive and prohibited for some religious cases such as an increased demand for Halal(Sameen *et al*, 2016).

Anchote is an underutilized tuber crop which is used only for limited purpose. The extraction of starch from anchote is one of an interesting area to increase the demand of the crop.

Therefore, research towards Extraction of Anchote (Coccinia Abyssinica) starch and Optimization of the yield by studying the effect of extraction method on the yield, physicochemical and functional properties of the starch, and also the application of starch in dairy processing industry for yogurt production was studied.

1.2 Statement of Problem

Anchote is an underutilized tuber crop that is used for only local consumption. It is a valuable crop consumed only as an indigenous food by local society. Anchote contains good nutritional content such as dietary fiber and protein. Anchote also contains edible minerals(Habtamu, 2014). This crop is not promoted well because of the standard product and process was not developed. Some recent studies report only the nutritional and anti-nutritional properties of Anchote. But standard product and method to extract starch was not developed. Consequently, was not well studied and utilized. With an increasing demand for starches in food industries, it is important to introduce anchote as a new alternative source of starch. The utilization of anchote for different food applications has good potential advantages for a number of reasons. Thus, utilization of anchote for different product development, can motivate the producer and gives raw material option for food industries. It could increase opportunities to expand the utilization of anchote. Anchote starch can be used as a stabilizer because it is organic and can represent synthetic stabilizers such as gelatin and Carboxyl Methyl Cellulose (CMC).

This study presented to determine the optimum method to extract optimum yield of starch from anchote (*Coccinia abyssinica),* characterization of starch and to evaluate its application as stabilizer in homemade yogurt production. This enables us the utilization of anchote for variety of food application and promote the crop.

1.3 Objective

1.3.1 General Objective

The general objective of this research is to extract and optimize Anchote (*Coccinia Abyssinica*) starch and, study its application as a stabilizing agent in yogurt production.

1.3.2 Specific Objectives

- ✓ To extract the starch and identify the best method for the optimum yield of starch by using design expert software.
- ✓ To determine the physicochemical and functional properties of Anchote starch
- ✓ To determine its application in yogurt processing as stabilizer and evaluate quality of yogurt (physicochemical and sensory analysis)

1.4 Scope of the Study

In this research, extraction of starch from Anchote (*Coccinia Abyssinica*), effect of extraction method on the yield, characterization of starch and application of Anchote starch in yogurt processing as stabilizer were studied and the produced yogurt was characterized.

1.5 Significance of the study

The significance of this study is to promote Anchote starch for different applications such as food processing industries (dairy industry). Also, it can play an important role in the country.

- ✓ Provide information about method of starch isolation from anchote tuber for researcher and processors
- ✓ Resource utilization and good option for food processing industries as raw material and food additive
- ✓ Representation of synthetic food additive (stabilizer)
- ✓ Provide job opportunity and income for local communities who are involved in agricultural practices of the crops and increase incomes

CHAPTER 2

LITERATURE REVIEW

2.1 History of Anchote

Anchote is a drought-resistant tuber crop indigenous to Ethiopia, originated and cultivated particularly in the Western Highlands region of Ethiopia such as Kellem Wollega, Eastern Wollega, Western Wollega, Jimma and Illu Abba Boora, and South-western parts of Ethiopia (SNNP Region). It is the tuber crop that belongs to the family *Cucurbitaceae* and pronounced by Afan Oromo as "Ancootee". The scientific name of anchote is *Coccinia abyssinica*. Anchote is an important tuber cultivated on about 300 hectares of the land in the Western part of Oromia. It mainly produced for local consumption as food during some celebration, holidays, and herbal medicine(Mengesha *et al.*, 2012; Yosef and Tileye, 2013). There are about ten species of Coccinia which are cultivated in Ethiopia. From these species, only anchote (*Coccinia abyssinica*) is cultivated and used for human food. Anchote is found both cultivated by the society as a regular root crop and also as wild. Anchote yields the total product of 150-180 quintals/hectare which is within the vary of the total product of potato, and sweet potato(IAR, 1986). One of the desired qualities of Anchote as a tuber crop is its good keeping quality since it can be stored in an underground pit and recovered when needed for processing and consumption, and providing food security in times of other crop failures or when drought occurs.

2.2 Taxonomy and Propagation of Anchote

Anchote (*Coccinia abyssinica*), has shot with simple stems and leaves. Fruits are red yellow at maturity and have an oval to cylindrical shape with 8.83cm length containing an average of 153 seeds. The stems are typically sympodial in growth(Masarat, 2018). Anchote has spherical to cone-shaped tubers which may vary with age, soil and Anchote type. Sometimes irregular shaped tubers may result because of poor land preparation and the presence of mechanical barriers, which will result in the development of irregular shaped tubers. The top portion of the tuber has the largest diameter with a rounded square in transverse section. In general, the plant has a runner growth habit with an irregular vine, which needs support for successful fruit development that provides sound seeds for future planting(Abera *et al.*, 1995).

Anchote is both propagated vegetative or from seeds. Therefore, it is an important crop during drought to get the seed of the plant(Mengesha *et al.*, 2012).

2.3 Economic Importance as Food, Herbal Medicine, and Income

Cereal crops are commonly used as a food source for the majority of the Ethiopian society. Tuber and root crops are not adopted as common food. But, their vital contribution to food security, financial income, and provision of resource base conservation and food energy is very greeted. Anchote is mainly used as a cultural food crop for the local communities found in the western part of Ethiopia. It is also recognized as an economic and social crop with an annual production yield of 25,000 tones(Masarat, 2018; Mengesha *et al*., 2012). Anchote is a valuable food supply and in step with native producers. It is herbal medicine to bits of help in fast mending of displaced joints and broken/fractured bones because of its high calcium and protein content than other root and tuber crops. It also believed that strengthen and healthier the lactating women(Habtamu, 2014).

Anchote is a traditional food in Oromo culture around Wollega. Among all root and tubers grown in the area, Anchote takes a special place in the customs of the Oromo society especially in Kellem Wollega, western Wollega, Eastern Wollega, and Horo Guduru Wollega. Anchote is served as a prestige dish in Oromo Culture during special ceremonies and on holidays. A delicious Anchote dish is prepared with clarified butter (Traditional ghee) from the tuber. It is also prepared in the form of stew locally called Anchote '*Ittoo*' in Afan Oromo on joyful events. Solely the '*Ittoo*' is prepared from sliced Anchote with sufficient locally made butter, buttermilk and cheese by seasoning with different traditional spices and then served with '*Injera*', around leavened bread made from tef (*Eragrostis tef*) (Zucc) Trotter which is called '*Chumbo*' in Afan Oromo(Habtamu and Kelbessa, 1997; Jale and Desalegn, 2016)

2.4 Traditional Processing of Anchote

Anchote tubers are, most of the time, consumed boiled. Boiling decreases both nutritional and anti-nutritional contents of foods by leaching and/or decomposition. But, boiling makes most foods palatable, increases digestibility and bioavailability of some nutrients, inactivates some anti-nutritional and enzyme inhibitors, and increases consumer preferences. Except for moisture, crude fiber, and iron, all contents were seen reduced after boiling. But, boiling after peeling caused more reduction in both nutritional and anti-nutritional contents (Table 2.1). Reduction in anti-nutritional such as Phytate, oxalate, tannin, and cyanide is desirable. Loss of nutrition is disadvantageous. So, boiling before peeling is recommended for anchote as this minimizes the nutritional losses and unnecessary moisture gain(Fekadu *et al.*, 2013).

2.5 Nutrient Content of Anchote

Anchote is also a stable food source with multiple medicinal qualities. It is an important food that contains high amounts of nutrients such as dietary fiber, protein, and minerals such as calcium, iron, zinc, and potassium which is rare in other roots and tuber crops(Girma and Dereje, 2015; Jale and Desalegn, 2016). The society believes and uses Anchote as herbal medicine to heal and fast repairing of broken/ fractured bones and displaced joints and also makes lactating mothers healthier and stronger without knowing the science behind calcium content of the Anchote. Also, anchote contains an active substance known as saponin. Because of this active substance, anchote juice is used for resistance diseases such as Tuberculosis, Gonorrhoea, and Tumour Cancer(Melkamu *et al.*, 2018). Although, it is used locally for different food purposes without mixed or mixed with other food materials. For examples anchote paste, anchote soup, Anchote slice, anchote juice, etc. Anchote also contains dietary fibres which give advantage to the human body, as they stimulate and accelerate the digestion of food in intestinal(Girma and Dereje, 2015b).

Table 2.1 Nutritional composition of raw Anchote tubers (based on wet weight basis)

Nutrients (g/100g)	Content (%)
Moisture	74.93
Crude protein	3.25
Total ash	2.19
Crude fiber	2.58
Crude fat	0.19
Utilizable carbohydrate	16.86
Gross energy(Kcal/100g)	82.12
Minerals (mg/100g)	
Ca	119.50
Fe	5.49
Mg	79.73
Zn	2.23
P	34.61

Source: Habtamu Fekadu, (2013): Habtamu and Kelbessa, (1997)

2.6 Sources of Starch

Starch is a complex carbohydrate naturally occurring as a polymer of α-D glucose. It is the major food ingredient for human beings and stored in leaves of green plants as the main energy source of higher plants. Starch is also stored in seeds, fruits, stems, roots and tubers of most plants. Starch granules are formed in amyloplasts of higher plants. They are also formed in chloroplasts where they serve as a temporary store of energy and carbon(Jayakody and Hoover, 2008). Commercial starches found in the market are mainly produced from different cereals, for example, wheat, maize, and rice. Some tuber crops such as potato, cassava, and sweet potato are also good sources of starches for different food purposes. Among these sources, corn starch accounts for about 80% of the world market. Also, starches are used as food additives in food processing industries for numerous applications such as stabilizer, thickener, gelling agent, and water retention(Zhu and Wang, 2013).

2.7 Applications of Starch in Food Industry

Food manufacturing industries frequently depend starches extracted from crops that are used as traditional sources of food in high demand with significant commercial implications. For example, flours and starches are the backbone of the baking industry such as breads, cakes, and snacks food processing industries. These products are our daily foods and cannot absent from our dish. Starch is an important ingredient in the formulations of the baking process accountable for the structure and the final product properties. It is also used as additives in small amounts to improve the sensory properties of many factory-made foods(Demelash, 2016). Some special products of starches such as maltose or glucose syrup which is produced by hydrolysis of starches and iso-glucose or fructose produced by isomerization are used in some food industries who produce products such as chocolates, sweets candy, cakes, pastries, desserts, jellies, sweetness or hygroscopicity (ability to hold water), and anti-crystallizing (Ascheri *et al.*, 2014).

Table 2.2 Industrial application of starch

Food types	**Use**
Frozen foods e.g yogurt	Resist gel breakdown during processing and storage Stabilizer due to high water-holding capacity.
Dressings, soups, and sauces e.g jellies and jam	Gelling agents in producing jellies (thickeners). Opacity agents to control consistency and texture.
Cereals and snacks	Oil retention agents to create crisp, hamper penetration of cooking oils leading to low fat-intake.
Confectionery	Manufacture of gums, pastes, and other types of sweets.

Source: FAO (1998)

Food additives such as stabilizers, thickening agents, and gelling agents are produced from starches and mainly used in dairy and juice processing industries(Ammar, 2018). Pasta, macaroni, noodles, instant foods, and fried foods are also produced from starches(Santana and Meireles, 2014).

Anchote is a good source of starch among the tuber crops next to potato and can be used in full or partial substitute with another starch source to produce different food products. For example, bread production with the addition of anchote flour in partial wheat flour can be used to improve some nutritional value of bread such as dietary fiber, protein, and minerals. It also reduces the dependence on wheat which is sometimes imported and saves the foreign currency for the country(Demelash, 2016). Not only in the bakery industry, starch is also used in the dairy industry as a stabilizer/thickener and has a capability to improve viscosity and sensory properties, and inhibit or decrease whey separation throughout storage, likewise as enhance the quantitative relation of total solids in factory-made dairy products(Ammar, 2018).

2.8 Classification of starch

Depending on its nutritional purpose starch can be divided into three categories: rapidly digestible starch (digested easily by the digestive system of the human body), slowly digestible starch (needs slight modification) and resistant starch (cannot digestible in the small intestine). Starch can be also divided into two groups (native starch and modified starch) based on the extraction process. Native starch is the starch isolated its sources such as cereal and tuber without any modification to the starch chain. Starches are extracted from the starch source and modified by an enzymatic, physical, and chemical process for more specialized uses (Ascheri *et al*., 2014; Lopez-Diago *et al*., 2018).

2.9 Starch Extraction Methods

Starch is a complex carbohydrate source that has an enormous economic and nutritional value. Different plant exhibits a different amount of starch yield and shows different physicochemical properties. The food industry and food processor need to find new starch sources to fulfil the need of their customers. For example, potato, sweet potato (Ipomoea batatas) and yam, cassava, and anchote are tuber crops used as potential as a source of starch(A.Surendra and Parimalavalli, 2014; Ascheri *et al*., 2014). Anchote flour is also used for bakery products as a source of starch such as bread biscuits and other snack food because it has a starch(Demelash, 2016). But it is not known that the industrial use of Anchote as a source of starch needs important consideration. Starch is isolated from roots and tubers by different methods. In general, we can divide the starch extraction method into two main groups; dry method and wet method. Among these methods, the wet method is the most effective one for the extraction of roots and tuber starch. For the wet method, different solutions are used(Ascheri *et al*., 2014).

The general extraction of starch from tuber crop is all most the same since they have a similar structure. For example, potato, sweet potato, cassava, yam is all root crop and they contain high moisture content. The tuber was selected and cleaned from dirty material, cut into pieces, chopped into slurry and homogenized with water. The homogenized mixture is filtered to remove impurities and non-starch components from the starch milk (A.Surendra and Parimalavalli, 2014). The filtered starch is separated by sedimentation as the starch settled at the bottom and the low-density materials suspended at the top. Then, the supernatant is discarded and the starch surface is washed carefully by distilled water to eliminate foreign materials. The surface of the starch is washed with distilled water before drying (Alves *et al.*, 1999). There are different procedures and methods to isolate starches from different sources because of their differences in structure and composition. Also, extraction media or chemicals can affect the starch yield and characteristics. Starch can be isolated from the tuber by blending the chopped tuber and water with a blending ratio of 1:10 to smooth slurry. Then adding of the different chemical solutions with different concentration, for example, Sodium metabisulphite or Sodium chloride or distilled water and mixing during slurrying. The homogenized slurry is filtered by sieve or cheesecloth to remove non-starch materials such as fiber and other foreign materials. After that sedimentation of starch is done by leaving the slurry from 4 hours to 12 hours or by centrifugation. The starch is dried to remove the moisture content which affects the shelf life and facilitates the microbial spoilage(A.Surendra and Parimalavalli, 2014; Ammar, 2018).

2.10 Functional Properties of Starch

The functional properties of starch are very important in ingredient formulation and new product development. Application of starch in food industries mainly depends on its functional properties such as swelling power, water absorption capacity, water solubility index, oil absorption capacity, viscosity, gelatinization, retrogradation, pasting properties, and freeze-thaw stability. Starches extracted from different sources show different functional properties(Ascheri *et al*., 2014; FAO, 2000). Amylose and phosphorous content can affect the functional properties of starches(Mohammadkhani *et al*.,1999). The influence of both amylose and phosphorous content affects the pasting and gelatinization properties of starches. These two parameters determine the functional characteristics of starch such as swelling power, water, and oil absorption capacity(Demelash, 2016).

Water absorption capacity (WAC) is an important functional characteristic of starches that measures the ability of food materials to absorb water. It is typically attributed to the protein content of the starch. WAC is an important property of starch used in food formulations such as dough formulation and handling in bakery products to improve dough consistency(Amani *et al*., 2004).

Oil absorption capacity of starch is also an important characteristic which predicts the capacity to absorb the oil during product processing(Sylvia and Maria, 2015).

Swelling power and solubility indices of the starch provide the evidence of interactions between the water molecules and the starch chains in the crystalline and amorphous regions The swelling characteristics of starch mainly depends on the presence of non-carbohydrate components such as lipids and protein, amylose and amylopectin structure, and amylose content. Non-carbohydrate components especially lipids inhibit the swelling power of starch. Starches with high solubility index have higher amylose leaching from it(Hoover, 2001).

Swelling power is an important functional property of starch which indicates the evidence of non-covalent bonding among starch molecules. The solubility index of starch granule depends on a number of factors such as swelling power, sucrose content, and inter associative forces between the starch molecules. Swelling and solubility of starch occurs when starch is mixed with water and heated resulting in a disruption of crystalline structure due to the breaking of hydrogen bonds (Moorthy, 2002).

Thus, swelling power and solubility indices of starch depends on temperature. As temperature increases the swelling power and solubility of the starch increase. This is due to the weakening of internal associative forces of the molecules that maintain the granular structure. The granule sizes of starch can also affect the swelling power and solubility of starches. Starch with large granule size has higher swelling power and lower solubility (Karim *et al*., 2007; Singh *et al.*, 2010).

2.11 Physicochemical Properties of Starch

2.11.1 Density

Density is an important physical property of starch. Bulk density, true density, and tapped density are among the important starch powder properties. Bulk density indicates the total volume occupied with the known mass of the starch. These properties can determine the bulk characteristics such as powder flow and compressibility. Starches with low bulk density flow poorly. This can affect the formation of a lubricant film during the mixing process. Carr's index and Hausner ratio are important and easy methods to predict the flow characteristics of the starch powders. Carr's index is commonly known as percent compressibility of the starch. According to this method the flow properties of the starch powder can be grouped as excellent (5-15%) Carr's index, good (12-16%) Carr's index, fair (18-21%) Carr's index, and poor (23-28%) Carr's index. Similarly, the Hausner ratio indicates the flow property of the starch granule. Good flow property of starch has the Value of Hausner ratio below 1.20 while the value above 1.50 indicates poor flow properties of starch(Manek *et al*., 2012). Generally, the higher density of starch is an important property that is desirable for the reduction in paste thickness. This property has greater ease of dispensability in infant foods(Amani *et al*., 2004).

2.11.2 Moisture content

Moisture content is the amount of free water bounded in starch powder. The property is an important concern in the food industry. Moisture content beyond the optimum range affects product quality such as shelf life and stability. Starch with higher moisture content has a short shelf life because of its exposure to enzymatic activity, chemical reaction, and microbial activity(M.Tsakama *et al.*, 2011). Based on the drying method the moisture content of the starch ranged from 6-16%. In some cereal starches dried by air-equilibrated dryer varies from 10-12%. Depending on the species of the raw material (some tuber and root crops) starches vary from 14-18%(Tester *et al*., 2004). Higher moisture content of the starch is the cause of subsequent quality deterioration by microbial spoilage and loss in starch quality. The optimum moisture content of starch powder for safe storage is 13% in most of the starch producing countries. Estimation of moisture sorption is needed to establish moisture activity and moisture content relationships for materials. Moisture content is an important property of the starch powder which plays a substantial role in the mechanical properties and powder flow(Moorthy, 2002).

2.11.3 Ash content

Ash content of the starch powder is an evidence of the existence of inorganic elements. It is an important property of starches and flour which is directly related to the raw material, soil type, stage of maturity and method of processing. Ash content is an indication of the starch purity. Starch can be grouped into different grades depending on their purity. The high purity of the starch depends on the absence of non-starch materials such as inorganic elements. Starch with high ash content has lower starch purity. British Pharmacopoeia recommended that the upper limit of the ash content of the starch is 0.6 % w/w(Abegunde *et al.*, 2012; Moorthy, 2002)

2.11.4 Protein content

Protein can also found in starch granules in small quantities or as residue. The quantities of protein can different for starches isolated from different sources and different extraction methods. For example, the protein content of cereal starch ~0.25% whereas, some tuber and root crop (e.g. potato) starch has 0.05%(Jobling, 2004). After starch extraction also there is a small amount of protein left in starch. Anchote tuber contains a high amount of protein content compared to other tuber and root crops such as sweet potato, potato, and cassava(Habtamu, 2014). Determination of protein in starch is very important to know the quality of starch. Higher protein content of starch shows the lower functional quality of starches(Ayalew e*t al.*, 2017).

2.11.5 Fat content

Cereal crops such as wheat, corn, and rice contain higher levels of lipid compared to root and tuber crops such as potato, cassava, and anchote which contains lower levels of lipids. This is due to the oil found in the germ of the cereal crops. Chemical composition of the starch depends on the raw material which is isolated from. Therefore, root and tuber crop starch contain a lower amount of lipids. Starch extracted from cereal crops contain 0.2-0.8% lipid whereas, root and tuber starch contains 0.1-0.2% lipids. The fat content of starch can affect functional properties such as swelling power, solubility, gelatinization, and water absorption capacity (Moorthy, 2002).

2.11.6 Fiber content

The fiber content of starch depends on the botanical origin, age or maturity of the crop, variety, and pore size of the sieve used for removal of the non-starch material. Fiber content of the starch can also an indication of starch purity(Charalampopoulos *et al.,* 2002).

2.11.7 Titratable acidity

Titratable acidity is an indication of the total amount of hydrogen ions available in the starch and expressed as mg lactic acid equivalent/gram of the starch sample. Titratable acidity is different from total acidity. Total acidity is the total amount of organic acids in the starch sample whereas, the titratable acidity of starch sample in the form of solution is an estimation of the solutions total acidity typically measured by reacting the acids present in the starch sample with an alkaline such as sodium hydroxide to the chosen endpoint close to neutrality, as indicated by an acid-sensitive color indicator(Chen *et al.*, 2003).

2.12 Application of Anchote Starch

Starches have many applications in the food and non-food processing industries. In the food industry starch is extensively used as the main ingredient and additives. Stabilizers are among the starch application in the dairy industry to improve the viscosity and enhance the whey separation during storage. They are applied in small amounts and can represent the synthetic stabilizers to improve the total solids and sensory characteristics of yogurt(Ammar, 2018). They can also improve secondary functional properties(Al-Kadamany *et al*., 2003).

2.12.1 Yogurt Preparation

Yogurt is a fermented dairy product produced for a wide variety of human consumption for a long period of time. Yogurt has significant nutritional and health benefits. They are various types of yogurts in the world. Stirred yogurt, set yogurt, plain yogurt, frozen yogurt, liquid yogurt, and sweetened yogurt are some examples. Preparation of yogurt includes the general steps such as standardization of milk component and blending of ingredients, pasteurization, homogenization, cooling, inoculate with starter culture, holding, cooling and packaging (Chandan, 2017).

2.12.2 Characterization of yogurt

Yogurt can be characterized for different physicochemical, functional, microbial and sensory qualities. But stabilizer can affect directly the total solids, syneresis and sensory quality of the yogurt(Ammar, 2018).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Fresh and non-defective tuber of Anchote was bought from the local market in Nekemte city, Ethiopia. The tubers were placed in a plastic bag to avoid moisture loss during storage prior to processing and analysis in the laboratory of the Department of Food Technology and Process Engineering, Wollega University.

3.1.1 Equipments and Chemicals

The major Engineering equipment required in this investigation such as laboratory blender, oven dyer, extractor (soxheletor unit), centrifuge, Muffle furnace, plastic containers, water bath, Analytical balance, Refrigerator, and UV-spectrophotometry were utilized from laboratory of Department of Food Technology and post-harvest management Jimma university and Department of Food Technology and Process Engineering, Wollega University. X-RD was utilized from the Laboratory of Department of Material Science and Engineering Jimma Institute of Technology.

The major chemicals, such as sodium metabisulphate, sodium chloride, and ammonia were analytical grade. Packaging materials such as polyethylene bags, plastic cups, and aluminium foil were purchased from the Jimma city market, Ethiopia.

3.2 Methodology

The aver all activities done in this research were highlighted in the following figure.

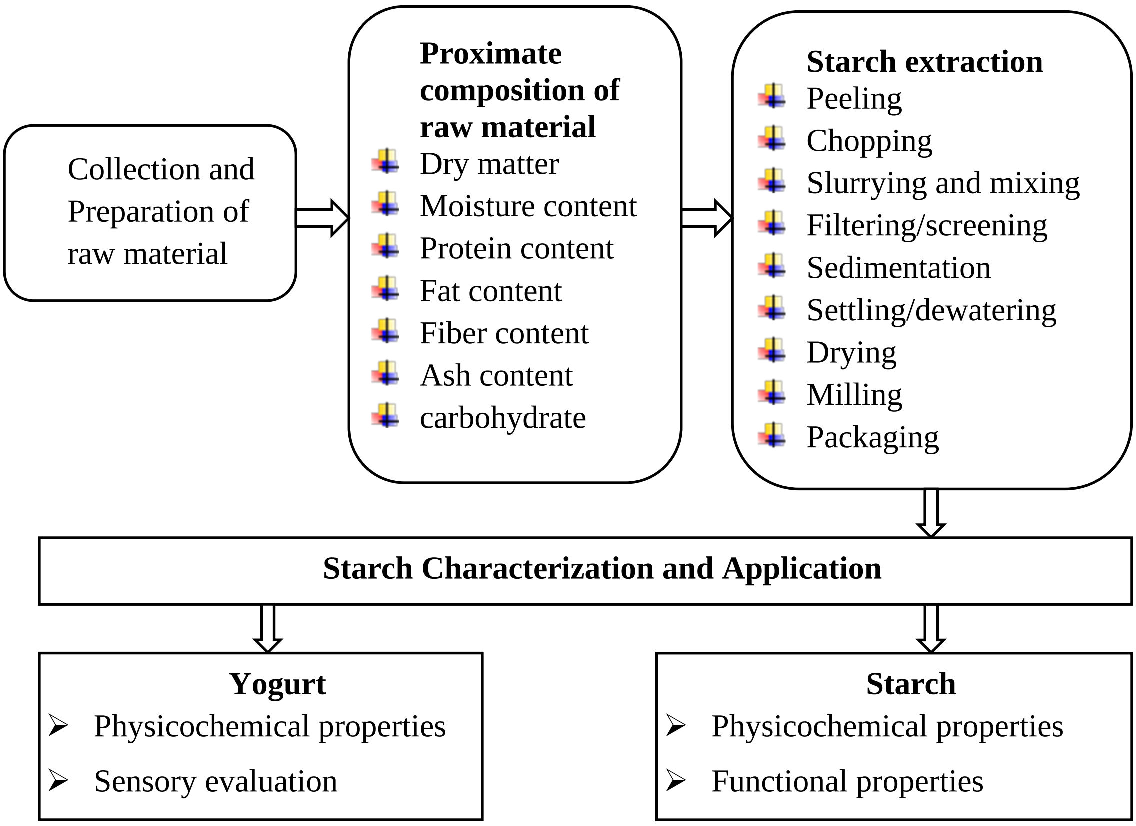

Figure 3.1 Framework of the experiment

3.2.1 Proximate Composition of Anchote tuber

Physical properties of anchote tubers were determined by visual inspection to check the damage and infected tuber. Then the proximate composition of the raw tuber such as dry matter, moisture, ash, protein, fat, fiber and carbohydrate content) of Anchote tuber was determined with the standard method of AOAC (2000).

3.2.1.1 Moisture content

The moisture content of the raw anchote tuber was determined by the standard method of AOAC (2000) with little modification. The moisture content in (%) was calculated by the following formula.

$$M.C\% = \frac{w_3 - w_2}{w_1} * 100 \quad (3.1)$$

Where: w_1 = initial sample weight, W_2 = weight if Petridish, and w_3 = weight of dried sample plus Petridish.

3.2.1.2 Ash content

The ash content of anchote tuber is an indication of the total inorganic element found in the tuber. It was determined by the standard method of AOAC (2000). The ash content of anchote tuber was calculated by the following equation.

$$\text{Ash content (\%)} = \frac{\text{weight of ash} + \text{crucible - weight of crucible}}{\text{initial weigth of sample}} * 100 \qquad (3.2)$$

3.2.1.3 Lipid content

The lipid content of the anchote tuber was determined by the standard method of AOAC (2000). The lipid content (%) was calculated by the following formula:

$$\text{Fat content (\%)} = \frac{\text{weight of flask + extract - weight of flask}}{\text{weight of sample}} * 100 \qquad (3.3)$$

3.2.1.4 Crude fiber

Crude fiber of raw anchote tuber was determined by the standard method of AOAC (2000). The % crude fiber found in anchote tuber was calculated by the following formula.

$$\text{\%Crude fiber} = \frac{\text{(weight of dry insoluble residue - weight of ash)}}{\text{weight of sample}} * 100 \qquad (3.4)$$

3.2.1.5 Protein content determination

The protein content of anchote tuber was determined by the Kjeldahl method by the standard method of AOAC (2000). The percentage of protein content was calculated by the following formula:

$$\text{Nitrogen (\%)} = \frac{14.01 * 0.1N * (TV - BV) * 100}{W * 1000} \qquad (3.5a)$$

$$\text{Protien (\%)} = \%N * 6.25 \qquad (3.5b)$$

Where: W= weight of sample

14.01 = Ammonia’s molecular weight

0.1 N = Titration solution’s normality

TV = Titre value

BV = Blank value

6.25= general nitrogen conversion factor

3.2.1.6 Determination of total carbohydrate

The percentage total carbohydrate content of anchote tuber was determined by the difference according to standard method AOAC (2000) by the following formula:

$$\%\text{Carbohydrate} = \left[1 - \left(\%\text{fat} + \%\text{protein} + \%\text{fiber} + \%\text{moisture} + \%\text{total ash}\right)\right] \text{ x}100 \qquad (3.6)$$

3.2.2 Experimental Design and Data Analysis

The experimental design and data analysis were performed by Design Expert 11.1.2.0 (State-Ease Inc., Minneapolis, MN, USA) software using Response surface methodology (RSM). Two independent factors and three levels were used as effective factors: one categorical factor (type of chemicals) and one numerical factor (concentration). Both have three levels. Type of chemicals (sodium chloride, sodium meta-bisulphite and ammonia) and concentration (0.5, 1 and 1.5%). The dependent variable (response variable) was the yield (%) of extracted anchote starch.

The optimization of the yield was done by using a response surface methodology. Each level has two replications and 14 runs were selected by D-Optimal design. The proximate composition of the raw anchote tuber, physicochemical and functional properties of starch were analyzed by ANOVA using statistical software Microsoft excel 2016 (Microsoft office TM Corporation, USA) and OriginPro 2019.

Table 3.1 Levels of independent variables for the starch extraction D-Optimal design

Independent variables	**Units**	**Coded symbol**	**Levels**	**L[1]**	**L[2]**	**L[3]**
Concentration	%	A	3	0.5	1	1.5
Type of chemical		B	3	$Na_2S_2O_4$	NaCl	NH_3

3.2.3 Starch Extraction

Anchote starch was isolated according to the method described by(Benesi *et al.*, 2004) with some modification. Fresh anchote tubers were thoroughly washed to remove surface soil, peeled and cut into small pieces about 1cm cubes. The small cubes were pulverized in high-speed blender into pulp. 1:5 v/v ratio of pulp to different concentration of chemicals solutions (0.50,1 and 1.50% concentration of sodium chloride, 0.50,1 and 1.50% concentration of sodium metabisulphite and 0.50,1 and 1.50% ammonia) and water for control was added to the pulp. Then the mixture was stirred for 5minutes and filtered with double folded cotton cloth. The filtrate was allowed to stand for 2hours for settling the starch and the supernatant was discarded. Then the respective solutions and water was added to the starch, stirred 5minute and stand for 2hours to remove the residue of the non-starch materials. The supernatant was discarded and the starch sediment was collected. The resulting starch was dried at 40℃ in the oven dryer overnight (12hours). Finally, the dried starch was milled to a fine powder using a laboratory grinding machine and packaged with polyethylene bags for analysis.

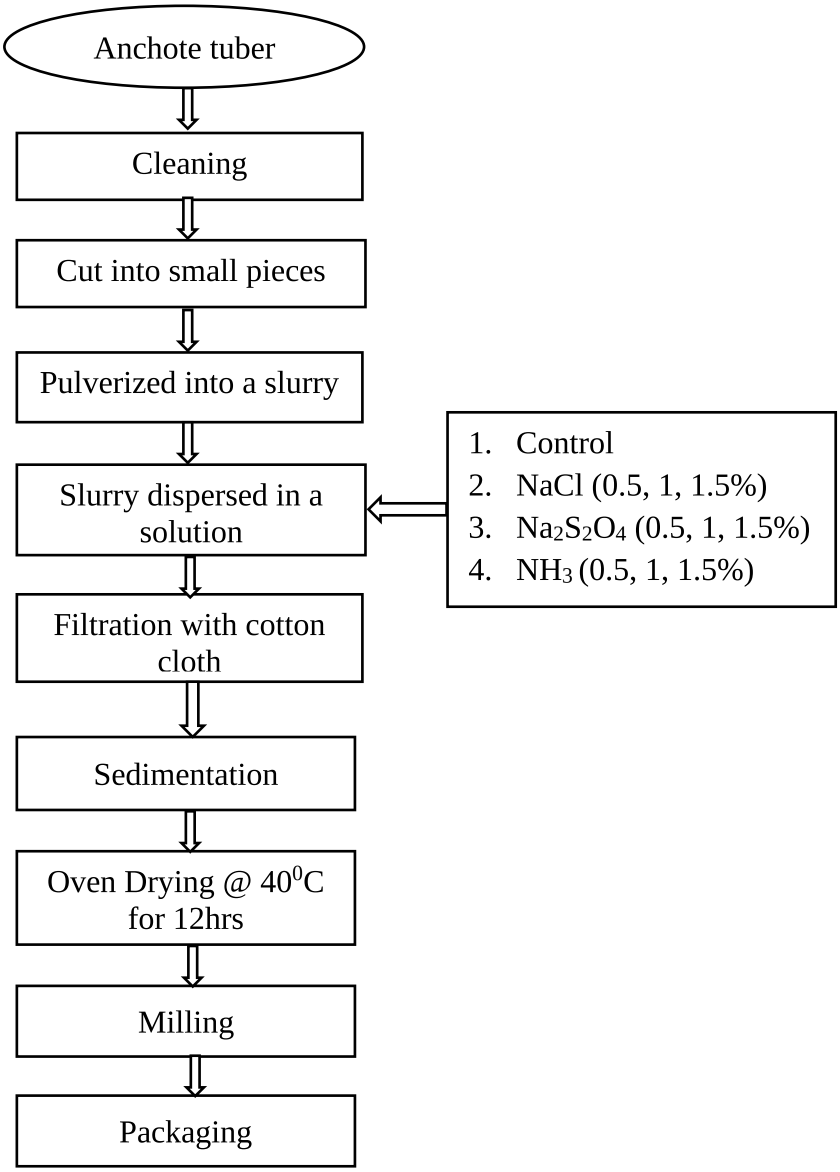

Figure 3.2 Anchote Starch extraction method process flow chart

3.2.4 Starch recovery

The yield of the starch extracted was expressed as a fresh weight basis (fwb) and dry weight basis (dwb) as equation 3.7 and 3.8 respectively. The starch yield on a fresh weight basis is the ratio of the weight of dry starch extracted (g) to the weight of peeled tubers (g).

The starch yield based on a dry weight basis is the ratio of weight (g) of starch extracted (fwb) to the dry matter content of the tuber.

$$\text{Starch (fwb)} = \frac{\text{Weight of dried starch}}{\text{Weight of peeled tubers}} * 100 \qquad (3.7)$$

$$\text{Starch yield dwb (\%)} = \frac{\text{Starch yield on fwb}}{\text{\% Dry matter}} * 100 \qquad (3.8)$$

3.2.5 Identification test

The starch identification test was described by the iodine test. The solubility of the starch in cold water and alcohol (%) was also done. 1g of starch powder was added to 2ml of distilled water and mixed to prepare a homogenized mixture. 15ml of boiling water was added and gently heated for 2 minutes. The iodine test is observed as any change in color as the slurry changed into jelly as well as further change during cooling of the slurry as iodine solution added.

3.2.6 Physicochemical properties of anchote starch

Physicochemical properties such as moisture, ash, fat, fiber, protein and total carbohydrate contents of anchote starch were determined with standard method AOAC (2000) as described in **section 3.2.1** with a little modification. Other physicochemical properties such as titratable acidity, pH and amylose contents were determined as listed below.

Bulk properties such as bulk density, true density, and tapped density were determined by the procedures described by(Obitte and Chukwu, 2007).

3.2.6.1 Bulk density

Bulk density of the starch was determined by filling 25g of starch sample to 50ml graduated glass cylinder and weighing on the balance. Then, bulk density is expressed as the ratio of mass (g) of the starch sample to volume occupied by the starch (ml) as follows.

$$\text{Bulk density} = \frac{\text{weight of starch}}{\text{bulk volume of starch}} \tag{3.9}$$

3.2.6.2 Tapped density

Tapped density of the starch powder was determined by adding 25g of starch sample into a 50ml graduated glass cylinder, tapped 50 times on a bench and the respective volumes recorded. The tapped density (g/ml) of the starch was determined by the following formula.

$$\text{Tapped density} = \frac{\text{weight of starch}}{\text{tapped volume of starch}} \tag{3.10}$$

3.2.6.3 True density

The true density of the starch was determined by the liquid displacement method. 1gm of starch powder was added into a 25ml graduated measuring cylinder containing 5ml of toluene. The true density of the starch (g/ml) was expressed as the ratio of the weight (g) of the starch to the displaced volume (ml) of toluene.

$$\text{True density} = \frac{\text{weight of starch}}{\text{displaced volume of tuluene}} \tag{3.11}$$

Then from densities of starch Carr's index and Hausner ratio were determined from the result obtained from bulk density and tapped density by the following relationship.

$$\text{Carr's index} = \frac{\text{Tapped density - Bulk density}}{\text{Tapped density}} * 100 \tag{3.12}$$

$$\text{Hausner ratio (HR)} = \frac{\text{tapped density}}{\text{bulk density}} \tag{3.13}$$

3.2.6.4 Crystallinity of starch

The crystallinity of the starch was expressed as X-ray powder diffraction taken with X-Ray diffractometer (XRD-7000). The diffractometer operating at the 2θ modes. The Cu target tube operated at 30Kv (25mA) in the range of 10-30^o of 2θ angle with single-crystal graphite monochromatic equipped with a microprocessor to analyze peak position and intensities. Measurements were made at room temperature and the resulting X-ray diffraction pattern and peak positions were analyzed graphically by using the OriginPro 2019 software. The crystallinity of the starch was determined by the peak positions(Jayakody *et al*., 2007).

3.2.6.5 Starch purity

The purity of starch extracted from the anchote tuber was estimated using the following relation:

$$\text{Starch Purity (\%)} = \frac{[\text{\% Carbohydrate }] \times 100}{(100 - \text{\% Moisture})} \tag{3.14}$$

3.2.6.6 Determination of pH

The pH of anchote starch was measured by using digital pH meter (pH METER pH-016) according to the method described by(Singh *et al*., 2010). 1% w/v of the starch sample was dispersed into distilled water, mixed for 5min and the pH value was noted.

3.2.6.7 Titrable acidity

The titrable acidity of anchote starch was determined as per the procedure of(Singh *et al*., 2010). 1gm starch powder was diluted with 20ml of distilled water and mixed gently to uniform dispersion. Then starch dispersion was titrated against 0.1N NaOH using phenolpht halein as an indicator. The acidity of the starch (%) was determined with the following formula.

$$\text{Titrable acidity (\%)} = \frac{T*N*V*E*100}{v*W*1000} \quad (3.15)$$

Were;

T = Titrable volume (ml),

N = Normality of alkali

E = Equivalent weight of reagent, (gm)

v = Volume of sample, (ml) and

W = Weight of sample taken (gm)

V = Volume made up (ml)

3.2.6.8 Amylose amylopectin ratio

The amylose content of the starch was determined according to the method described by(Williams *et al.*, 1958) with some modification. 0.1gm of starch sample was added to the flask. 1ml of 96% ethanol and 9ml of 1M NaOH were added. Heat is applied to solubilize the mixture on a boiling water bath for 10 minutes. 10ml of distilled water was added to 1ml of the solubilized mixture. Then 0.5ml of the diluted mixture was transferred to 0.1ml acetic acid (1M) and 0.2ml of iodine test solution (0.2gm I_2 + 2.0gm KI in 100ml of distilled water). 10ml of distilled water filled, the dark-blue solution was formed and keep for 20minutes until the development of the color completed. The absorbance of the solution was read on UV-spectrophotometer after overtaxing the solution for 5minutes. As standard corn starch with 70% amylose was used to estimate the amylose content of anchote starch as the following equation.

$$\text{Amylose content (\%)} = \frac{\text{\% Amylose of standard x Absorbance of sample x 100}}{\text{Absorbance of standard}} \quad (3.16)$$

The amylopectin ration can be calculated by the following formula.

$$\text{Amylopectin (\%)} = (100 - \text{amylose content}) \quad (3.17)$$

3.2.7 Functional properties of anchote starch

3.2.7.1 Water absorption capacity (WAC)

The water absorption capacity of anchote starch was determined according to the method described by(Adebayo *et al.*, 2010) with little modification. 2gm starch sample was added into centrifuge tubes. 20ml of distilled water added and mixed well by shaking for 5minutes on the shaker. The mixture was centrifuged at 1500rpm for 30 min and the supernatant was discarded. The tube was allowed to drained at 45° angle for 10 min and WAC was calculated by the following formula.

$$\text{WAC}(\text{g H}_2\text{O g}^{-1}\text{ starch}) = \frac{\text{mass of wet starch - mass of dry starch}}{\text{mass of dry starch}} \tag{3.18}$$

3.2.7.2 Oil absorption capacity (OAC)

OAC of anchote starch was determined according to the method described by(Adebayo *et al.*, 2010). 1g of starch sample and 10ml of oil was transferred into a centrifuge tube and stand for 30minutes. The mixture was centrifuged at 1500rpm for 30minutes and the upper layer (supernatant) was decanted. Oil absorption capacity was calculated by the following formula.

$$\text{OAC}(\text{g Oil g}^{-1}\text{ starch}) = \frac{\text{mass of wet starch - mass of dry starch}}{\text{mass of dry starch}} \tag{3.19}$$

3.2.7.3 Swelling Power (g/g) and Solubility Index (%)

The swelling power and solubility index of the starch powder was determined according to the method described by(Leach *et al.*, 1959). 10ml of distilled water and 2g of starch powder were added to a centrifuge tube. The mixture was heated at 30, 50,70 and 90°C in a water bath for 30min. The samples were cooled to room temperature and centrifuged at 3000rpm for 15 minutes and the supernatant was drained to pre-weighed Petri-dish for the solubility index determination. The mass of the swollen starch was noted and the swelling power of the starch was determined according to equation 3.20. Finally, the supernatant was dried for 5hours at 105°C in the oven dryer and the solubility of the starch was calculated as equation 3.21.

$$\text{Swelling power}(\text{g / g}) = \frac{\text{weight of the wet sedemented(g)}}{\text{weight of the dry starch(g)}} \tag{3.20}$$

$$\text{Solublity}(\%) = \frac{\text{weight of dried supernatant(g)}}{\text{weight of the dry starch(g)}} * 100 \tag{3.21}$$

3.2.8 Preparation of Yogurt

Yogurt is a semi-solid and preserved milk product which is produced at home and in the dairy industry. It is prepared by fermentation of milk by lactic acid bacteria such as *Lactobacillus bulgarius* and *Streptococcus thermophilius.* Homemade yogurt can be prepared according to the method described by Jian Yang (2016). Fresh cow milk was filtered and extracted anchote starch (0, 0.5, 1, and 1.50%) were added for each. The mixture was heated to 95℃ for 15minutes and stirring to pasteurize the milk. The pasteurized milk was cooled to 42℃, 4% w/w of commercial yogurt added and mixed gently. The prepared milk was then transferred into clean food-grade plastic jar and incubated at 42℃ for 12hours. Then after fermentation the yogurt is stored at 4℃ and the quality of yogurt was analyzed(Tamime and Robinson, 1999; Verbeken *et al.*, 2006).

Table 3.2 The ratios of Anchote starch with milk to produce yogurt

Treatments	Extracted Anchote Starch %
Y_C	-
Y_0	0
Y_{As1}	0.5
Y_{As2}	1
Y_{As3}	1.50

Where Y_C *= commercial yoghurt,* $Y_{0=}$ *without anchote starch,* Y_{As1}*= 0.50% Anchote starch,* Y_{As2}*= 1% Anchote starch and* Y_{As3}*= 1.50% Anchote starch*

3.2.9 Quality Analysis of Yogurt

The yogurt produced was analyzed for physicochemical properties and sensory analysis to determine the effect of the addition of anchote starch as a stabilizer. Quality of yogurt such as pH, titratable acidity, and syneresis were determined.

3.2.9.1 Determination of pH

The pH of the yogurt was measured by using electronic digital pH meter (pH METER pH-016) after pH calibration with buffer solutions to pH 7.

3.2.9.2 Titrable acidity

The acidity of the yogurt sample was determined by titration as the amount of 0.1N NaOH solution (mL) used to neutralize 10g of yogurt samples, by using phenolphthalein as an indicator to achieve a pink color.

$$\text{Titrable acidity (\%)} = \frac{9 * 0.1 * \text{ml of NaOH}}{\text{initial yogurt weght}} \qquad (3.22)$$

3.2.9.3 Syneresis

Determination of the syneresis of the yogurt was conducted according to the method described by(Keogh and O’Kennedy, 1998). Cooled (4℃) yogurt sample was homogenized, 30g of the sample was transferred to a conical flask and left for 2hours at 4℃ for stabilization. Then, the prepared sample was centrifuged at a temperature of 10℃ for 15 minutes at 3000rpm. The whey was drained, weighed and the syneresis was expressed as the ratio of the separated whey to the initial weight of the sample.

$$\text{Fresh wey(\%)} = \frac{\text{weight of whey}}{\text{initial weight of yogurt sample}} * 100 \qquad (3.23)$$

3.2.9.4 Sensory analysis

The sensory analysis of manufactured yogurt was carried out by 9-point hedonic scale method (9: extremely like, 8: like very good, 7: like good, 6: like moderately, 5: neither like nor dislike, 4: dislike moderately, 3: dislike fairly, 2: dislike very much, 1: dislike extremely). Organoleptic characteristics such as appearance, color, taste, flavor, odor, and overall acceptability of the yogurt were tested by 10 untrained panelists that comprised undergraduate and teaching staff members of Food Technology and Process Engineering Department, Institute of technology, Wollega University according to the method described by(Sameen *et al*., 2016).

CHAPTER 4

RESULTS AND DISCUSSION

In this chapter, the results of the experiment were discussed and presented. The maximum starch extracted by 1.50% concentration of sodium chloride solution was discussed. Proximate composition of anchote tuber such as ash content, moisture content protein content, fat content fiber content, and total carbohydrate content was discussed. Although functional properties and physicochemical properties of anchote starch were discussed. Finally, the application of starch as stabilizer/thickener in yogurt manufacturing, physicochemical properties and sensory evaluation of yogurt was also discussed.

4.1 Proximate Composition of Anchote Tuber

The proximate compositions of raw anchote tuber such as dry matter, moisture, total ash, crude protein, crude fat, crude fiber, and carbohydrate contents were determined by the standard method of AOAC (200). The proximate composition of anchote tuber were shown in Table 4.1.

4.1.1 Moisture Content

The moisture content of anchote tuber was 76.41% in Table 4.1. This result was comparable with the previous work of (Habtamu, 2014) which was 74.93. The small difference may be due to the maturity, accession, and season of the tuber. The yield of anchote starch depends on the moisture content of the tuber. The moisture content of root and tuber crops was 70-80% and 16-24 starch content(FAO, 1990).

4.1.2 Ash Content

The ash content of raw anchote tuber was 2.04% which is comparable with the previous finding of (Habtamu and Kelbessa, 1997;Habtamu, 2014) 2.0% and 2.19% (Table 4.1). Total ash content is directly proportional to the inorganic element content of the tuber.

4.1.3 Fat Content

The mean total fat content of raw anchote tuber was 0.16% which was less than the previous work of(Habtamu, 2014).

4.1.4 Fiber Content

The mean fiber content of raw anchote tuber was 2.97% which is greater than that of previous work of (Demelash, 2016) 2.57%. This small difference may due to the maturity of the tuber, variety and season of harvesting(Girma and Dereje, 2015).

4.1.5 Protein Content

The protein content of anchote tuber was 3.27% which is comparable with the previous work of(Habtamu *et al*., 2013) 3.25%. The higher protein content can affect the yield and functional properties of starch.

4.1.6 Total Carbohydrate

The total carbohydrate content of anchote tuber was 26.87% which is greater than the previous finding of (Habtamu and Kelbessa, 1997) 25.50%. The higher carbohydrate content is an indication of the starch content of the tuber because starch is the complex carbohydrate.

Table 4.1 Proximate composition of raw anchote tuber

Chemical properties	**Value (%)**
Moisture content	76.41
Ash content	2.04
Fat content)	0.16
Fiber content	2.97
Protein content	3.27
Total carbohydrate	26.87

4.2 Starch Recovery and Identification

4.2.1 Identification of the Starch

The yield of recovered starch was shown in table 4.3. The identification test result showed that the anchote starch was insoluble in cold water and alcohol (95%) at room temperature. This characteristic is an indication of the starch quality and purity. The iodine test of the starch was a positive result that indicates the starch amylose forms the dark blue color complex with iodine test solution. This result confirms the identity of materials obtained from anchote tuber is starch(Konstantinos, 2008).

4.3 Starch Yield Optimization

The results of anchote starch extracted were analyzed using statistical software Design Expert 11.1.2.0 (State-Ease Inc., Minneapolis, MN, USA). The response surface method was used for ANOVA and 2FI model was suggested to determine the significance of the results at ($p<0.05$). The maximum starch recovered from anchote tuber was 20.86% in 1.5% concentration of sodium chloride solution and 16.71% for control table 4.2. The starch yield on a dry weight basis (dwb) of anchote tuber is 67.82% and 55.07% for 1.50% concentration of sodium chloride solution and control respectively.

Table 4.2 Design summary for anchote starch yield optimization

File Version	11.1.2.0		
Study Type	Response Surface	Subtype	Randomized
Design Type	D-optimal	Runs	14
Design Model	2FI	Blocks	No Blocks
Build Time (ms)	13.00		

This indicates that the addition of chemical solution can remove non-starch polysaccharide (NSP) and proteins which reduce the yield and purity of the starch. NaCl isolation can increase the yield of starch by breaking the bond between starch and protein molecule. Starch-protein association broken down, the starch molecules were settle down by gravity and the protein molecules were floating as a supernatant.

Finally, the supernatant was removed by decantation(Lopez-Diago *et al*., 2018).

Table 4.3 The experimental values using D-Optimal design of anchote starch yield (%)

Run	**Factor 1** **A: Concentration (%)**	**Factor 2** **B: Type of chemical**	**Response 1** **Yield (%)**
1	0.5	$Na_2S_2O_4$	17.83
2	0.5	NH_3	16.81
3	1.5	$Na_2S_2O_4$	18.9
4	1.5	NH_3	17.32
5	1.5	NH_3	17.12
6	1	NaCl	19.61
7	1.5	NaCl	20.86
8	1	$Na_2S_2O_4$	18.28
9	0.5	NH_3	16.85
10	0.5	NaCl	18.56
11	0.5	NaCl	18.39
12	1	NH_3	16.93
13	1	$Na_2S_2O_4$	18.32
14	1	NaCl	19.75

Table 4.4 ANOVA for Response Surface 2FI Model for anchote starch yield

Source	**SS**	**DF**	**MS**	**F-value**	**p-value**	
Model	19.49	5	3.90	542.65	< 0.0001	Significant
A-Concentration	3.07	1	3.07	426.72	< 0.0001	
B-Type of chemical	16.04	2	8.02	1116.12	< 0.0001	
AB	1.65	2	0.8263	115.02	< 0.0001	
Residual	0.0575	8	0.0072			
Lack of Fit	0.0116	3	0.0039	0.4225	0.7453	not significant
Pure Error	0.0458	5	0.0092			
Cor Total	19.55	13				

SS = Sum of Square, DF = Degree of Freedom, MS = Mean Square

From ANOVA table 4.4 the suggested model was highly significant with a p-value of <0.0001. This indicates that the model used to fit response variables was adequate. The relation the relationship between independent variables (type of chemical and concentration) and dependent variable (yield of starch) was statistically significant ($p < 0.05$) with a $p < 0.0001$. This shows that the type of chemical and concentration can affect the yield of starch during extraction. This may be due to the different chemical can have the ability to remove the non-starch component from the surface of the tuber. Also, the concentration of the chemical in the solution is another factor that can affect the yield. In the case of the present study, sodium chloride is the best chemical solution with a concentration of 1.5%. The yield of the starch was increasing with increasing concentration of the solution to 1.5%. The F-value of "Lack of Fit" 0.4225 implies non-significant when compared to the pure error and the suggested model was adequate. The interaction between the variable was also significant ($p<0.05$).

Table 4.5 Fit Statistics

Std. Dev.	0.0679	R^2	0.9981
Mean	18.24	Adjusted R^2	0.9970
C.V. %	0.3719	Predicted R^2	0.9940
		Adeq Precision	91.1763

The Predicted R^2 of 0.9940 is in reasonable agreement with the Adjusted R^2 of 0.9970; i.e. the difference is less than 0.2. Adequacy precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 91.176 indicates an adequate signal. This model can be used to navigate the design space.

The correlation coefficient (R^2) shows the quality of the developed model and has a value of 0.9971. For a good statistical model, the R^2 value should be close to one which ensured a satisfactory adjustment of the model to the experimental data. This indicated that there was approximately 99.71% of the variability in the dependent variables (starch yield). Therefore, there was a good agreement between experimental and predicted values.

The residual analysis can be used to check the fitness of the model. The component of variation, which roughly fitted to the model is usually considered as residuals. They perform according to the normal distribution feature. Graphical visualization of the normality of the residual was evaluated by the normal probability plot. Figure 4.1 shows the normal plot of starch yield. It describes how the data are spread approximately in a straight line and describe the relationship between the experimental data and predicted values of the response variable.

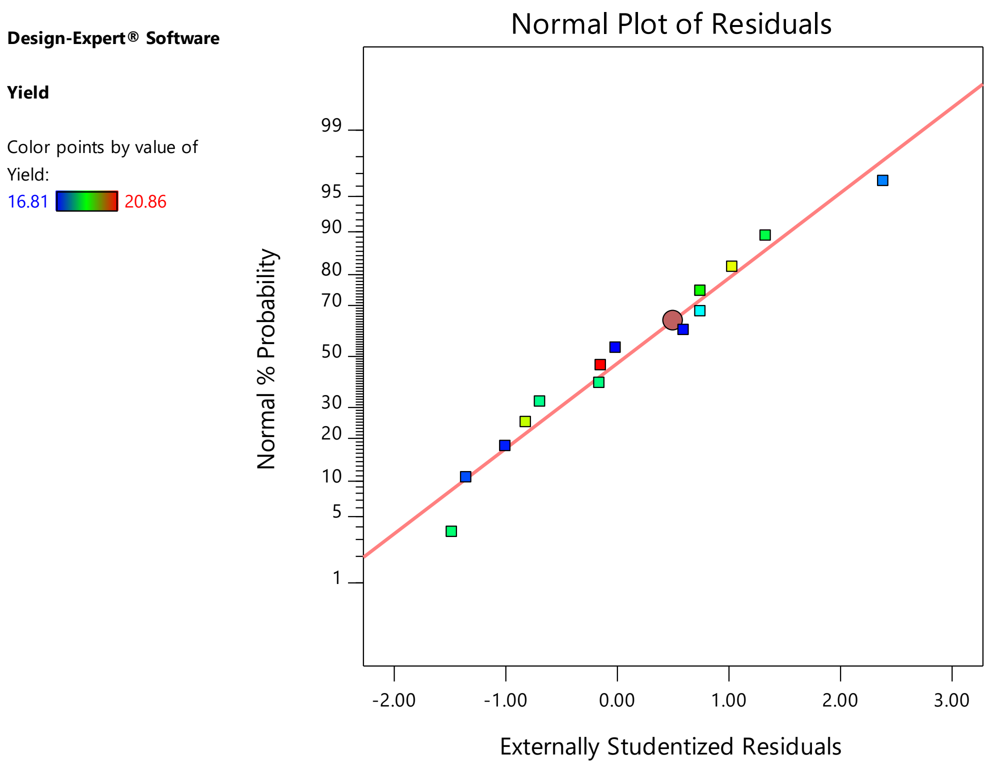

Figure 4.1 Normal probability plot of anchote starch yield

The optimum conditions of anchote starch yield was determined by two-dimensional (2-D) plots. The interaction plot indicates the effect of the independent variables and their interaction on the response variable. It indicates the yield of anchote starch was increased with the concentration NaCl to 1.5%. The optimum concentration for maximum anchote starch yield was NaCl with 1.50% concentration.

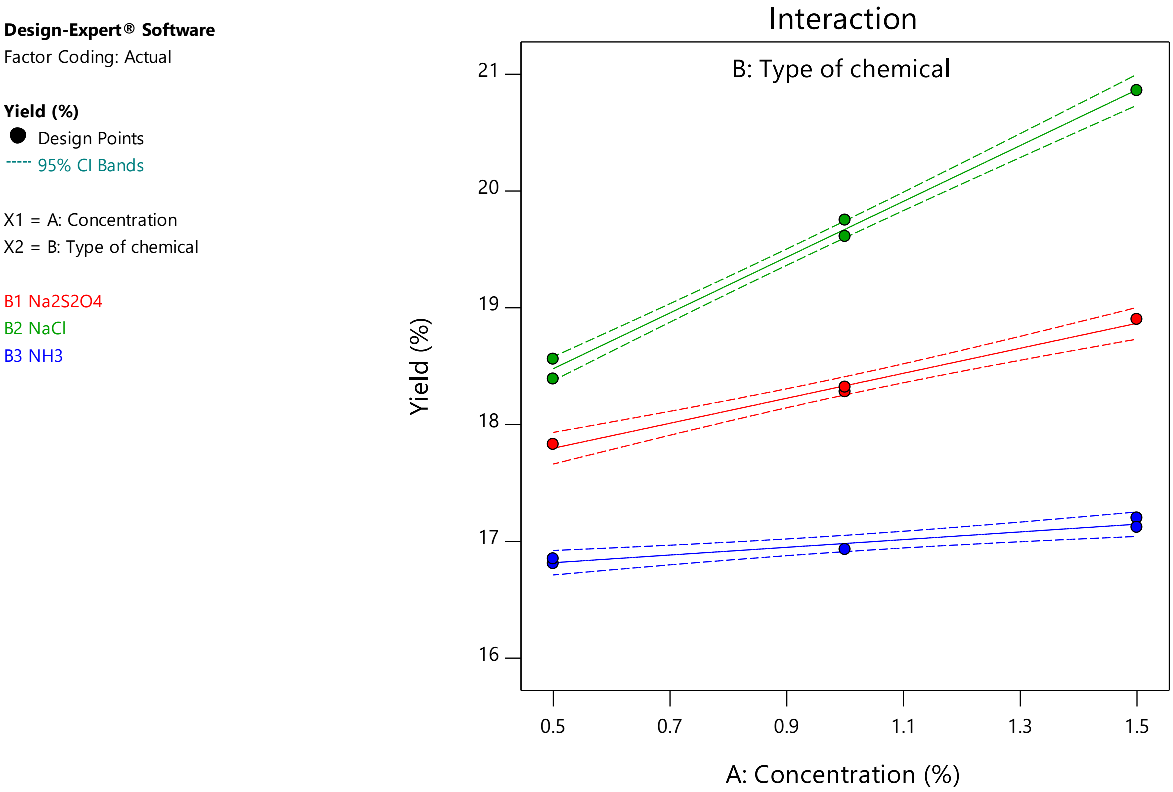

Figure 4.2 Interaction plot of anchote starch yield

The residual calculated against the order of experimentation was expressed as residual versus run plot figure 4.3. It indicates the existance of correlation that how the experimental run was randomized.

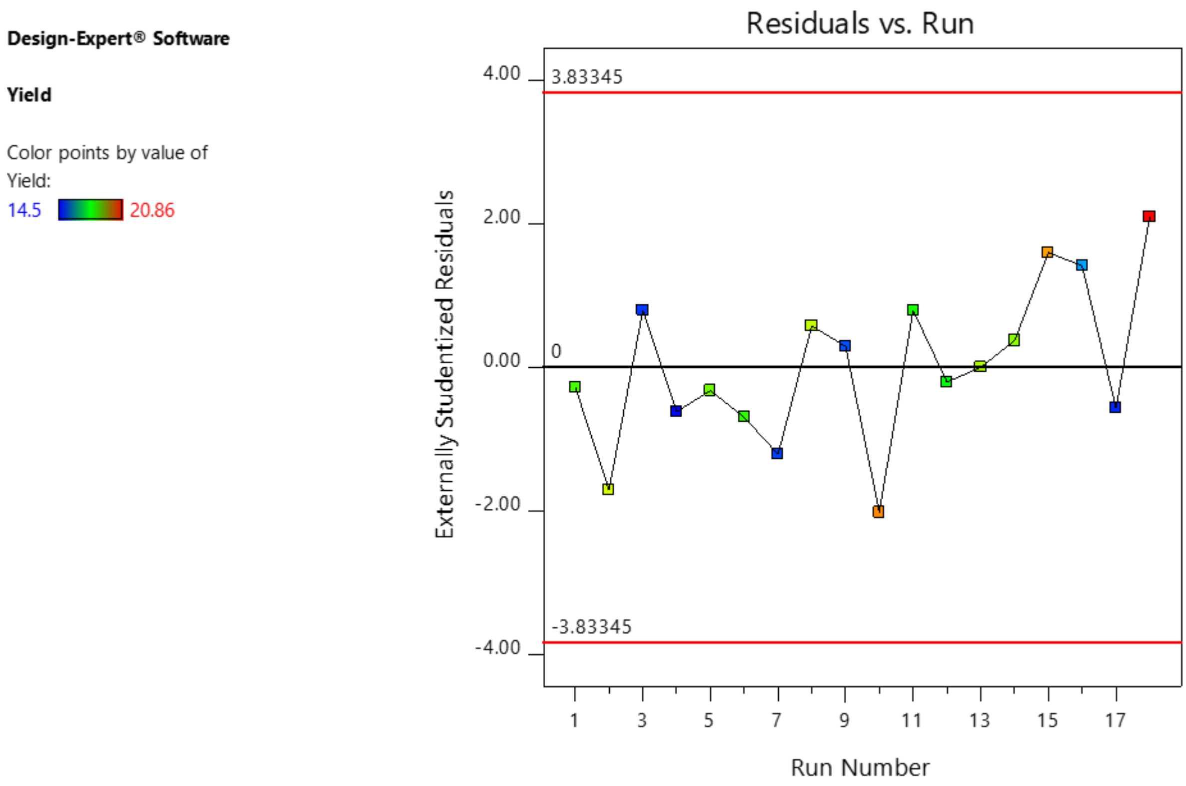

Figure 4.3 Residuals versus run order plot of anchote starch yield

How precisely the suggested model fitted was evaluated with predicted versus actual plot Figure 4.4. The point above the straight line is an indication of the predicted value was greater than the actual value vice versa. The closeness of the points to a straight line shows how the predicted value and actual values were closer to each other.

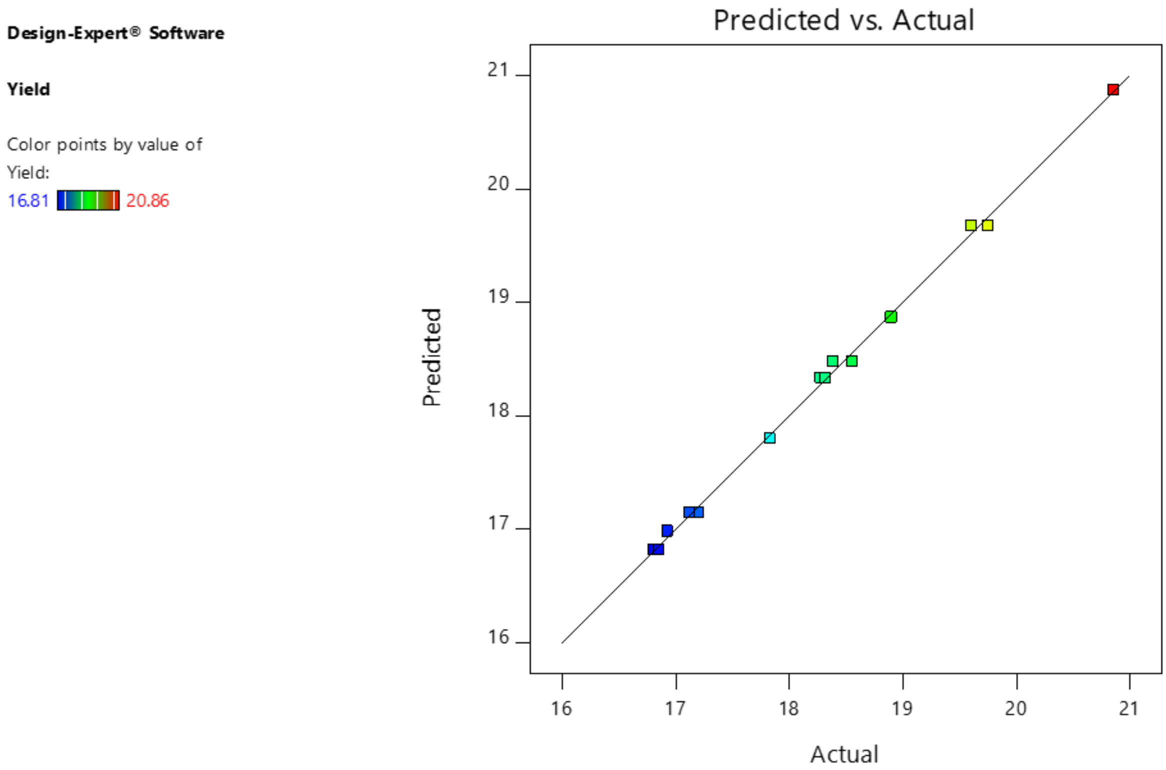

Figure 4.4 Predicted versus actual plot of anchote starch yield

4.3.1 Development of regression model equation

The empirical relationship between the independent variables (type of chemical and concentr ation) response function (yield) was evaluated by the response surface method. The prediction of the response variable was made by the equation of coded factors. The comparative impact of the factors was distinguished by the coded equation by comparing the coefficients. D-optimal design was used to determine the mathematical relationship between independent variables and response (dependent variables) in terms of coded and actual factors.

The results showed the yield of starch extracted from anchote tuber depend on both independent variables and the interaction of the variables. Depending on the above equation the response variable depends on the concentration of sodium chloride. Sodium chloride at higher concentrations (1.5%) had a higher yield. This may be due to the capability of sodium chloride solution to release the non-starch molecules from the surface and free the starch molecules to settle. Also, it can breakdown the starch-protein bond and the protein molecules easily removed from the starch.

Final equation in terms of coded factors for anchote starch yield

$$\text{Yield} = +18.34 + 0.6414A - 0.0046B[1] + 1.34B[2] - 0.1064AB[1] + 0.5529AB[2] \qquad (4.1)$$

Where; A= concentration (%) and B= type of chemical

Final equation in terms of actual factors for anchote starch yield

Type of chemical: NaCl

$$\text{Yield} = +17.28429 + 2.38857\text{Concenteration} \qquad (4.2)$$

Table 4.6 Solutions for 9 combinations of categorical factor levels

Number	Concentration	Type of chemical	Yield	Desirability	
1	**1.500**	**NaCl**	**20.867**	**1.000**	**Selected**
2	1.000	NaCl	19.673	0.707	
3	1.500	Na2S2O4	18.867	0.508	
4	0.500	NaCl	18.479	0.412	
5	1.000	Na2S2O4	18.332	0.376	
6	0.500	Na2S2O4	17.797	0.244	
7	1.500	NH3	17.147	0.083	
8	1.000	NH3	16.982	0.042	
9	0.500	NH3	16.817	0.002	

The maximum yield of starch was obtained at 1.5% concentration of NaCl solution.

4.4 Physicochemical Properties of Starch

Chemical properties of Anchote starch such as moisture, ash, fat, fiber, protein, carbohydrate contents, amylose content, starch purity, pH, and titrable acidity of anchote starch were studied and discussed. Also, physical properties of the starch (bulk density, tapped density, true density, and crystallinity) were discussed in Table 4.5.

4.4.1 Moisture content

The moisture content of starch was 12.14 %. This result was found in the recommended range **(**10-15%) of moisture content for root and tuber starches. The safe storage life of some starches is < 13% for some starch producing countries(Tian *et al.*, 1991)**.** Moisture content of anchote starch fall within the recommended range of moisture content. The higher moisture content has an adverse effect on the starch such as microbial damage and starch quality deterioration (Moorthy, 2002).

4.4.2 Ash content

The ash content of anchote starch isolated with 1.5%NaCl solution was 0.30%. Total ash content is directly proportional to the inorganic element content of the tuber. Hence the samples with high percentages ash contents are expected to have high concentrations of various mineral elements, which are an advantage to speed up metabolic processes and improve growth and development for our body. The upper limit of ash content recommended by the British Pharmacopeia is 0.6 % w/w. Ash level may also be regarded as a measure of the quality or grade of the starch and often a useful measure in identifying the validity of food. It can also measure the mineral status of a starch(Abegunde *et al.*, 2012).

4.4.3 Fat content

The fat content of anchote starch isolated was 0.04 for 1.5% NaCl solution. The fat content of the root and tuber crop is very small or negligible. It can be higher in cereal starches because of the lipid found in the germ. The fat/lipid content of anchote starch is in the range of typical root or tuber starch 0.05-0.1%(Jobling, 2004).

4.4.4 Fiber content

The total fiber content of anchote starch isolated with 1.5%NaCl solution was (1.64%). Fiber content in food have an advantage in food such as hypoglycaemic and hypocholesterolaemia activity. But they can affect the color and affect the quality of the starch. A higher amount of the fiber and ash is an indication of poor-quality grade of the starch FAO (2000).

4.4.5 Protein content

The protein content of anchote starch extracted was 0.53%. Protein is an important component of food material. High protein content has an adverse effect on starch characteristics such as the water absorption capacity of the starch molecules and granule surface and gelatinization (Abegunde *et al*., 2012).

4.4.6 Total carbohydrate

The total carbohydrate content of the starch was 85.35. It is an important characteristic of starch which is indirect measure starch purity. Higher content of carbohydrate shows the good quality for starch. Starch with higher carbohydrate content greater than 96 % w/w starch was recommended for pharmaceutical applications (Abegunde *et al*., 2012).

4.4.7 Starch purity

The purity of starch shows the percentage of total carbohydrate content free of moisture content found in the starch. The purity of anchote starch is 97.09 and 97.14% for control and 1.5%

NaCl solution respectively. This result shows significance ($p<0.05$) difference and can be affected by the fiber and ash content of the starch.

4.4.8 Determination of pH

The pH is an indication of the alkalinity or acidity of the starch. It affects the performance of the starch in food processing applications. The pH value of anchote starch extracted with control and 1.5% NaCl solution was found 7.25 and 7.32 respectively. Thus, the pH values of the starch obtained in this study in agreement with the standard pH of alkaline treated starches 5.0-7.5 FAO (2000).

4.4.9 Titratable acidity

The titratable acidity of anchote starch extracted with control and 1.5% NaCl solution was found 0.158 and 0.160% respectively. The titratable acidity is an indicator of freshness in the starch.

Table 4.7 Physicochemical properties of anchote starch

Chemical properties	**Anchote starch**	
	Control	**NaCl (1.5%)**
Moisture content (%)	12.31	12.14
Ash content (%)	0.25	0.30
Fat content (%)	0.05	0.04
Fiber content (%)	1.63	1.64
Protein content (%)	0.62	0.53
Total carbohydrate (%)	85.14	85.35
Amylose content A.C (%)	17.18	17.42
Amylopectin (%)	82.82	82.58
Starch purity S.p (%)	97.09	97.14
pH	7.25	7.32
Titratable acidity (%)	0.158	0.160

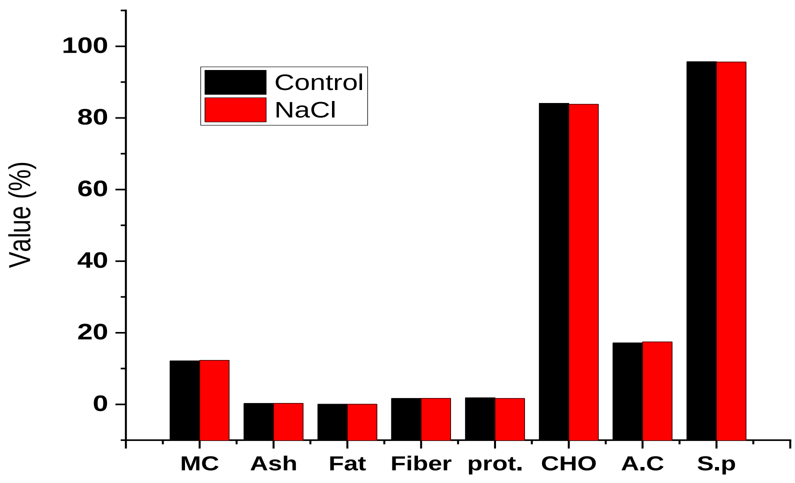

Figure 4.5 Chemical properties of anchote starch

4.4.10 Amylose content

The amylose content of the starch is used for the determination of other molecular components known as amylopectin. The amylose/amylopectin ratio of root and tuber crops has been vary based on the botanical source. Starch amylose hinders the swelling of the granule and disintegrating potential of the starch by forming the starch-lipid complex(Hoover, 2001).

The amylose content of anchote starch for control and 1.5% NaCl solution was 17.18 and 17.42% respectively. There is significance ($p<0.05$) different between amylose content of starch isolated with control and 1%NaCl solution. This result shows that anchote starch contains a lower amylose/amylopectin ratio and it can be used as disintegrant and thickeners (Wickramasinghe *et al*., 2009).

4.4.11 Bulk properties of the starch

Flow characteristics of the starch powder is very important for industrial operations like mixing, transportation, and packaging(Tay *et al.*, 2017). Bulk density, tapped density, true density, Carr's index and Hausner ratio of the starch was discussed. Anchote starch granules results in bulk density of 0.47 and 0.46, true density 1.52 and 1.50 and tapped density 0.68 and 0.65, Carr's index 24.24 and 23.97 and Hausner ratio 1.33 and 1.32 for control and 1.5% NaCl solution respectively. Starches with higher density have high diluent power. This can reduce the volume of the starch granule and improve the association and flow(Aulton, 2001).

Table 4.8 Bulk properties of the starches

Bulk Properties	**Starch**	
	Control	**1.5 %NaCl**
Bulk density (g/cm^3)	0.47	0.46
True density (g/cm^3)	1.52	1.50
Tapped density (g/cm^3)	0.68	0.65
Carr's index (CI)	24.24	23.97
Hausner ratio (HR)	1.33	1.32

Carr's index and Hausner ratio of anchote starch were 23.97 and 1.32 respectively. Carr's index is the prediction of the starch compressibility and the value above 23% was an indication of low compressibility. The Hausner ratio of the starch shows the flow property of powders and the value above 1.2 was an indication of low flow property of the granule (Muazu *et al.*, 2011). Carr's index is a simple test to evaluate bulk and tapped densities of the powders and the rate at which they are packed down. HR indicates the flow properties of the powder.

4.4.12 Crystallinity of starch

Crystallinity of the starch is an indication of the order structure of amylopectin molecules inside the granule. The X-ray powder diffractogram of anchote starch was shown in Fig.4.6 Starch having different crystallinity pattern have different packing arrangements(Hong et al., 2016; Moorthy, 2002). Depending on their diffraction pattern starches can be divided into three categories. A-type starches are cereal starches having strong diffraction peaks at about 15° and 23° of 2θ angle and an unsettled doublet at around 17° and 18° of 2θ angle. B-type starches are tubers and high amylose starches which exhibit the strongest diffraction peak at around 17° of 2θ angle and a few small peaks at around 15°, 20°, 22°, and 24° 2θ angle. The C-type starches were legumes, roots, and some fruit starches which shows the property of both A and B-type(A. Surendra & Parimalavalli, 2014). The crystallinity of the starch powder was measured by X-ray diffractograms (Model XRD-7000). XRD is used for the investigation of powder crystal structures at the atomic level. The X-ray patterns of anchote starch show the diffraction peaks at 15.5, 17.2, 22 and 24.2° of 2θ angles for both control and 1.5% NaCl solution. This similarity shows the extraction method con not affect the crystallinity. Both results of starch showed the sharp strong diffraction peak at 17° 2θ of angles and few small peaks at 2θ values of 14.5, 15.5 and 19.5°. The previous work of(Surendra and Parimalavalli, 2014) report that potato starch showed diffraction peaks with 2θ values of 17, 22 and 24° and sweet potato starches showed peaks at the 2θ diffraction angles around 10, 11,15,17,20 and 23°. From these observations, anchote starches revealed a B-type diffraction pattern that is comparable with potato starch. This result shows that the isolation method did not affect the diffraction pattern of anchote starch. A-type starches show higher levels of crystallinity and higher gelatinization temperatures whereas B-type starches show lower levels of crystallinity and lower gelatinization temperatures(Builders and Arhewoh, 2016).

Table 4.9 X-ray diffraction data of the anchote starches

Anchote starch	**Strongest peaks**							
	Peak 1		**Peak2**		**Peak 3**		**Peak 4**	
	angle (2θ°)	Intensity	angle (2θ°)	Intensity	angle (2θ°)	Intensity	angle (2θ°)	Intensity
Control	15.5	1274	17.2	1590	22	1190	24.05	1054
NaCl	14.52	1263	17.12	1470	21.64	1108	24.24	947

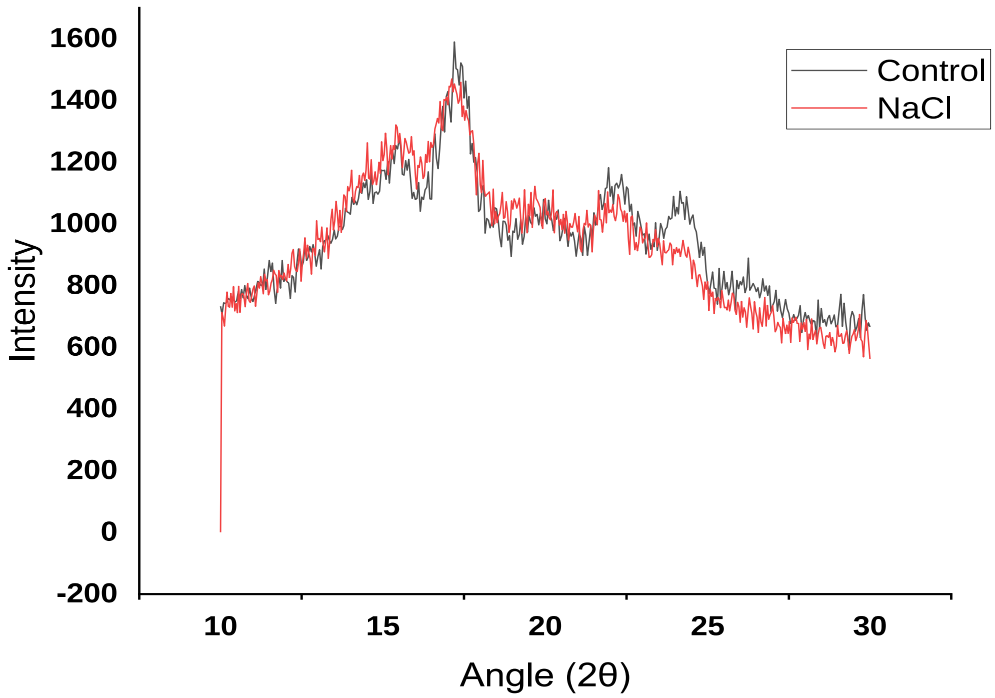

Figure 4.6 X-ray diffractograms of anchote starch

4.5 Functional Properties of Starch

The functional properties of anchote starch extracted are given in Table 4.8- 4.9.

4.3.3.1 Water absorption capacity (WAC)

Water Absorption Capacity (WAC) of anchote starch was 0.16 and 0.17ml of water/ g starch for control and 1.5% NaCl solution respectively. Water absorption capacity is influenced by the protein content of the starch. It is an indication of protein-water interaction in food. Higher water absorption capacity results in weak interactive forces of the starch component(Riley *et al*., 2006). Water absorption capacity may be due to the loose structure of starch and removal of fat exposes the water binding sites of amino acids (Resio *et al.*, 2000).

4.3.3.2 Oil absorption capacity (OAC)

The oil absorption capacity of anchote starch was 0.15 and 0.17ml of oil/g starch for control and 1.5% NaCl solution. OAC is affected by the protein content of the starch. Protein contains both hydrophilic and hydrophobic parts. The hydrocarbon chains of lipids can form hydrophobic interaction with non-polar amino acid side chains. WAC and OAC can express the industrial interest of the starches in the formulation of food products(Resio *et al.*, 2000).

Table 4.10 Water absorption capacity and Oil absorption capacity of anchote starches

Properties	**Starch samples**	
	Control	NaCl
WAC (ml/g)	0.16	0.17
OAC(ml/g)	0.15	0.17

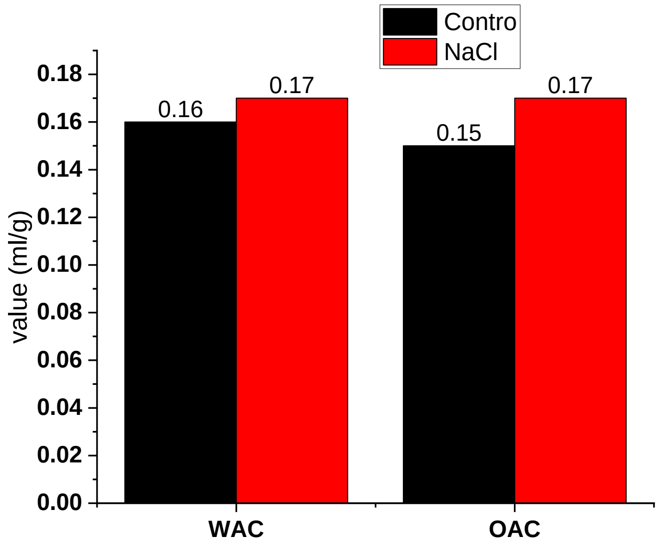

Figure 4.7 Water absorption capacity and Oil absorption capacity of anchote starches

4.3.3.3 swelling power and solubility index

Swelling power and water solubility index of the starch was tested at different range of temperatures from 30 - 90℃ (30, 50, 70 and 90°C). They are also related to the water absorption capacity and source of the starch. From the result, both swelling power and solubility of anchote starch increased with increasing temperature. Statistically, there is a significant ($P<0.05$) difference observed between control and 1.5% NaCl solution in swelling power and solubility index. As temperature increase to 90°C both swelling power and solubility of the starch increase. The swelling power can be affected by the protein content of the starch. When starch suspension in water was heated, the hydrogen bonds of the starch breakage and its crystalline structure disrupted. The hydrogen bonds linked to the exposed hydroxyl groups of amylose and amylopectin. This can cause an increase in starch granule swelling power and solubility (Hoover, 2001).

Table 4.11 Swelling power and Solubility of anchote starch at different temperature

Temperature (ºC)	Starch	
	Control	NaCl
	Swelling power(g/g)	
30	1.91	1.90
50	2.54	2.53
70	8.11	8.10
90	11.66	11.65
	Solubility (%)	
30	0.32	0.31
50	0.91	0.90
70	4.80	4.81
90	15.42	15.43

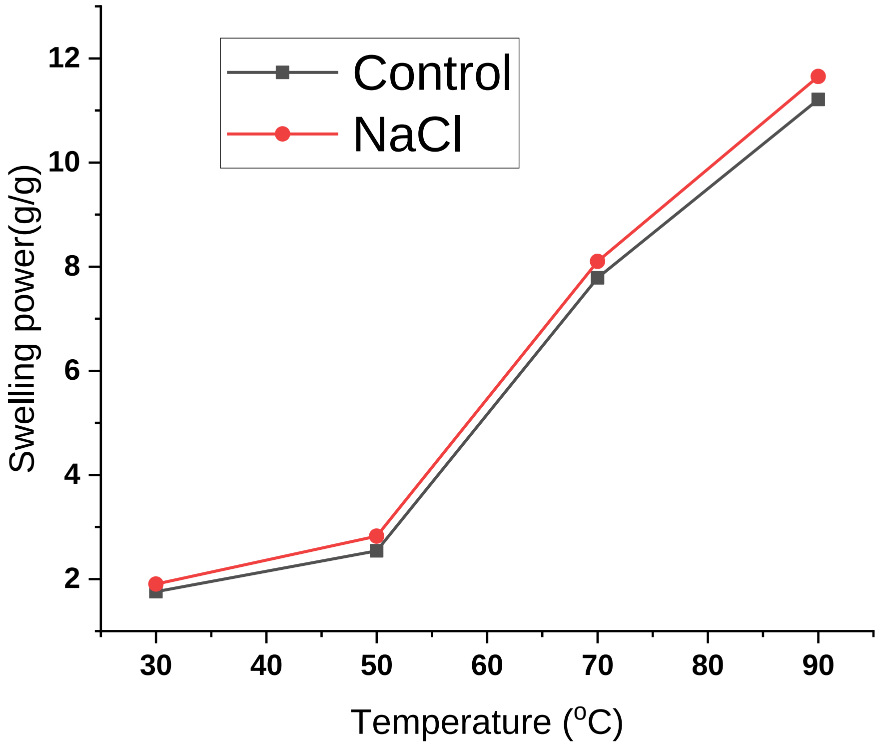

Figure 4.8 swelling power plot of anchote starch

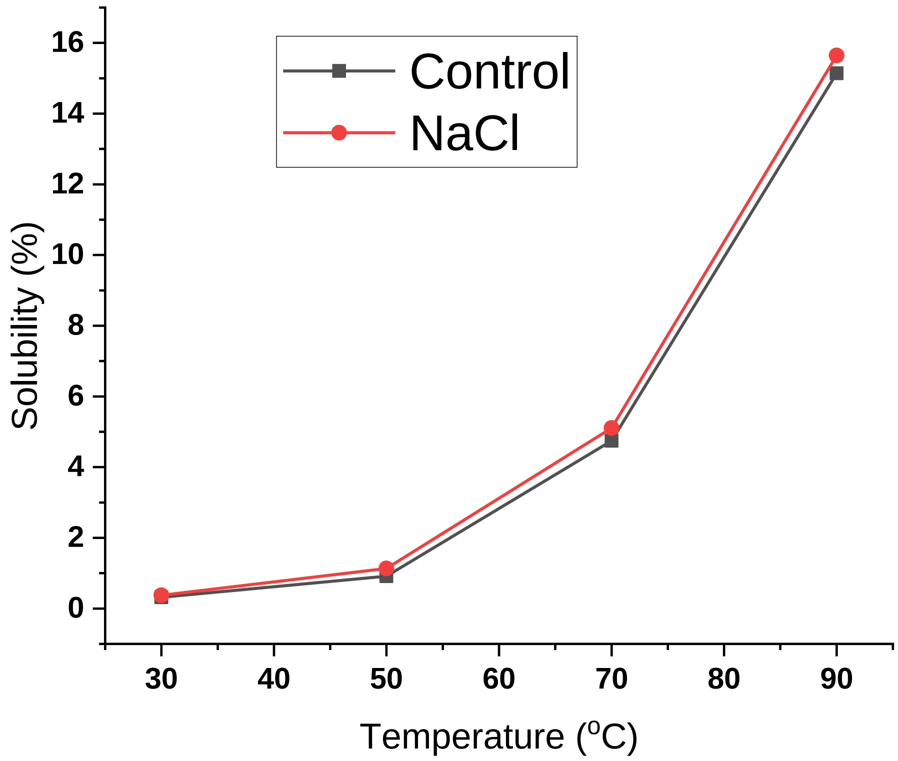

Figure 4.9 Solubility plot of anchote starch

4.3.4 Application of Starch

The optimized anchote starch was used for yogurt production with different ratios as a stabilizer. The yogurt was prepared by the method explained in **section 3.2.5.** The prepared yogurt sample was stored at a temperature of 5℃ for quality determination.

4.3.5 Quality Analysis of Yogurt

The manufactured yogurt was studied for different physicochemical characteristics such as pH, Titratable acidity, syneresis, and sensory evaluation or Organoleptic characteristics such as Appearance, color, taste, flavor and overall acceptability the yogurt samples were determined.

4.3.5.1 pH of yogurt

The pH of yogurt prepared were in Table 4.10. The addition of anchote starch had a significant effect on the pH of yogurt samples. The pH value of the yogurt was increased with increasing the addition ratio of the starch. Statistically, there were significant (p < 0.05) differences yogurt samples Y_0, Y_{As1}, Y_{As2}, and Y_{As3}. This may be due to the high pH value of the starch. But the results were in the range of the codex standard (CODEX STAN 243-2003) for yogurt pH which 4 - 4.5. Also, the pH of the yogurt samples was decreased with increasing storage time. These results were comparable with the previous study of (Ammar, 2018) which shows the pH of the yogurt decreased as storage time increases. This is due to lactic acid produced during storage.

The conversion of lactose into lactic acid was affected by the concentration of starch(Hassan *et al.*, 2011). Yogurt prepared with the addition of 1.5% of anchote starch showed the best pH value.

Table 4.12 pH in yogurt stored at 5°C for 15 days

Storage Period (Days) @ 5°C	pH of yogurt			
	Y_0	**Y_{As1}**	**Y_{As2}**	**Y_{As3}**
1	4.37	4.39	4.40	4.43
5	4.34	4.36	4.38	4.41
10	3.87	3.92	4.02	4.22
15	3.50	3.64	3.73	4.15

4.3.5.2 Titratable acidity of yogurt

The titratable acidity of the yogurt was an indication of the presence of lactic acid which is produced during fermentation. The statistical analysis showed that there was a significant ($p<0.05$) difference between the yogurt prepared without adding the starch and the stabilized yogurt. The titratable acidity of yogurt decreased with increasing concentration of the starch. The acidity of the yogurt was increased as the storage time increase. This result was comparable with the previous work of (Al-Kadamany *et al.*, 2003; Sameen *et al*., 2016) who reported that the acidity of yogurt prepared by the addition of sweet potato and taro starch was increased with storage time and decreased as the concentration of the starch. The previous study of the yogurt stabilized by potato starch also showed the addition of the high amount of starch can affect the acidity of the yogurt by absorbing the water. This could inhibit the metabolization of lactose sugar by bacteria and thus reducing the quantity of lactic acid produced(Ammar, 2018). The result of titratable acidity of yogurt was with the codex standard (CODEX STAN 243-2003) range of the yogurt acidity >0.3%. The higher acidity can lower the pH of the yogurt and increase the syneresis.

Table 4.13 Titratable Acidity in yogurt stored at 5°C for 15 days

Storage Period (Days)	**Titratable Acidity (%)**			
	Y_0	**Y_{As1}**	**Y_{As2}**	**Y_{As3}**
1	0.89	0.84	0.81	0.78
5	0.91	0.85	0.83	0.80
10	0.97	0.95	0.87	0.90
15	1.23	1.02	0.96	0.91

4.3.5.3 Syneresis of yogurt

Syneresis or whey separation is the presence of whey on the surface of the yogurt during storage because of its shrinkage and results in whey loss. It can be caused by a change in the formulation of yogurt with low total solids content and other factors such as a decrease in pH, high acidity, high incubation temperatures or inadequate storage temperature(Al-Kadamany *et al.*, 2003; Sameen *et al.*, 2016). There was a decreasing value of syneresis with increasing concentration of starch. But there was an increasing value of the syneresis as storage time increases. This result was comparable with the previous finding of(Mwizerwa *et al*., 2017) who reported the addition of cassava starch to enhance the syneresis of the yogurt. The proportions of anchote starch and the storage time had a significant effect ($p<0.05$) on yogurt syneresis. Yogurt prepared with 1.5% anchote starch gives the lowest syneresis. For all samples, the syneresis was decreasing with an increasing proportion of anchote starch (0.5-1.5%) and storage time from 1 to 15 days. Yogurt prepared without anchote starch (Y_0) and yogurt prepared with 0.5% (Y_{As1}) anchote starch had significantly ($p<0.05$) higher syneresis compared to the yogurt samples Y_{As2} and Y_{As3}. Yogurt sample Y_{As3} showed the best result of syneresis.

Table 4.14 Syneresis of Yogurt stored at 5˚C for 15 days

Storage Period (Days)	**Syneresis (%)**			
	Y_0	**Y_{As1}**	**Y_{As2}**	**Y_{As3}**
1	42.32	25.73	23.48	20.78
5	43.50	28.27	25.54	24.34
10	45.74	32.45	29.12	27.04
15	47.18	37.72	33.38	30.17

4.3.5.4 Sensory evaluation

Organoleptic characteristics of the yogurt such as Appearance, color, flavor, taste, and overall acceptability were determined. Commercial (Y_C) yogurt was used to evaluate the effect of the addition of anchote starch on the sensory property of the yogurt. Commercial yogurt sample Y_C and yogurt samples Y_0 and Y_{As1} flavor and overall acceptability showed the difference. The appearance and overall acceptability of the yogurt samples Y_0 and Y_{As1} were liked moderately. and for Y_{As2}, and Y_{As3} like good. There was no difference in color between commercial yogurt and all yogurt sample. The result of the appearance of the Y_C, Y_{As2}, and Y_{As3} were showed liked good. Generally, the yogurt prepared with 1% and 1.5% anchote starch showed good sensory quality.

Table 4.15 Mean scores sensory evaluation of yogurt

Treatment	Appearance	Color	Flavor	Taste	Overall acceptability
Y_C	7.50	7.80	7.00	8.00	7.50
Y_0	6.30	7.00	6.60	7.20	6.60
Y_{As1}	6.90	7.10	6.33	7.00	6.30
Y_{As2}	7.20	7.00	6.00	6.00	7.00
Y_{As3}	7.30	7.40	6.60	6.50	6.70

Scores on the hedonic scale: *9-point hedonic scale method (9: extremely like, 8: like very good, 7: like good, 6: like moderately, 5: neither like nor dislike, 4: dislike moderately, 3: dislike fairly, 2: dislike very much, 1: dislike extremely).*

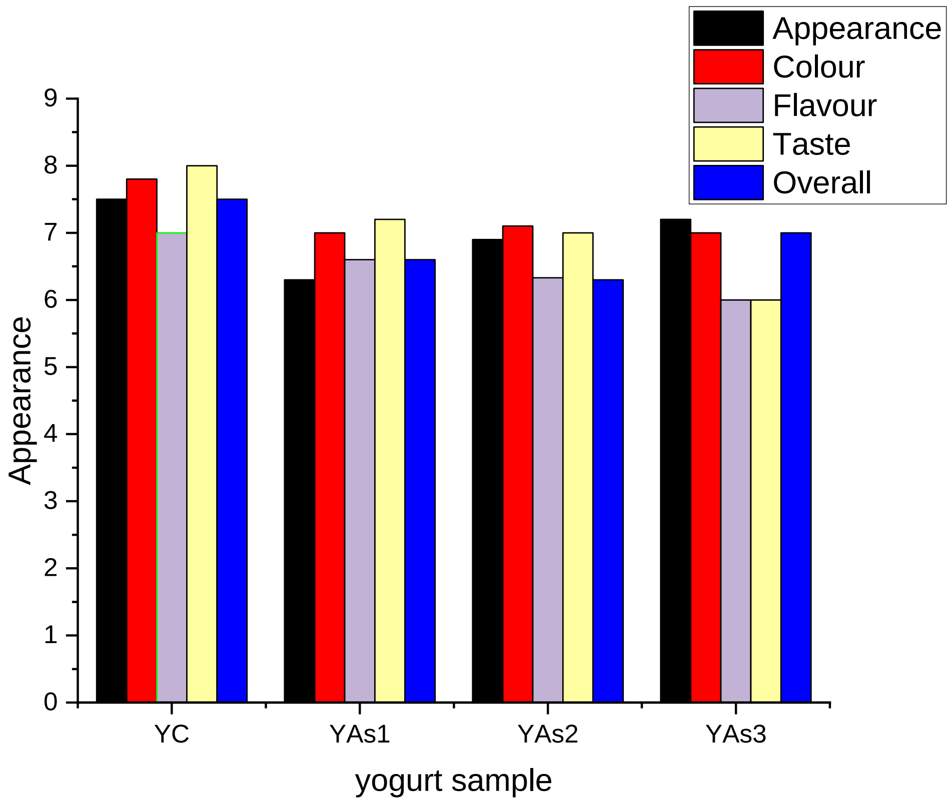

Figure 4.10 sensory plot of yogurt

The statistically there was no significant difference ($p > 0.05$) between Y_C, Y_0, Y_{AS1}, Y_{AS2}, and Y_{AS3} and Y_G for all sensory parameters except for flavor of the yogurt. The result of the study shows that there was a significant ($p < 0.05$) difference between the yogurt sample with different concentrations and control for flavor. This may due to the flavor of the anchote starch which was developed from the raw material. Yogurt sample with 1.50% anchote starch shows good sensory evaluations. The results also showed that addition 1.50% extracted anchote starch gave better result for overall acceptability of the manufactured yogurt which was comparable with the previous work of (Ammar, 2018) which reports that yogurt prepared with potato starch with 0.75 and 1% was the best result for sensory evaluation and syneresis.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study has been carried out to investigate the extraction of starch from anchote tuber which is underutilized crop, optimization, and characterization of the starch. Then the optimum and characterized starch was investigated for yogurt manufacturing as a stabilizer to represent the synthetic one. The optimum yield of the starch was produced with 1.5% NaCl solution which was 20.86% fresh weight basis or 67.82% dry weight basis.

The physicochemical and functional properties of anchote starch also show the good results for food application. The moisture content, ash content, fat content, protein content, total carbohydrate contents were within the standard range. The XRD result shows that of anchote starch has crystalline property. The extracted anchote starch was applied to yogurt manufacturing with different proportions (0.5,1,1.5%). Then the yogurts prepared were investigated for different physicochemical analyses and sensory evaluations.

The physicochemical analyses yogurt such as pH, titratable acidity, and syneresis showed good results. Yogurt prepared with 1.5% anchote starch showed the best result of pH value between 4-4.5. The addition of a stabilizer has an advantage on yogurt quality such as syneresis or whey separation and stability during storage. The addition of 1.5% anchote starch gives the best result with lower syneresis and good stability of yogurt. The sensory evaluation also indicates that the addition of a 1% anchote starch gives the best result. Only the flavor and taste give the lower score and but the overall acceptability of the yogurt is good.

5.2 Recommendation

From this study, I have recommended the following points.

1. Further study on feasibility and commercial viability of anchote starch production
2. Other food application of anchote starch and product development should be studied
3. Mineral content and thermal properties of the starch should be studied

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APPENDICES

Appendix –A Tables

Table A:1 Sensory Evaluation Table

Sensory attributes	Samples code:								
	9	8	7	6	5	4	3	2	1
Appearance									
Colour									
Taste									
Flavor									
Overall acceptability									
Remark (if any)									

Date _______________time______________________name and sign______________

Scores on the hedonic scale: *9: extremely like, 8: like very good, 7: like good, 6: like moderately, 5: neither like nor dislike, 4: dislike moderately, 3: dislike fairly, 2: dislike very much, 1: dislike extremely.*

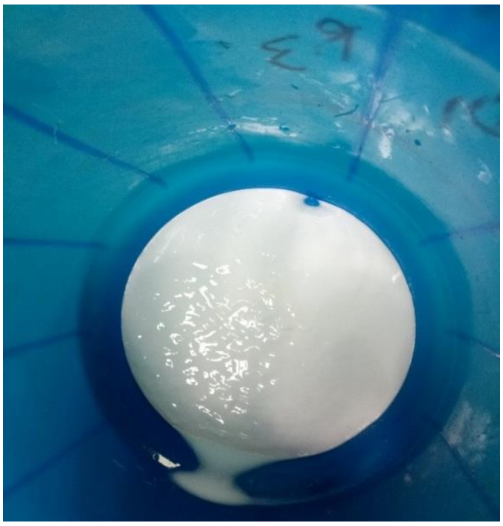
Appendix-B Photos taken during practical work

Raw anchote tuber set for sell

Peeled anchote tuber

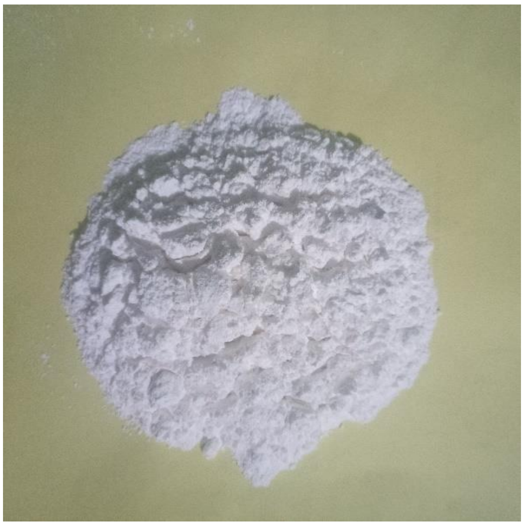
Sliced tuber

Milling and blending



Filtration

Wet starch



Drying in oven dryer

Starch powder

Ash content determination

Ash