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Investigating the Efficiency Of Phenolic Compounds Extracted From Potato Peel As A Preservative Agent For Local Cow Butter

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INVESTIGATING THE IFFICIECY OF PHENOLIC COMPOUNDS EXTRACTED FROM POTATO PEEL AS A PRESERVATIVE AGENT FOR LOCAL COW BUTTER

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BY

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INVESTIGATING THE IFFICIECY OF PHENOLIC COMPOUNDS EXTRACTED FROM POTATO PEEL AS A PRESERVATIVE AGENT FOR LOCAL COW BUTTER

By

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A Thesis

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Bahir Dar, Ethiopia

DECLARATION

I, the undersigned, declare that the thesis comprises my own work. In compliance with internationally accepted practices, I have dually acknowledged and refereed all materials used in this work. I understand that non-adherence to the principles of academic honesty and integrity, misrepresentation/ fabrication of any idea/data/fact/source will constitute sufficient ground for disciplinary action by the university and can also evoke penal action from the sources which have not been properly cited or acknowledged.

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ABSTRACT

This research mainly studies the effect of potato peel phenolic compound on cow butter as a preserving agent. It was extracted from potato peel (solanium tuberosom species) waste which delays spoilage and oxidation of most oil and lipid containing foods. It inhibits these foods from microbial spoilage and oxidation reaction formation causing undesirable and toxic product formation. This phenolic compound is extracted from the peel by the method of maceration extraction with ethanol solvent. The percentage yield obtained was 10.42 ± 0.03% and the presence of major phytochemicals (phenol, flavonoid and tannin) were checked. The total phenolic compound was determined by Folin–Ciocalteau methodology with uv-spectrophotometr and the total flavonoid content was determined by the method of aluminum chloride colorimetric method. From this, a total phenolic content of 2.9468± 0.03 mg GAE/g of dry extract and a total flavonoid content of 3.6885±0.02 mg Quercetin equivalent /g of dry extract were obtained. The potato peel extract was then applied on cow butter with different concentrations (0.2%, 0.3% and control) and two storage temperatures (20^{o}C and 45^{o}C) for a total storage time of 21 days. So that the microbiological (aerobic total bacterial count and yeast-mould count) and chemical parameters (peroxide value and free fatty acid value) were analyzed. The result indicated that from the two storage temperatures storing a butter sample at 20^{o}C and an extract concentration of 0.3% showed better preservation for aerobic total bacterial count, peroxide value and free fatty acid value than other treatments done here. For yeast-mould count storing butter sample at a temperature of 45^{o}C and 0.3% extract concentration shows lower number than the rest treatment. But storing the butter sample at high temperature (45^{o}C) without extract caused high number of bacteria and high formation of peroxide value and free fatty acid value.

Keyword: - potato peel phenolic extract, cow butter, preservation effect

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ABBREVIATIONS

AOAC	association of official analytical chemist
AOCS	American oil chemist society
BHA	butylated hydroxyl toluene
BHT	butylated hydroxyl toluene
CGA	chlorogenic acid
FFA	free fatty acid
GAC	Gallic acid
OS	oxidative stability
PG	propyl gallet
PPE	potato peel extract
PP	potato peel
PPP	potato peel powder
ROS	reactive oxygen species
TBHQ	ter-butyl hydroquinone
UV	ultra violet

1. INTRODUCTION

1.1 Background

Potatoes (Solanum tuberosum) are, after maize, wheat, and rice, one of the most cultivated crops around the world (Silva-Beltrán et al. 2017). Ethiopia is one of the principal potato producing countries in Africa and probably displays a unique position for having the highest potential area for cultivating potatoes (Anon., 2000). Central, Eastern, North-Western and Southern regions, constitute approximately 83% of the potato farmers in the country. Most of the available agricultural land is suitable for potato production (Teshome 2016).

Many researchers suggest that Potatoes possess many health benefit properties like antihypertensive (Vlachojannis et al., 2010), anticancer (Yang et al., 2006) and antimicrobial effects (Jin et al., 2009). Both the pulp and peel parts have beneficial properties; however, the peel is often discarded as a by-product during potato food processing. Phenolic are concentrated in the peel and adhesive tissues and found to be low in the Tube (Singh and Saldaña 2011). Previous reports have shown that potato peels (a waste by-product of potato processing) have antioxidant activity in several assay systems (Singh and Rajini 2008) and the aqueous extract of PP is rich in various phenolic acids, including hydroxycinnamic acids and flavonoids, which have strong antioxidant capacity (Albishi et al., 2013) and offer therapeutic effect, including protection of erythrocytes, without having any mutagenic effects (Singh and Rajini 2008).

Oil or lipid oxidation is a free radical chain process leading to the deterioration of oil and lipid containing materials (Sashidhara et al., 2006). In foods, these reactions can lead to rancidity, loss of nutritional value from the destruction of vitamins (A, D and E) and essential fatty acids and the possible formation of toxic compounds and colored products. Oxidation reactions may involve highly reactive molecules called free radicals. Free radicals are molecules that have lost an electron and try to replace it by reacting with other molecules. This causes the substance to break down. In our bodies, this break down may be a primary cause of Cancer. However, addition of some suitable antioxidant in fats and oils retard the oxidation process (Zia-Ur-Rehman et al., 2004). In order to overcome the stability problems of oils and fats, synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and ter-butyl hydroquinone (TBHQ) have been used as food additives. But, recent reports revealed that these compounds may be implicated in many health risks, including cancer and carcinogenesis (Yousr et al., 2017).

Phenolic compounds are the main class of natural antioxidants (Atoui et al., 2005). Therefore, as sources of natural antioxidants much attention is being paid to plants and other organisms. Thus interest in natural antioxidant, especially of plant origin, has greatly increased in recent years (Jayaprakasha and Jaganmohan Rao 2000). Potato peel contains phenolic acids, the largest part consists of chlorogenic acid (CGA), other phenolics, Gallic acid (GAC), caffeic acid (CFA) and protocatechuic acid (PCA), are present in potato peel in low amounts.

In this research study, it is assuming to extract phenolic compound from potato peel and see the effect of antioxidant on cow butter preservation.

1.2 Statement of Problem

Cow butter which is highly fat containing dairy product is easily spoiled and oxidized so that needs to preserve from spoilage and oxidation reaction formation. The present trend to preserve butter in most residents is use of herbs and spices. but they are costly, not easily accessible everywhere at all the time, there is a need to searching for other natural source that is more economical, safe and rich in antioxidant compounds.

After potato processing, a large amount of waste is generated in the form of peels and trimmings, causing handling and storage problems. The potato peel is a good source of phenolic compounds. As Phenolics exhibit many health benefits like anti-mutagenic, anti-carcinogenic, anti-glycemic, anti-cholesterol and anti-microbial properties that avoid neurodegenerative and cardiovascular diseases it is a good way of changing the non-used part in to a usable one for inhibiting fat and lipid containing food products.

1.3 Research Objectives

1.3.1 General Objectives

The main objective of this thesis work is investigating the efficiency of phenolic compound on cow butter as a preservative agent by extracting it from potato peel.

1.3.2 Specific Objectives

- To extract the phenolic compounds from potato peel using ethanol as a solvent and analyze the extracted compound.

- To analyze some characteristics parameter (total bacterial count, yeast-mould count, peroxide value and free fatty acid value) of cow butter by adding the extract on it.

1.4 Scope of the Study

This research, as described in the objective, basically studies the preserving properties of phenolic compound on cow butter by extracting it from potato peel solanum tuberosom species with maceration extraction and ethanol solvent. In addition to applying on cow butter, some extract parameters including percentage yield, phytochemical screening test, total phenol and flavonoid quantity were also measured. To see its preserving property some microbiological and chemical characteristic parameters including total bacterial count, yeast-mould count, peroxide value and free fatty acid values were analyzed for 21 days with two storage temperature (20^oC and 45^oC) in the chemical & food engineering faculty laboratory of Bahir Dar institute of technology. The necessary chemicals, materials and equipment were collected in the laboratory from available places.

1.5 Significant of the Study

The study basically tends to solve the problem of cow butter oxidation and microbial growth on it. Microbiological growth and oxidation reaction formation of lipid and oil containing food and toxic formation which causes human health problem can be reduced by using this easily available natural extract. It also minimizes the waste formation from the potato peel as it changes to usable product.

2. LITERATURE REVIEW

2.1 Introduction

Potato (Solanum tuberosum L., Solanaceae) is one of the basic crops grown worldwide and plays an important role in human nutrition. It is the major dietary sources of phenolics and of a number of antioxidants (Kolasa 1993). (Weshahy and Rao 2009) says in addition to direct consumption, processed potato products, such as French fries, chips, mashed potato, crisps, starch, potato flakes, flour and puree can be used.

In processing, 10–25% of the raw product is discharged as waste. Peels are the major waste of potato processing that is perishable and cause many management problems in terms of disposal and sanitation (Mohdaly, et al., 2013) . Thus, new aspects concerning the use of these wastes as by-products for further production of food additives or supplements with high nutritional value have gained increasing interest because these bioactive are high-value products and their recovery may be economically attractive (Oreopoulou and Russ 2007).

Recent reports revealed that synthetic antioxidants e.g., butylated hydroxyanisole [BHA], butylated hydroxytoluene [BHT], tert-butyl hydroquinone [TBHQ]) may be implicated in many health risks, including cancer and carcinogenesis. Due to these safety concerns, there is an increasing trend among food scientists to replace these synthetic antioxidants with natural ones, which are supposed to be safer (Marinova et al., 2001).

Food lipids undergo a variety of chemical reactions, such as accelerated oxidation, thermolysis, and polymerization under heat exposure(Marinova et al., 2001). The oils with higher contents of unsaturated fatty acids, especially polyunsaturated fatty acids, are more susceptible to oxidation. It is, therefore, important to evaluate the oxidative stability (OS) of oils and fat as affected by processing and storage conditions (Mohdaly et al., 2013). Lipid oxidation could not only affect the nutritional and flavor quality of foods, but also produce free radicals which are responsible for health problems and accelerated aging (Park et al., 2014). thus free radicals also have high capacity to attack cell molecules in the body and further interfere with the cell structure and affect their functions (Lobo et al., 2010).

In recent years' studies have shown the potential of using potato peels as a source of natural antioxidants. Therefore these anti-oxidants are used to increase the storage life of several food products, including soybean oil (Zia-Ur-Rehman et al., 2004) and processed lamb meat (Kanatt et al., 2005). In industrial processes they can extend shelf life of the food and cosmetics and prevent degradation of the rubber and gasoline (Gao 2014).

2.2 Food Oxidation

A chemical reaction that transfer Electron from a substance to an oxidizing agent which is achieved by the free radical chain reaction is termed as oxidation. It causes food quality losses during preparation processing and storage that leads the development of deterioration and rancidity of oil and lipid containing food products. The loss of nutritional value like vitamins, aroma, test and texture degradation comes from these lipid oxidations

(Sashidhara et al. 2006). This makes the addition of antioxidants in most lipid containing foods.

Lipid oxidation product is basically facilitated by factors like oxygen, light, temperature, relative humidity and some metals (Y. S. Lee et al., 2004). Oxygen uptake in fried foods such as potato chips is the lipid oxidation mechanism. Lipid oxidation in meat and dairy products is the major causes of spoilage and deterioration of organoleptic properties leading to off-flavor development, color degradation and nutritive loss (Kansci et al., 1997).

2.3 Lipid Oxidation in Foods

In many foods the quality development like flavor, texture and color is because of the presence of lipids. But the high unstability to oxygen attack leads to undesirable odor and flavor compound formation. Lipids may be classified as saturated or unsaturated depending on their bond structure as saturated lipid implies all carbon atoms bound to as much hydrogen as possible and that of unsaturated fatty acids have mono-unsaturated or poly unsaturated double bonds between carbon atoms. Foods such as oil, fish, and meat and dairy products with high level of unsaturated fats are easily exposed to oxidation as their double bond is easily attacked to oxygen. This reaction leads the formation of free radicals and oxidation products that limits the shelf life of many lipids and lipid containing products. In food industry it is the major economic concern as it lowers the nutritional quality as well as flavor, and textural behavior damage which are unacceptable for consumers. (Ballard, 2008).

Undesirable color and textural development comes from the interaction of secondary oxidation product malondialdehyde (MDA) and 4-hydroxynonenal (4-HN) with protein or amino acids. Darkening of food comes from condensation reaction between oxidation product and protein which is undesirable color change. Many key nutrients in milk like vitamin B2 (riboflavin) and vitamin C (ascorbic acid) destroy light induce lipid oxidation or photo oxidation (Ballard 2008).

2.3.1 Mechanism of Lipid Oxidation

Food lipid Oxidative deterioration comes mainly because of auto-oxidative reactions and accompanied by various secondary oxidative and non-oxidative reactions. The cause for Auto oxidation of unsaturated fatty acids is a free-radical chain reaction mechanism (fig. 2.1) which involve initiation (formation of free radicals), propagation (free-radical chain reaction), and termination steps (formation of non-radical species) (Akyol et al., 2016).

A. Initiation

The initiation step is the step where fats and lipids containing unsaturated fatty acids with labile hydrogen atom react with oxidizing agents like singlet oxygen, transition metals and free radicals, which results the formation of lipid free radicals ($R^{\cdot}$). These unstable free radical immediately react with oxygen to form lipid peroxyl radicals ($ROO^{\cdot}$).

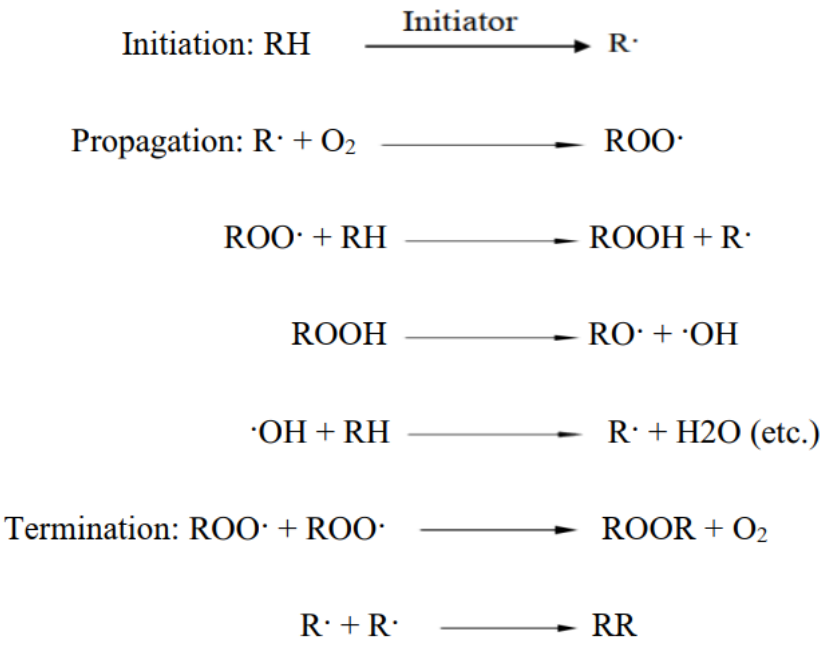
B. Propagation

At this stage the unstable lipid peroxyl radical abstracts a hydrogen atom from another unsaturated fatty acid and form a lipid hydroperoxide (ROOH) and another $R^{\cdot}$ which causes

for the formation of free radical. Again the unstable lipid hydroperoxide further degraded into aldehydes, ketones, acids and alcohols. These are responsible for the development of off-flavours and odours and further reactions with other food constituents like proteins and free amino acids (Akyol et al., 2016).

C. Termination

In the last stage free radicals bind with each other to give the non-radicals species and the lipid oxidation cycle completed here.



Source (Singh 2010)

2.4 Free Radical Formation

Free radicals are formed when weak bonds split which are very unstable and react quickly with other compound by attracting the electron in order to be stable. The compound that lost its electron again becomes free radical and the process continue which cause the disturbance of many fatty foods. Many external factors may contribute for the formation of free radicals in the body including herbicide, cigarette smoking, pollution and radiation so that antioxidants are needed to control such free radical formation in the body or any substance that forms oxidation (Hamid et al., 2010).

2.5 Antioxidants

Antioxidant may be defined as any compound that inhibit or delay the oxidation of other molecule by interacting with free radicals to make them stable unless which causes cell damage leading the occurrence of disease in the body like cancer. These antioxidants by oxidizing themselves remove free radical intermediate and inhibit other oxidation reaction using as a reducing agent. Food antioxidants also play a role in physiological and dietary antioxidants such as ascorbic acid, proteins, flavonoids and other phenolic compounds there by supplementing the body's natural resistance to oxidative damage (Sies 1997) and the behavior of antioxidant (Walter and Purcell 1979).

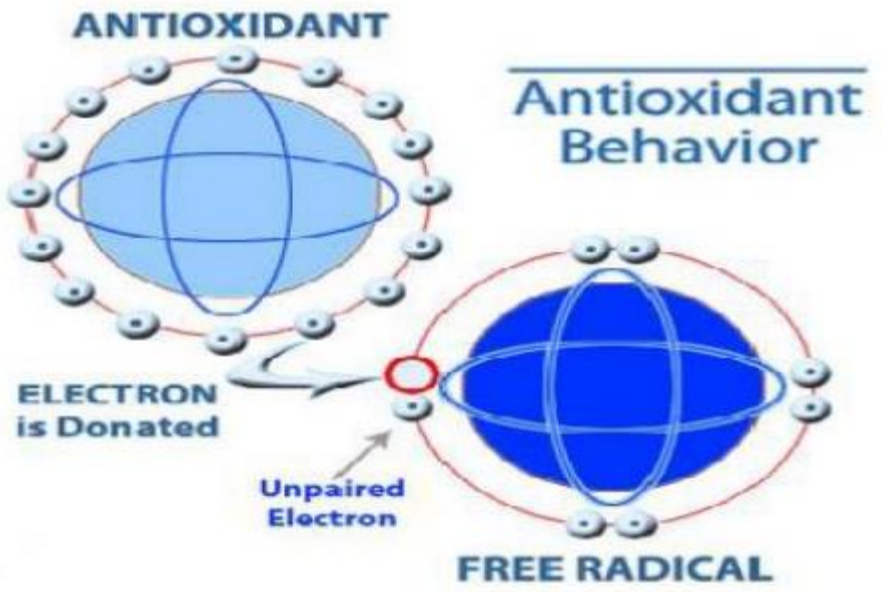


Figure 2. 1 Antioxidants Donating an Electron to Free Radical Molecule

Antioxidants interact the damaging effect of free radical in the tissue these protect the body from different disease like heart diseases, cardiovascular, cancer etc. fruits and vegetables with several antioxidants against these different diseases. Dietary antioxidants like α-tocopherol, α-carotene, and ascorbic acid protect cell from damage. Studies show that multiple hydroxyl group phenolic are most efficient in preventing lipid and low density lipoproteins (LDL) oxidation (Moure et al., 2001).

2.5.1 Classification of Antioxidant

Generally, antioxidant is defined as natural or synthetic compound which can act in many ways, like scavenging reactive oxygen species or their precursor, inhibiting the reactive oxygen species (ROS) formation and binding desired metal ion to the catalysis of ROS generation (Gilgun-Sherki 2002).

Natural antioxidant

Natural products like plant or animal are the major sources of natural antioxidant. They extend the shelf life of many oil, fat and fat soluble products by retarding their oxidation which causes the formation of unpleasant flavors and odors. Especially oils, fats, and products with a high fat content are susceptible to oxidation and require the addition of antioxidants. Most of the time processing may lose some natural antioxidant from plant or animal source. So that supplementation will be mandatory unless the product may lost its acceptable quality parameter. It has been reported that plant polysaccharides or their derivatives have strong antioxidant activities and considered as novel potential source (J. Lee et al., 2008).

The major components of polyphenol antioxidants includes: phenolic compound, flavonoid and tannin.

A. Phenolic Compounds

Phenolic compounds found in almost all plants especially vegetables, fruits and beverages are the major sources of these compounds (Balasundram et al., 2006). Phenolic compounds include phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, quinones, and others. The antioxidant activity of phenolic compounds is due to their ability to scavenge free radicals by donating hydrogen atoms or electrons. These compounds have high range of physiological properties including anti-allergenic, anti-artherogenic, anti-inflammatory, antimicrobial, antioxidant, cardioprotective and vasodilatory effects. They

also protect from pathogens, parasites and predators as well as contribute to the color of plant (Huang et al., 2010).

B. Flavonoid Compounds

According to the report of (Garg et al., 2001), the broad spectrum of biological activities within the group and the multiplicity of actions displayed by certain individual members make the flavonoids group one of the most intriguing classes of biologically active compounds which is called "bioflavonoids". Flavonoids are the most potent anti-oxidative compounds of plant phenolic which occur in vegetable, fruits, berries, tea leaves and herbs (Skrede and Wrolstad 2002). Chemically, flavonoids and isoflavonoids are one-electron donors and derivatives of conjugated ring structures and hydroxyl groups having the potential function as antioxidants in cell culture in vitro, or in cell free systems (Sun et al., 2002).

Flavonoids are very important constituents of plants because of the scavenging ability conferred by their hydroxyl groups that contribute directly to anti-oxidative action and known to be good natural antioxidants. Flavonoid compounds occur in particularly all parts of plants including fruits, vegetables, nuts, seeds, leaves flowers and bark. Bioflavonoids were found to be essential components in correcting this bruising tendency and improving the permeability and integrity of the capillary lining that include hesperidin, citrin, rutin, flavones, flavonoids, catechin and quercetin (Garg et al., 2001).

Flavonoids have the general structural backbone of C6-C3-C6. The two C6 units are of phenolic nature. Flavonoids can be further divided as flavan-3-ols, flavones, flavanones

and flavonols (Figure 2.3). The most common flavonoid includes Quercetin, myricetin, Catechins, etc. (Shewakena 2015).

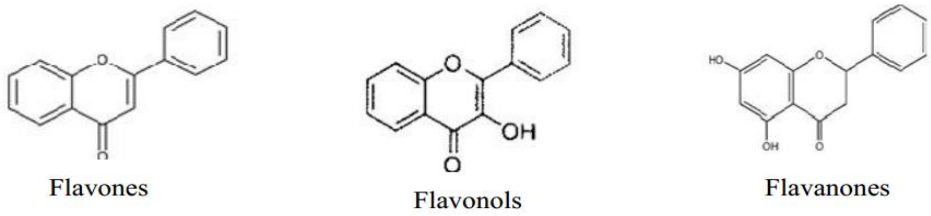


Figure 2. 2 Examples of Flavonoids

C. Tannin

Tannins are the other basic class of polyphenol which can be classified into two major groups as hydrolysable tannins (hydrolyzed with acids, bases or enzymes) and non-hydrolysable (condensed) tannins like polymers of catechin and leucoanthocyanidin that are not readily hydrolyzed by acid treatment and responsible for the characteristics astringency of the vegetables and fruits which feels the mouth dry and pucker like that of red wine, strong tea or un-ripened fruit. The bitter test of hop is also comes from tannin (Reis Giada 2013; Shewakena 2015).

A. Pentagalloyl glucose (gallotannins); B. Procyanidin (polymer of catechins)

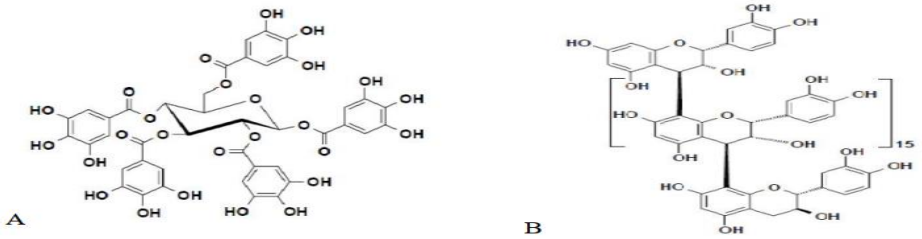


Figure 2. 3 Hydrolysable and Non- Hydrolysable Tannins

Source of natural antioxidants

In recent years, because of adverse health effect, many research has been focused on identifying sources of natural antioxidants that can be used to replace their synthetic antioxidant. The main sources of natural antioxidants are of plant origin. Some animal sources like shrimp have also been found. The most common plant sources are cereals, citrus fruits, cocoa and coffee beans, herbs and spices, oilseeds, olives, onion and garlic, potato, tea, as well as some miscellaneous products (De Rosenzweig Pasquel and Babbitt 1991)

Table 2. 1 Some Phenolic Compounds with Sources

Antioxidants	Precursor	Source
Phenolic acid	Caffeic acid	Coffee, tea
	Gallic acid	Land plants and aquatic plant(myriophyllum spicatum)
	Chlorogenic acid	Potato, bambo, peach, prunes
Stillbenes	Resveratrol	Grape seeds and peel
Others	Curcumin	Turmeric

Sources: (Milbury and Richer 2013)

Synthetic Anti-Oxidants

Synthetic antioxidants have been widely used to extend the shelf-life of various food materials. The current preference for synthetic antioxidants can be attributed to their proven effectiveness in a variety of food systems and their relative low cost when compared to natural antioxidants. The most commonly used synthetic antioxidants in the food industry include BHT, BHA and TBHQ. The use of PG has been limited by its tendency to cause undesirable color changes. BHT and BHA are hydrophobic phenolic antioxidants that inhibit free-radical initiated chain reactions (Gao 2014). Protection against lipid oxidation may occur as a result of the formation of a BHT radical, which is thought to have a lower reduction potential than that of lipid peroxyl radicals. BHA is commonly used in combination with BHT or PG which creates a synergistic effect. TBHQ is less volatile than BHA and BHT but is stable at higher temperatures. Due to the stability of TBHQ at elevated temperatures, it has proven to be more effective in polyunsaturated vegetable oils (Ballard 2008).

Although synthetic antioxidants are extremely effective at slowing oxidation, there have been recent consumer concerns over potential adverse health effects associated with these compounds. Studies have reported that BHT and BHA cause a wide range of health problems including, enlarged liver, increased liver microsomal enzyme activity and conversion of some ingested materials into toxic and carcinogenic substances, especially if they are present in excessive amounts. Many processors wish to avoid adding synthetic antioxidants to foods to eliminate these health concerns and to be able to state on the label that the product is “all natural”(Ballard 2008).

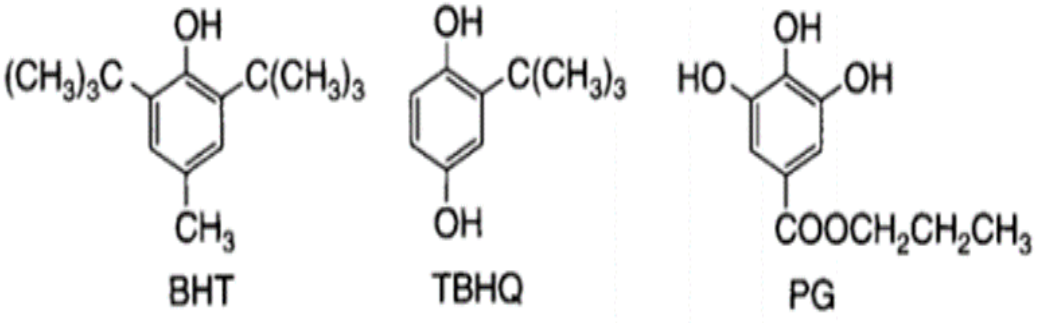


Figure 2. 4 Chemical Structures of Synthetic Antioxidants

2.5.2 Antioxidant Mechanism of Action

Two principle mechanisms of action for antioxidants are chain breaking mechanism and reactive oxygen species or reactive nitrogen species initiator where the first one the primary antioxidant donate electron to present in the system like lipid radicals and the second one is by quenching chain initiator catalyst.

Free radical chain reactions

- **Initiation stage**

1) $RH \longrightarrow R^{\bullet} + H^{\bullet}$

2) $R^{\bullet} \longrightarrow R^{\bullet} + O_2 \longrightarrow ROO^{\cdot}$

3) $2ROOH \longrightarrow ROO^{\cdot} + RO^{\cdot} + H_2O$

❖ **Propagation Stage**

1) $R^{\bullet} + O_2 \longrightarrow ROO^{\bullet}$

2) $ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet}$

3) $RO^{\bullet} + RH \longrightarrow ROH + R^{\bullet}$

❖ **Termination Stage**

1) $R^{\bullet} + R^{\bullet} \longrightarrow R—R$

2) $R^{\bullet} + ROO^{\bullet} \longrightarrow ROOR$

3) $ROO^{\bullet} + ROO^{\bullet} \longrightarrow ROOR + O2$

4) Antioxidants + O_2 ⟶ oxidized antioxidants (Hamid et al., 2010)

2.5.3 Action of Antioxidant in the Body

Unsaturated fatty acids are formed when fats are in contact with oxygen during free radical chain reaction and this gives rise to free radicals. Hydro peroxide break down to yield radicals that abstract a hydrogen atom from another molecule and become a hydro peroxide producing further radicals. The antioxidants added to it, will neutralize the free radicals by donating one of their own electrons ending the reactions.

A. Health Benefits of antioxidant for Human

As it is described before antioxidants could exhibit antibacterial, antiviral, ant carcinogenic, anti-inflammatory and vasodilatory performance, they have been used for protection of food and cells from oxidative degeneration (Mattila & Hellstrom , 2007). Antioxidant like phenolic acids are reported to have the ability to inhibit the growth of human cancer cells and decrease the possibility of hepatoma and human immunodeficiency virus (HIV) replication (Finotti, et al., 2012). The Function and Health Benefit of Common Antioxidants (Milbury and Richer 2013) are shown in table below.

Table 2. 2 Function and Health Benefit of Common Antioxidants

Name	Function	Health benefit
Vitamin A	Combing with protein opsin to form rhodopsin	Inhibition of cancer, HIV and dermatological disease
Vitamin C	Rendering harmful free radical reactions harmless	Preventing or delaying food spoilage (enzymatic browning reaction)
Vitamin E	Breaking chain by preventing lipid oxydation	
selenium	Defending enzymes	Reducing the odds of prostate cancer
carotenoids	Converting to vitamin A	Decreasing the risks of cancers and eye disease
phytochemicals	Modulating cell metabolism and direct quenching of radicals	Increasing capillary strength and decreasing the risk of diseases

2.6 Microbial activity of phenolic compounds

According to the report by (Awad and Ahmed 2003) an increased number of hydroxyle groups on phenol group are thought to be related to their relative toxicity to micro-organism. Phenolic compounds possessing a C3 side chain and containing no oxygen are classified as essential oils and used as antimicrobial.

Flavones and flavonoid phenolic compounds are known to be synthesized by plants in response to microbial infection and their antimicrobial activity is by making complex with soluble proteins and bacterial cell walls. Flavonoids may also disrupt microbial membranes (Awad and Ahmed 2003) .The author also suggests that, as tannin are found in most plant part; bark, wood, leaves, fruits and roots, their antimicrobial activity is probably by making irreversibly complex with nucleophilic amino acids in proteins which inactivate protein

2.7 Ways of Extraction for phenolic compounds

Extraction is use of standard procedures to separate medicinally active portions of plant which are soluble in the selective solvents from insoluble cellular residues (Azwanida 2015).

Several methods used for the extraction of phenolic compound from plants. From thus one of the most known methods is maceration extraction which uses different organic solvents like water, ethanol, ether and methanol. It enables to improve the efficiency, quality of the extract, extraction time and consumption of solvent (Viera, V.B et al., 2017).

According to the report by (Azwanida 2015), different extraction methods used to extract phenolic compound and some of these are discussed below:

Maceration extraction: it involved soaking powdered plant materials with a selective solvent and allowed to stand at room temperature for a period of minimum 3 days with frequent agitation in order to breaking the plant's cell wall and release the soluble phytochemicals followed by filtration. This method is easiest and simple but takes large volume of solvent.

Soxhlet extraction or hot continuous extraction: finely ground sample is placed in a porous bag or "thimble" which is made from a strong filter paper or cellulose and placed in the thimble chamber of the Soxhlet apparatus. Extraction solvents is heated in the bottom flask, vaporizes into the sample thimble, condenses in the condenser and drip back. After reaching to the siphon arm, the liquid content emptied into the bottom flask again and the process is continued.

This method uses low extraction solvent than maceration extraction.

Microwave assisted extraction (MAE): it uses microwave energy to facilitate partition of analytes from the sample matrix into the solvent. It can be considered as selective methods which favour polar molecules and solvents with high dielectric constant.

The advantage of this technique is that, reduced extraction time and solvent volume than maceration & Soxhlet extraction methods. However, this method is limited to small-molecule phenolic compounds.

Ultrasound-assisted extraction (UAE) or sonication extraction: involves the use of ultrasound energy ranging from 20 kHz to 2000 kHz causing to increases the surface contact between solvents and samples and permeability of cell walls.

The advantage of UAE is mainly reduction in extraction time and solvent consumption. But, ultrasound energy more than 20 kHz may have an effect on the active Phytochemicals through the formation of free radicals.

Phenolic compounds were usually extracted with organic solvents from plant material, such as methanol, acetone, ethanol and ethyl acetate (Dai & Mumper, 2010). However, very polar phenolic acids (benzoic, cinnamic acids) could not be extracted completely with pure organic solvents, and mixtures of alcohol–water or acetone–water are recommended.

The Folin–Ciocalteu assay is also used for the determination of the total content of plant food phenolic compounds (Singleton, et al., 1965). Higher extraction yields of phenolics are achieved by milling the sample into smaller particle sizes which facilitate enzymatic action and extraction (Giao, et al., 2009) . Spectrophotometry is one of the relatively simple techniques for quantification of plant Phenolics. The Folin-Denis and Folin-Ciocalteu methods were the two widely used spectrophotometric assays to measure total Phenolics in plant materials for many years.

Lowering particle size increases surface contact between samples and extraction solvents and powdered samples with smaller particle size show better surface contact with extraction solvents. for efficient extraction to occur, the solvent must make contact with the target analytes and particle size smaller than 0.5 mm is ideal for efficient extraction

(Azwanida 2015). Because of the advantage that to get high extraction yields of phenolics, this thesis study extract the phenolic compound with lower particle size (0.5 µm).

2.8 Application of Antioxidant in the Food Industries

Antioxidants have a wide range of applications in different sectors including food, medical, polymer etc.

As most of the dairy product producers are from rural areas of the country, the scarcity of electricity to store their product in a refrigerator which elongate the shelf life time by some time and the highest price and availability of spices is also difficult (Asha et al., 2015).

The report by (Tola et al., 2018) suggested that, although 80% of the butter produced used for human consumption, most of the hot and humid areas of Ethiopia made the dairy product easily spoil unless cooled or treated with preservatives.

Table 2. 3 Commercial Use of Antioxidants to Increase Shelf Life of Lipid-Containing Foods

Antioxidant	Function	List of Food Products
Artificial		
Butylated Hydroxy methylph-enol (BHT)	Free Radical Terminator	Chewing gum, potato flakes, sweet potato flakes, shortenings, enriched rice, dry breakfast cereals, meats, animal fats
Butylated hydroxyanisole (BHA)	Free Radical Terminator	Beverages, ice cream, ices, candy, baked goods, chewing gum, gelatin desserts, soup bases, potatoes, glaceed fruits, potato flakes, sweet potato flakes, dry breakfast cereals, dry yeast, dry mixes for desserts, lard, shortening, unsmoked dry sausage, shortening
Natural		
Rosemary extract	Free Radical Terminator	Meat, fried foods, egg powder, mechanically deboned turkey meat
Ascorbic Acid	Reducing Agent	Sliced peaches, frozen fish dip, dry milk, beer ale, flavoring oils, apple juice, soft drinks, fluid milk, candy, artificially sweetened jellies and preserves

Source (Cottone 2009)

In foods of vegetable origin, lipids are usually more unsaturated than that of animal origin which implies the initiation rate of oxidation reactions is higher and natural antioxidants originally present in foods are more rapidly consumed than in animal source such as lard, tallow and other animal fats. On storage edible oils become rancid. The type of rancid off-flavor depends mainly on their fatty acid composition and the presence of minor

components. Including the addition of antioxidants, edible oil producers prolong the shelf life by different techniques. Appropriate level of antioxidants must be considered during the addition of natural antioxidants (Terefe 2016). Like these edible oil products, fat products like butter needs to prolong their shelf life.

Table 2. 4 Some materials in which potato peels were used as an antioxidant ingredient

Food	Potato type	Criteria
Processed lamb meat	Potato peel (solanium tuberosom cv.'kufri chandramukhi'	TBARS and carbonyle content
Fish rape-seed oil mixture and in oil-in water emulsion	Potato peel (solanium tuberosom cv.'sava' and 'bintje')	Peroxide value, anicidine value, tocopherol concentration and sensory evaluation
Soybean oil, sunflower oil	Potato peel (solanium tuberosom cv. 'Diamond'	Peroxide value, p-anisidin
Minced horse mackerel Ttrachurus trachurus)	Potato peels (solaniumtuberosum'sava' variety)	Peroxide value, volatiles, carbonyl compounds, protected against the loss of a-tocopherol and tryptophan and tyrosin residues
Ground salmon	Potato peels and tubers ('purple', innovator', 'Russet' and 'yellow')	TBARS
Sunflower oil	Potato peel (solanium tuberosom cv. 'Diamond'	primary and secondary oxidation products
Soybean oil	Potato peel (Agrea)	Peroxide, totox and p-anisidin values

Source (Akyol et al., 2016)

2.8.1 Application of Natural phenolic compound on Dairy Food Product

Ethiopians have been using milk and milk products like butter as part of their diet starting from longer times ago. In most of African countries including Ethiopia, the indigenous dairy product is traditionally produced and consumed from different milk sources such as cow, goat, camel, buffalo and sheep. In Ethiopia butter locally called “Kibe” is mostly produced by churning fermented and soured cow milk (locally called “Ergo”). This soured milk is obtained by adding fresh milk to the milk already existed in to traditional container or bottle gourds. Because of the inefficiency of traditional materials and methods of dairy processing, the quality of dairy products including butter is substandard which causes for the occurrence of spoilage when it stored at room temperature for a long time. Locally produced butter, which is semisolid at room temperature, has a pleasant odor when fresh, but with an increase in storage time, changes will occur in odor and taste, unless refrigerated or further processed into traditional ghee (nitir kibe) by boiling with spices (Alganesh and Yetenayet 2017). For this reason treating the dairy products by the natural antioxidants play a role to extend the shelf life of these products.

Butter is an emulsion of water in oil which is an important product in the dairy industry because of its particular sensory attributes and nutritional value. The quality of butter have been the object of numerous studies. The main problem affecting butter quality during storage is rancidification which is a process caused by lipolysis (release of free fatty acids). Lipase which is an indigenous milk enzyme, causes hydrolysis of triacylglycerol which causes for the accumulation of free fatty acid. This free fatty acid accumulation results for the formation of off-flavor or rancid, bitter, unclean, soapy or astringent. Oxidation of the

fatty acids that affect the flavor and lowers the nutritional quality of butter forming serious problems and economic losses in the dairy and food industries. A high content of unsaturated fatty acids in milk fat increases the risk of oxidation and production of off-flavours (Méndez-Cid et al., 2017).

2.9 Traditional methods of butter preservation

Dairy product producers use various traditional preservatives and preservation techniques for extending shelf life of the product mostly by acidifying (milk fermenting), reducing moisture content (melting) and salting included. But as the salt used to preserve in most smallholders is the ordinary coarse salt, the hygiene and quality of it affects the butter quality adversely and increases the total solid (Alganesh and Yetenayet 2017).

Adverse health effect of synthetic antioxidants push for developing antioxidants of natural origin. Because of the reason that use of herbs and spices as main crop are costly, not easily accessible (available) everywhere at all the time, there is a need to searching for other natural source that is more economical, safe and rich in antioxidant compounds. natural extract Addition in dairy products is a newly emerging area which has a vast potential (Méndez-Cid et al., 2017).

2.9 .1 Development of Peroxides in Butter

Peroxide value (PV) indicates the extent of primary oxidation products in lipids and oils that can be measured by the ability to liberate iodine from potassium iodide which indicates the quantity in mg of active oxygen contained in 1 g of lipid (Méndez-Cid et al., 2017).

2.9.2 Free Fatty Acids

Free fatty acids (FFA) are formed by hydrolysis, cleavage and oxidation of double bonds (Asha et al., 2015).

2.10 Factors Affecting Antioxidant Activity

The quality of natural extracts and their antioxidative performances depends on the quality of the original plant, the geographic origin, climatic condition, date of harvesting, storage time, environmental and technological factors which affect the activities of antioxidants from residual sources (Terefe 2016).

3. MATERIALS AND METHODS

3.1 Raw Materials Used

The main raw materials used in this study were potato peel and cow butter.

Potato peel

Fresh red type potato (solanium tuberosom) was purchased from Bahir Dar market where it comes from Hamusit which is 39 km far away from Bahir Dar city. The potato harvested at the age of 90 days. The peels were used for extracting the polyphenolic compound which is a natural preservative and applied on cow butter.

Cow Butter

The butter was bought from Bahirdar city local cow milk processer. Like most Ethiopia butter producers, it was made traditionally by churning sour milk, locally called Ergo, which has been collected over 4 days. The churn was smoked with dried wood chips locally called ‘abalo’ which believed to impart good flavor. As soon as it produced, it was taken to Bahir dar university food microbiology laboratory for microbiological and chemical analysis. After stored overnight in refrigerator at 4 ^{o}**C**, it was then ready for analysis.

3.2 Chemicals and Equipment Used

In order to accomplish this research, the following main chemicals and instruments were used

Table 3. 1 Chemicals and equipment used

Name of chemical or equipment	Uses
Freeze dryer	For potato peel drying
Mortar and mill	For milling of dried potato peel
Sieve	For sieving of milled potato
Magnetic stirrer	For mixing of milled potato peel with solvent
Rotary evaporator	For antioxidant extraction and recovery of solvent
Drying oven	For removing of remaining solvent and storage of butter sample during analysis
UV-visible spectrophotometer	For phenolic compound quantification
Autoclave	For sterilization of chemical and equipment during microbial analysis
Safety hood	For minimizing external contaminant during sample pouring in to petridish
Incubator	For sample storing during microbial and chemical analysis
Ethanol (97%)	For extraction of phenolic compound
Potato dextrose agar (PDA)	For yeast and mold growth during microbial analysis
Plate count agar (PCA)	For total aerobic bacteria growth
Colony counter	For counting of microbial colony
Water bath	Maintained at 45 ^{o}C for microbiological analysis

3.3 Study Design

This research methodology followed the experimental type which designed to answer the research questions and achieve its objectives based on experimental findings. The overall activity and research process were shown in the study flow chart fig. 3.1 below.

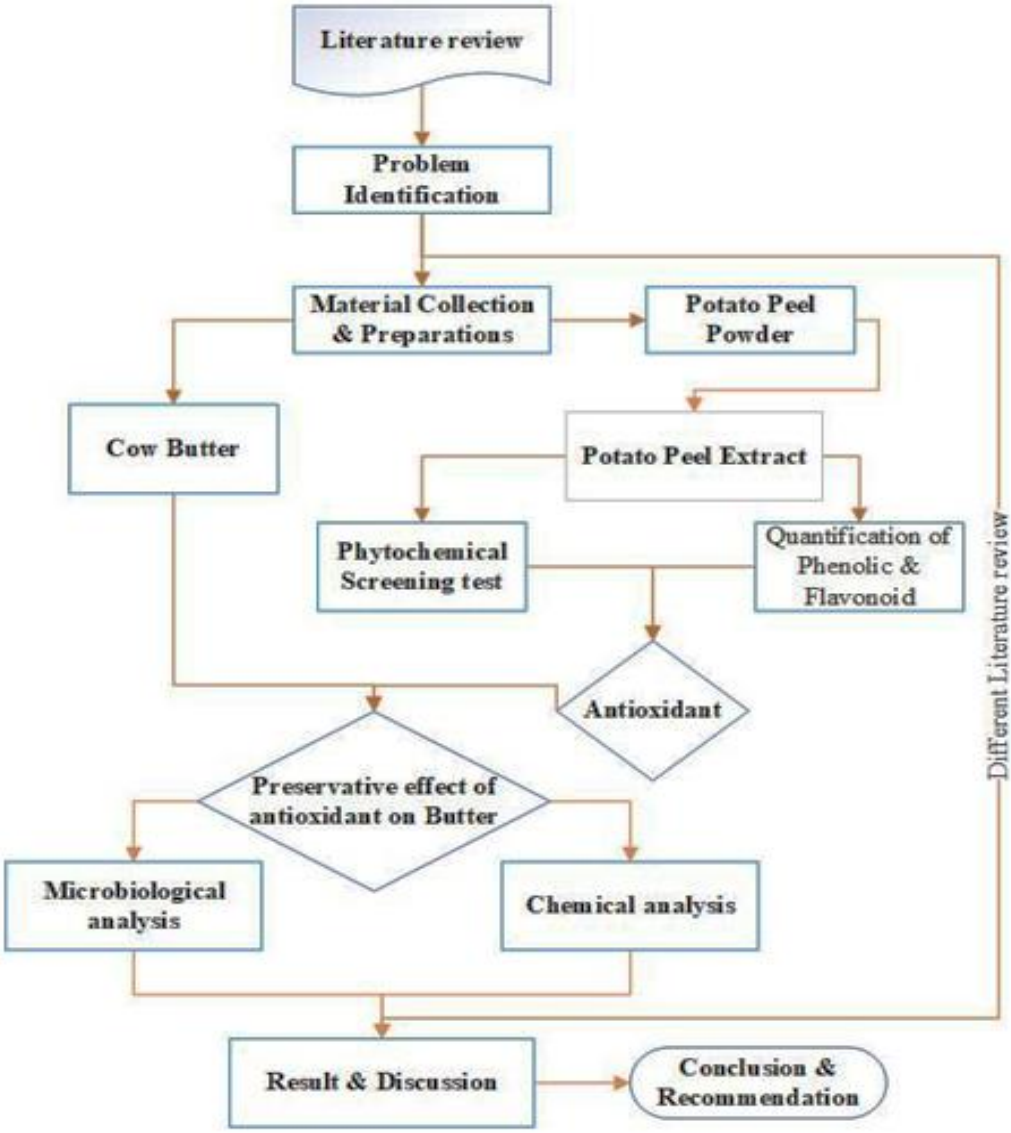


Figure 3. 1 Study Design Flow Chart

3.4 Potato Peel Extract Preparation

The collected Red type fresh potato were thoroughly washed with tap water and remove all foreign matter. The raw potato was peeled manually with a knife with approximately 1mm thickens and then freeze dried for 3 days using a Vertis freeze drier model (LyoCapsule 7-vial Freeze dryer) to avoid degradation of phenolic compound by heating with high temperature. The dried sample is then grinded with mortar and mill and sieved with a 0.5 µm sieve size. The grinded potato peels were sealed in plastic bag and stored in a refrigerator at 4 °C until used for the next analysis. Fig.3.2 shows picture of fresh, dried and powder form of potato.

Figure 3. 2 Potato Peel Extract preparation

The extract preparation was done by using the method described by (Mohdaly et al., 2010) except pp dried by freeze dryer. The lower-polarity solvents, particularly hexane, petroleum ether, and diethyl ether, showed much lower ability to extract phenolic compounds compared with the higher-polarity solvents. For use in the food industry ethanol would be a more appropriate solvent as it is more polar than water. So that at the end of extraction as the antioxidant is applied on the food product, ethanol was used as a solvent for extraction. Thus 10 ml of solvent was mixed with 1 g of powder in the volumetric flask, the mixture then stirred with a magnetic stirrer and allowed for shaking on a shaker of model (Heidolph unimax 2010, Germen), for 3 days at room temperature and then the extract filtered through wathman No 42 filter paper for removal of peel particles followed by evaporating in a rotary evaporator for a temperature of 38°C in order to minimize the phenolic compound degradation with high temperature and finally the remaining solvent removed through evaporation by using drying oven model (Type,M40-VF,MPM instruments, Bernareggio-Italia) with 20°C. Finally the extracted compound sealed in air tight plastic jar and stored in a refrigerator until the parameters characterized and applied on the cow butter.

3.5 Percentage Yield of the Extract

Percentage yield of the potato peel extract was obtained by using the method described by (Terefe 2016) which enable to determine the quantity of sample for extraction purpose.

Percent Yield= [EPP/PPP] ×100

Where: - EPP: - weight of potato peel dry extract after extraction

PPP: - weight of dry potato peel powder before extraction

3.6 Phytochemical Screening Test

As the name indicates phytochemical is the name for plant chemicals. These chemicals may be either primary or secondary metabolite where the first indicate plant component which are essential for growth and reproduction. Their absence in the plant affect the plant negatively. The secondary metabolite are not as essential as the primary metabolites which benefit the plant by imparting color, flavor, aroma etc. (Jayswal et al., 2018).

Qualitative phytochemical test of the sample was done according to the method described by (Jayswal et al., 2018). Standard solution of the plant extract was used for screening of major phytochemicals such as flavonoids, phenols and Tannins.

Standard solution preparation: Stock solution was prepared by dissolving 1g of ethanolic PP extract in 100 mL of ethanol.

3.6.1 Test for Flavonoids:

Alkaline Reagent Test: (It was done with the method described by (S Jayswal et at., 2018)) where 1 mL stock solution was taken in a test tube and added few drop of dilute sodium hydroxide (NaOH) solution. A few drops of 10% hydrochloric acid were added. There was formation of intense yellow color indicating presence of flavonoid. See fig.4.2.A.

3.6.2 Test for Phenols:

Ferric Chloride Test:(It was done with the method described by (S Jayswal et al., 2018)) where 1 mL stock solution was taken in a test tube and added few drop of 10% ferric

chloride ($FeCl_3$). Dark green color was observed, which indicates the presence of phenols in the sample. See fig.4.2.B.

3.6.3 Test for Tannins:

Chloride test: (It was done with the method described by (S Jayswal et al., 2018)) where 1ml of the stock solution was taken in to the test tube with 10% of ferric chloride ($FeCl_3$) addition in the test tube containing the sample and stirred well. Intense greenish black color formed which indicates the presence of tannins. See fig.4.2. C

3.7 Quantification of Total Phenol compound in the PP extract

The total phenolic content was measured by using the Folin-Ciocalteau reagent (FCR) method proposed by (Singh and Saldaña 2011) Where, 0.1 mL sample mixed with 7.9 mL of distill water following the addition of 0.5 ml Folin–Ciocalteau reagent (where its preparation shewed below) and allowed to withstand for 5 min. Sodium carbonate (20% w/v; 1.5 mL) was then added to the mixture. After shacking, the mixture was incubated for 90 min. The Folin-Ciocalteau reagent is sensitive to reducing compounds including polyphenols, thereby producing a blue color upon reaction. The absorbance was then measured at 765 nm using a UV-spectrophotometer where different concentrations of Gallic acid (GAC) used for the standard curve measurement. Finally the results expressed as milligrams of Gallic acid equivalents per g of potato peel powder.

3.7.1 Preparation of Folin-Ciocalteau Reagent

It was done according to the method described by (Singh and Saldaña 2011) where 10g of sodium tungstate ($Na_2WO_4.2H_2O$), and 25 g of sodium molybdate ($Na_2MoO_4.2H_2O$) was dissolved in 70 ml of distilled water in a flask. 0.5 ml of concentrated phosphoric acid and 10ml of concentrated hydrochloric acid was added consecutively and then refluxed for 10 hours. Then it was cooled and 15 g of lithium sulphate, 5 ml of distilled water and 1 drops of bromine was added and allowed to stand for 2 hours. It was then boiled for 15 minutes to expel excess bromine and cooled. Then it was filtered and diluted by 100 ml of distilled water.

3.7.2 Preparation of Sample for Total Phenol Determination

It was done according to the method described by (Singh and Saldaña 2011) where 0.1 g of dry extract was dissolved in 100 ml of methanol to prepare stock solutions of 1000 mg/L. by taking 0.1ml from stock solution, a 5ml sample with 20 mg /L was prepared in triplicate. A series of 20 mg/L, 40 mg/L, 60 mg/L, 80 mg/L and 100 mg/L concentration of methanolic solutions with Gallic acids was also prepared and used as a standard for calibration curve.

3.7.3 Determination of Total Phenolic Content

It was done according to the method described by (Singh and Saldaña 2011) where 1ml Standard Gallic acid solution was taken from each sample in a test tube. 5 ml FCR (10 times diluted) added in each test tube. 4 ml sodium carbonate (7.5%) also added and

allowed to stand for 30 minutes. Absorbency of the sample then read from UV-vis spectrophotometer (PerkinElmer UV-Vis spectrometer, Lambda 35) at 765 nm (data shown in appendix B1). In a similar way sample absorbance also measured in triplicate and total phenol value was obtained from the regression equation and expressed as mg Gallic acid equivalent per gram of sample using the formula,

$C = cV/m$

Where: - C = total content of phenolic compounds in mg GAE /g sample,

c = the concentration of gallic acid (mg/l) established from the curve,

V = volume of extract (0.1 ml) and

m = the weight of pure plant ethanolic extract (0.1 g)

3.8 Determination of Total Flavonoid Content

The total flavonoid content of extract was determined by the aluminum chloride colorimetric method described by (Viera et al., 2017). In a 20 ml volume test tube, initially 1 ml of extract was added followed by the addition of 300 µL of 5% $NaNO_2$ (Sodium nitrite). 5 minutes later, 300 µL of 10% $AlCl_3$ (aluminum chloride) was added and after 6 min, 2 ml of 1 M NaOH (sodium hydroxide) was added. Finally 2.4 ml of distilled water was added. After measuring absorbance of sample at 510 nm using a UV-Vis spectrophotometer (PerkinElmer UV-Vis spectrometer, Lambda 35), it was compared with a quercetin standard curve of concentrations: 0 µg/ml, 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml and 250 µg/ml to determine total flavonoid content and expressed as quercetin

equivalents (QE) per gram of powder on a dry weight basis. concentration verses absorbance table showed on appendix B2.

3.8.1 Reagent Preparation:

it was prepared according to the method described by (Viera et al., 2017) .

Preparation of 5% sodium nitrite: in 100 ml volumetric flask, 5 grams of $NaNO_2$ was dissolved with small amount of distilled water. Then the final volume was made up to the mark with distilled water.

Preparation of 10% Aluminum chloride: in a 100 ml volumetric flask, 10g of aluminum chloride was dissolved with a little distilled water and finally the flask was filled with distilled water up to the mark.

Preparation of 1 M sodium hydroxide: To obtain 1M of this quantity, initially 4 grams of NaOH was taken in 100 ml of volumetric flask and dissolved with little distilled water. Then the final volume was made up to the mark by adding the required amount of distilled water.

Standard quercetin acid solution Preparation: 0.2 grams of quercetin was dissolved into 200 ml distilled water and a concentration of 0.001 gram/ml formed which is the stock solution. Then different concentration solution (0 µg/ml, 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml and 250 µg/ml) was obtained by performing a serial dilution which is used for preparing a calibration curve.

Blank sample Preparation: Blank consists of all the reagents, but the extract or quercetin/ gallic acid standard solution was substituted by 1 ml of distilled water.

3.9 Application of Extract on Cow Butter

The preservative effect of potato peel extract was applied on cow butter which is a dairy food product with high fat content and easily spoiled within a short time unless treated or refrigerated. So that, use of natural plant extract will solve these problems and extend the shelf life by some time and one of such sources of phenolic compound is potato peel extract.

3.9.1 Factors for Application of Extract on Butter and Storage Conditions

The butter samples which were bought from Bahir Dar city of local producer was then treated as, control (free from extract), incorporated with 0.2 % and 0.3 % PPE (in weight bases) stored at two storage temperatures 20 °C (T1) & 45 °C (T2) for 21 storage days. The effect of preservative were analyzed with intervals of 4 days for microbiological analysis (total aerobic bacteria & yeast-mould count), chemical analysis (peroxide value and free fatty acid value) of butter samples were analyzed on 1^{st}, 5^{th}, 9^{th}, 13^{th}, 17^{th} and 21^{st} days of storage with duplicate measurement.

Here during application of extract on cow butter as the PPE were extracted at an optimum condition of sample size 0.5µm, solvent type ethanol and sample to solvent ratio 1 g: 10 ml, the quantity and quality of main phenolic compounds were checked and finally it was applied on cow butter. It was applied on the butter sample by dissolving on a water bath

adjusted at a temperature of 46°C for uniform mixing and then it was stored to its storage temperature. Factors taken during application were then extract concentration, storage temperature and time which play a role for storage condition of butter. The extract concentration is the main factor as it was applied at different weight based percentage value it affect the stored sample. Like this, storage temperature also affect the butter sample where at different temperature occurrence of different reaction formed and growth of both bacteria and yeast-mould also affect by temperature. Similarly Sample storage time affect all parameters as the butter is an easily perishable dairy product within a short time unless treated. So that time is the other factor during analysis.

The reason for choosing extract concentration of 0.2 % and 0.3 % is that as the extract applied on was found to be very effective in retarding lipid oxidation in mackerel mince Samples concentrations 0.24 % and 0.48 % (weight base) resulted in low levels of peroxide value and carbonyl compounds (Igor & Ruta, 2015).

3.9.2 Microbiological Analysis of Cow Butter with the Preservative Extract

In this study, two microbial quality parameters, the total aerobic plates count and yeast-mold counts, were conducted according to Bacteriological Analytical Manual (BAM). The reason for choosing these parameters is that the dairy product including butter mainly affected by these main microbes (Alganesh and Yetenayet 2017).

A. Total Aerobic Bacteria

The potato peel extract was incorporated in to butter sample with a concentration of 0.2 % PPE, 0.3% PPE and a control without any additive were prepared at a base of 5 g butter for

all treatment with two storage temperatures (20 °C and 45 °C). During analysis, the samples were aseptically transferred to stomacher bag and homogenized with 40 ml of peptone water. It was then allowed to melt on a water bath adjusted at 46°C for uniform mixing of extract and the `plate count agar (PCA), which is used as an agar media, was cooled with this bath temperature after autoclaved and before pouring to petri-dish. One ml of homogenized sample was added into sterile test tube containing nine ml peptone water. The serial dilution of up to 10^{-5} were done and mixed thoroughly. One ml of the sample from appropriate decimal dilution was placed on a petri-dish and then molten agar medium (10-15 ml) was poured onto the petri-dish and then it was rotated on its place to distribute the sample uniformly (pouring technique). After solidification, it was incubated for 48 hours at 35°C. Finally, colony count was made using colony counter. It were calculated by multiplying counts on the dish by 10^n, where n stands for the consecutive number of the original sample dilutions. See Appendix C1.

B. Yeast-Mould Count

Samples of butter with a potato peel powder concentration of 0.2%; 0.3% and a control without extract were prepared for a storage temperatures of 20°C; and 45°C. Potato dextrose agar (PDA) media was autoclaved for 15 min at 121°C and tempered in water bath of 45°C temperature. The sample was serially diluted in peptone water and volumes of 0.1 milliliter of appropriate dilutions were plated on Petri-dishes. By using potato dextrose agar, serial dilutions of the suspension up to 10^{-4} were also prepared by spreading technique. The sample was uniformly distributed on plate using spreader with the help of flame to minimize contamination from sample to sample. After solidification, the Petri dish

plates were then incubated at 25°C for 3 to 5 days. Colonies with a blue green color was counted as yeasts and moulds and were counted using colony Counter as colony forming units/g of butter (cfu/g). See Appendix C 2.

3.9.3 Chemical Analysis of Cow Butter with the Potato Peel Preservative *Extract*

The chemical parameters (peroxide value, and free fatty acid value) were measured according to the method of CD 8- 53 (AOAC. 1980. Official Methods of Analysis, 13'hEd. Association of Official Analytical Chemists, Washington, DC.).

A. Peroxide Value

For peroxide value determination, 5 gm of butter sample was dissolved in 10 ml chloroform and mixed with 15 ml glacial acetic acid and 1 ml saturated KI and it was kept in the dark for 5 minutes at room temperature. On shaking vigorously 75 ml distilled water was added and then 1 ml of 1% starch solution also added. The resulting solution was titrated with 0.001N sodium thiosulphate until the color become clear. The same procedures were also applied on blank sample. Finally the peroxide value was calculated as

$$PV = [(V_f - V_i)\ N/M]$$

Where: V_f is the amount of volume difference of $Na_2S_2O_3$ used for titration (ml),

V_i is the amount of $Na_2S_2O_3$ used for the blank (ml)

N is the normality of $Na_2S_2O_3$ titrated against 0.001 N sodium thiosulphate solution until the yellow color almost disappeared and

M is the amount of sample (g).

PV is the peroxide value and expressed as gram equivalent of oxygen per kg of sample.

B. Free Fatty Acid Value Determination

For free fatty acid value determination 5 gm of butter and 30 ml of ethanol were mixed and few drops of phenolphthalein indicator were added. The mixture was then warmed to promote dissolution and titrated with 0.001N KOH until the pink color stable at least for 15 seconds.

$$\%\ \mathrm{FFA}\ = \frac{(\mathrm{Vf}\ - \mathrm{Vi}) \times \mathrm{N} \times 56}{\text{weight of butter(g)}}$$

Where; % FFA: percentage free fatty acid

Vf: volume of KOH used for sample titration

Vi: volume of KOH used for blank titration

N: normality of KOH used for titration

4. RESULT AND DISCUSSION

4.1 Preservative Extract

After 24 hours soaking of potato peel powder in ethanol solvent at ambient temperature and removal of the precipitate by filtering with filter paper, the solvent was removed by using rotary evaporator as shown in the first figure 4.1. The rotary evaporator enables to separate extract from solvent and recover the solvent. Finally the remaining solvent removed by using drying oven and the extract becomes concentrated which was a semi solid. As it is seen in the second picture of (Fig. 4.1) below.

Figure 4. 1 Potato Peel Extract

4.2 Percentage Yield of the Extract

The result for percentage yield of the extract sample obtained by using the above method of maceration extraction with ethanol solvent was 10.42 ± 0.03% where it is the mean plus or minus standard deviation. When it was compared to previous works by (Mohdaly et al.,

2010) with similar extraction solvent this result was better than 10.15 ± 0.33 where it was mean plus or minus standard deviation. The reason may be the potato peel drying system where in this thesis freeze drying was performed and PPP soaked for more time than this one and enables to extract more solute. (Samarin et al., 2012) reported that, even though the yields were higher during extraction by water than other alcoholic solvents (methanol, ethanol, hexane and acetone), but the total phenolic compounds obtained was lower which was not preferred for high antioxidant activity extraction. These authors also show the order of extracting ability from high to low as methanol > water > ethanol > acetone > hexane. According to the report by (Hassan 2015; Kanatt et al., 2005), methanol is not preferred solvent for extracting antioxidant used in food product because of its toxicity. The variation for percentage yield of extract will come from different extraction methods, extraction solvent type, plant variety, geographic location of plant, time of ripening, temperature etc. all these factors play a role.

4.3 Phytochemical Screening Test

From many secondary metabolite (alkaloids, terpenes, flavonoids, lignin, steroids, curcumins, saponins, phenolic, tannins), testing the presence of basic components that play a role in preservative activity (phenol, flavonoid and tannin) were done for this thesis study. The test was performed to identify by color change. The color change during these phytochemical tests showed on Fig 4.2. For this qualitative test, the result showed their presence in the potato peel extract making it better preservative for fat or lipid containing food products. According to the report by (S Jayswal et al., 2018), in addition to food preservation, these plant components have also health benefit for human being. (Huang et

al., 2010) reported that the presence of these phenolic compounds have the properties of anti-allergenic, anti-artherogenic, anti-inflammatory, antimicrobial, antioxidant, cardio-protective and vasodilatory effects. According to the report of (Salawu et al., 2015), these phytochemicals are beneficial to humans under disease induced oxidative stress.

Figure 4. 2 Phytochemical Screening Test Result

Therefore during application of extract on cow butter preservation effect was because of presence of these main components.

Table 4. 1 Phytochemical Screening Test Result

Phytochemical	Result of color change
Phenol	+
Flavonoid	+
Tannin	+

Where '+' sign indicates presence of sample in the extract

4.4 Total Phenol Content

The total phenolic content was determined in terms of gallic acid equivalent by using standard curve equation, which obtained from concentration versus absorbance table (appendix B1), The equation obtained as:

$y = 0.0079x - 0.0586$, with $R^2 = 0.9475$

Using this equation, the total phenol content was found 2.9468± 0.03 mg GAE/g where it was mean plus or minus standard deviation of dry extract. In a previous study ethanolic pp extract, using ultrasonic extraction technique, the potato peels has reported to contain total phenolic content of 0.280 ± 0.00521mg GAE/g of dry extract. According to the report by (Mohdaly et al., 2010) who use a similar extraction method (maceration) and ethanol solvent the total phenol was 2.74 ± 0.03 mg GAE/g dray extract. (Kanatt et al., 2005) suggests that, the variation for total phenolic may come from color and variety of the potato, selection of the solvent and the extraction method affected the concentration. The other report suggested by (Hassan 2015) also says that the variation may come from geographical location, season and storage condition. In this research as the potato to extract the compound was obtained from Ethiopia and the above from other country, the variation may come from geographical location and also soil type.

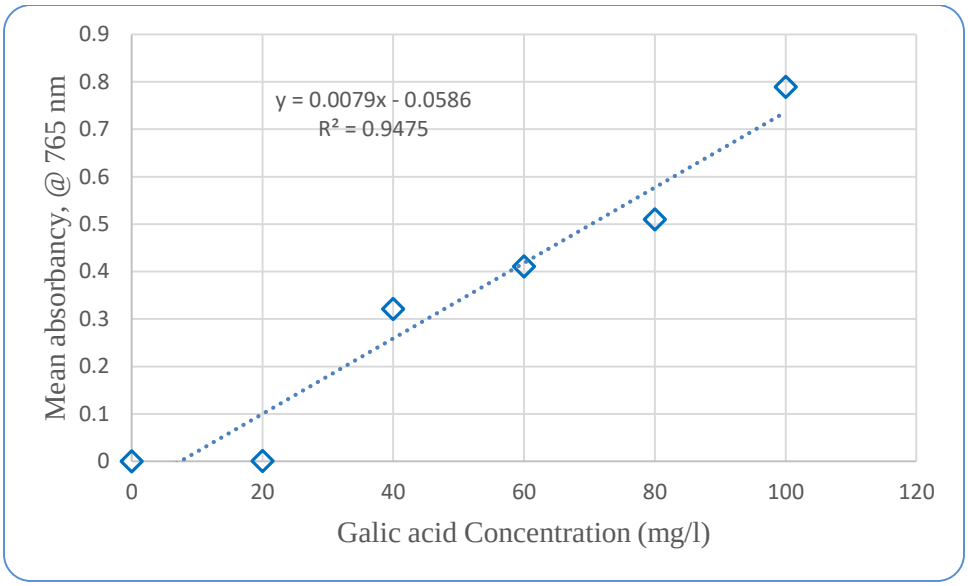


Figure 4. 3 Calibration Curve for Total Phenol Graph

4.5 Total Flavonoid Content

The author (Mohdaly et al., 2010) reported that, in addition to the broad spectrum of chemical and biological activities, flavonoid possess radical-scavenging properties which are important for food oxidation stabilization. This implies that the higher the quantity of flavonoid gives a better product preservation. The total flavonoid content was determined in terms of Quercetin standard from standard curve equation y=0.0007x+0.0259 (Fig 4.4), the total flavonoid content was found to be 3.6885±0.02 mg Quercetin equivalent /g of dry extract which is mean plus or minus standard deviation. In the study reported previously by (Mohdaly et al., 2010) the total flavonoid content was found 0.81±0.04 mg Quercetin equivalent /g dry extract. This potato peel extract had more total flavonoid content than sugar beet pulp and sesame cake which were 0.91±0.02 and 0.29±0.01 mg Quercetin equivalent/g dry extract respectively with a similar extraction method and ethanol solvent like this one.

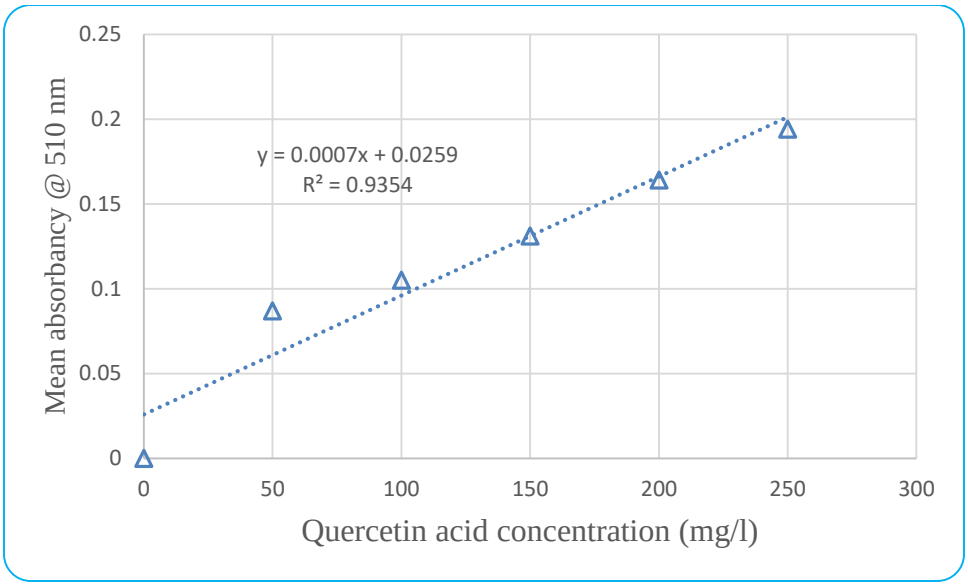


Figure 4. 4 Calibration Curve for Total Flavonoid Determination

4.6 Preservative Effect of the Extract

4.6.1 Microbiological Analysis

The microbiological test of cow butter by the natural potato peel extract was for 21 storage days and the test was performed by the gap of 4 days interval for a total of 6 times. The microbiological tests done in this study were total aerobic bacteria count and yeast-mould count. The results of each butter treatment for both parameter were expressed by the logarithm of colony forming units obtained by direct count of colonies on each serial dilution per ml of sample inoculated to plate count agar medium.

A. Total Aerobic Bacterial Count

The average logarithmic total aerobic bacterial count of the sample is shown in table on appendix B and in Fig.4.5 below. According to the result of the first day, for different

concentrations and temperatures the resulting bacterial counts were not that much different. But as the time increase, the bacterial count also increase too. As the figure showed, from all six time measurements, the total number of aerobic bacterial count for the blank (butter sample without preservative) with higher temperature (45 ^{o}C) was higher than the rest. The reason for this may be because of the higher storage temperature and free from extract. Up to the 9th day, the next higher logarithmic bacterial count value was that of 0.2% antioxidant concentration and 45 ^{o}C (C1T2) but as the time increases after 9th day the logarithmic value of control at T1 (C0T1) becomes higher than C1T2. The reason is that as the time goes, even though the temperature T1 is lower than T2, as C0T1 was control without extract its total bacterial load increased through time. This indicates that, the extract at T2 (45 ^{o}C) also shows some antioxidant effect than that of control with T1 (20 ^{o}C). As shown on appendix C2 ANOVA table, as the p-value lower than 5%, both concentration versus temperature and time showed a significant effect.

The order of total aerobic bacterial count after 11th day arranged as

C0T2>C0T1>C1T2>C2T2>C1T1>C2T1 from higher logarithmic aerobic total bacterial count to lower one.

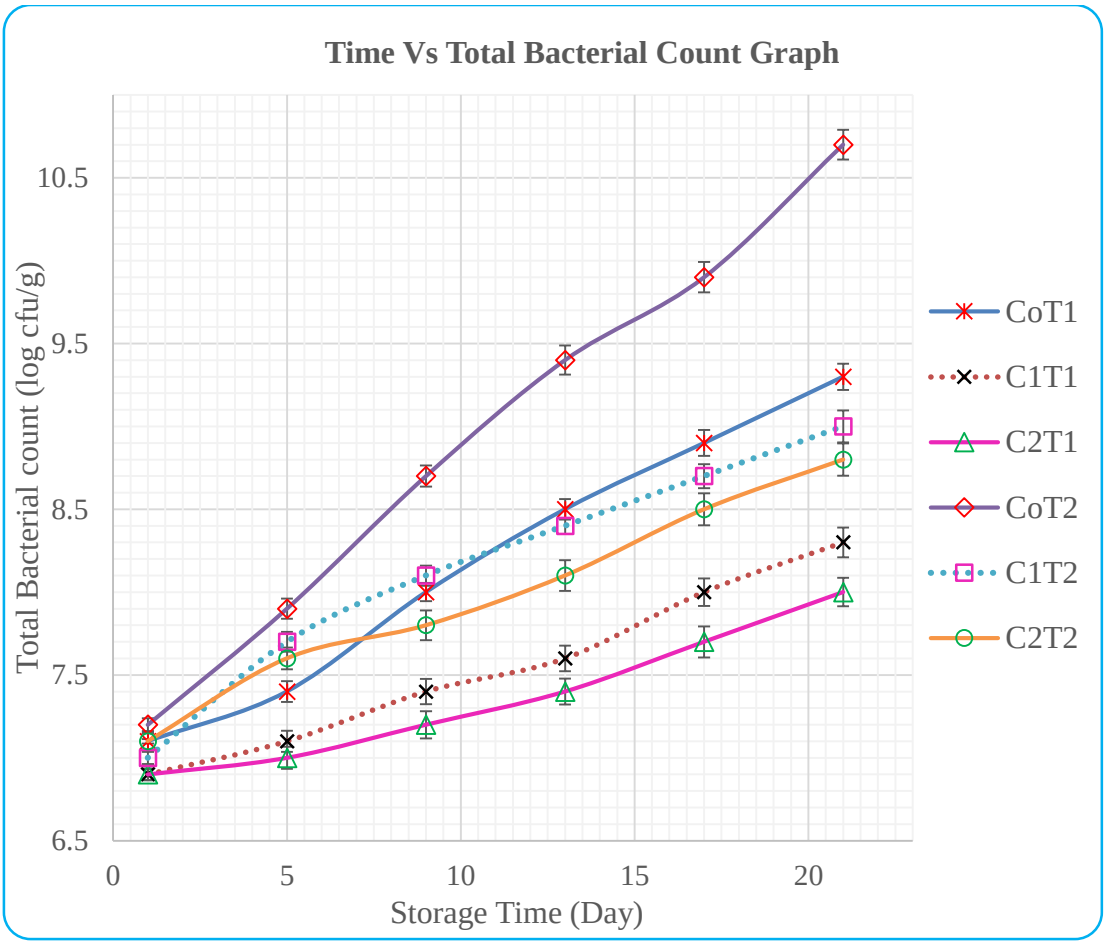


Figure 4. 5 Average Bacterial Count ± Standard Deviation of Butter Stored at Different Temperature and Extract Concentration

[CO: control; C1:0.2% PPE; C2: 0.3% PPE; T1: 20 ^{o}C; T2: 45 ^{o}C]

B. Yeast and Mold Count

The values of mold and yeast count for control butter sample were higher than potato peel crude extract treated butter samples in all three weeks and two storage temperatures. The number of yeast- mould were increased significantly during each week. As shown in Fig. 4.6, both 0.2% and 0.3% potato peel crude extract treated butter samples showed lower value of aerobic mold and yeast count for all storage time than the control at both

temperatures. But the control which is out of extract showed higher number of yeast-mould count for both temperature throughout the storage time. As shown on appendix C3, the ANOVA indicates that, both time and concentration with temperature had a significant effect on butter sample throughout the time as the p-value lower than 5 %. Clearly the result indicates that the extract had antimicrobial effect against the growth of yeast and mold in butter for both storage temperatures.

Generally a 0.3% potato peel extract with a 45^{o}C storage temperature was better than others. According to the report by (Tola et al., 2018), the mean yeast and mould count for untreated butter sample at initial preservation time was 6.70 log cfu/g that was greater than the value obtained in this thesis result where initially it was 6.02 log cfu/g. the author also suggested the maximum tolerable limit of yeast and mold regulated by Ethiopian standard authority (ESA) which is 1 log cfu/g. But both results are beyond the acceptable limit. Many reasons may put for this as poor hygiene of milk utensils, unclean water used for cleaning purpose, contaminants from cow dung, lack of knowledge in milk processor, storage condition (temperature, humidity) etc. May cause for low quality of butter produced. Here in the study it is proved that the extract limit the growth of yeasts and moulds was compared with that of the control throughout the storage time (21 days) and shows the preservation effect and can be used as a preservative.

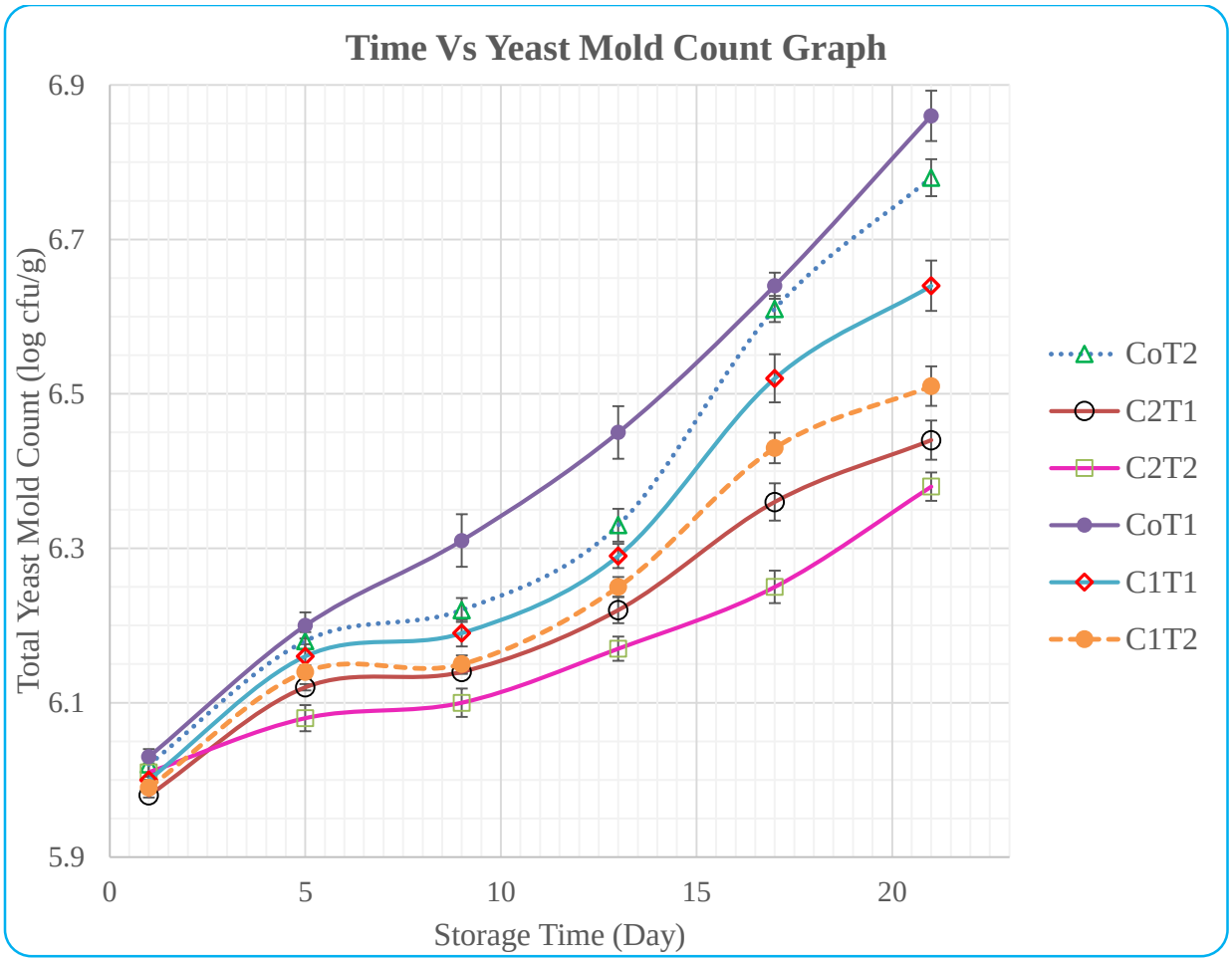


Figure 4. 6 Average Yeast Mould Count ± Standard Deviation of Butter Stored at Different Temperature and Extract Concentration

[CO: control; C1:0.2% PPE; C2: 0.3%PPE; T1: 20 ºC; T2 45 ºC]

4.6.2 Chemical Analysis

A. Peroxide Value

According to the report by (Asha et al., 2015), peroxide value is an indicator of primary oxidation products quantitatively one mg of active oxygen within one gram of lipid which can be measured by its ability to liberate iodine from potassium iodide. As shown in the

Fig. 4.7, the treatment creates variation on butter preservation. As it was done with ANOVA analysis (table showed on appendix E1), all the treatments including blank shows a significant effect with p value less than 0.05 throughout the storage period. Individually the blank sample also shows a significant effect on both temperatures with time. But the blank sample with higher temperature (45^{o}C) showed higher peroxide value throughout the time than all other treatments which indicates higher primary oxidation product formation than other treated samples and a control at 20 ^{o}C. The increase in PV for blank sample with lower temperature (20 ^{o}C) was lower than the blank with higher temperature (45^{o}C). This is because of storage temperature as the two temperature showed significant difference (p value less than 0.05). During the entire 21 storage days, butter sample treated by 0.3% potato peel extract and storage temperature of 20^{o}C followed by sample treated with 0.2% potato peel extract and 20^{o}C storage temperature were more effective. At 45^{o}C butter sample treated by 0.3% potato peel extract exhibited a greater inhibitory action than 0.2% potato peel extract. Generally the resulting data shows treating butter by a concentration of 0.3% potato peel natural extract and a storage temperature of 20 ^{O}C were better than the rest treatment tested on this work. One reason for this may be at higher temperature, there is higher oxidation reaction formation and also lower distraction of phenolic compound which used to preserve foods. The report presented by (Soulti and Roussis 2007) also supported that some phenolics may be taken into account as antioxidants in butter during storage.

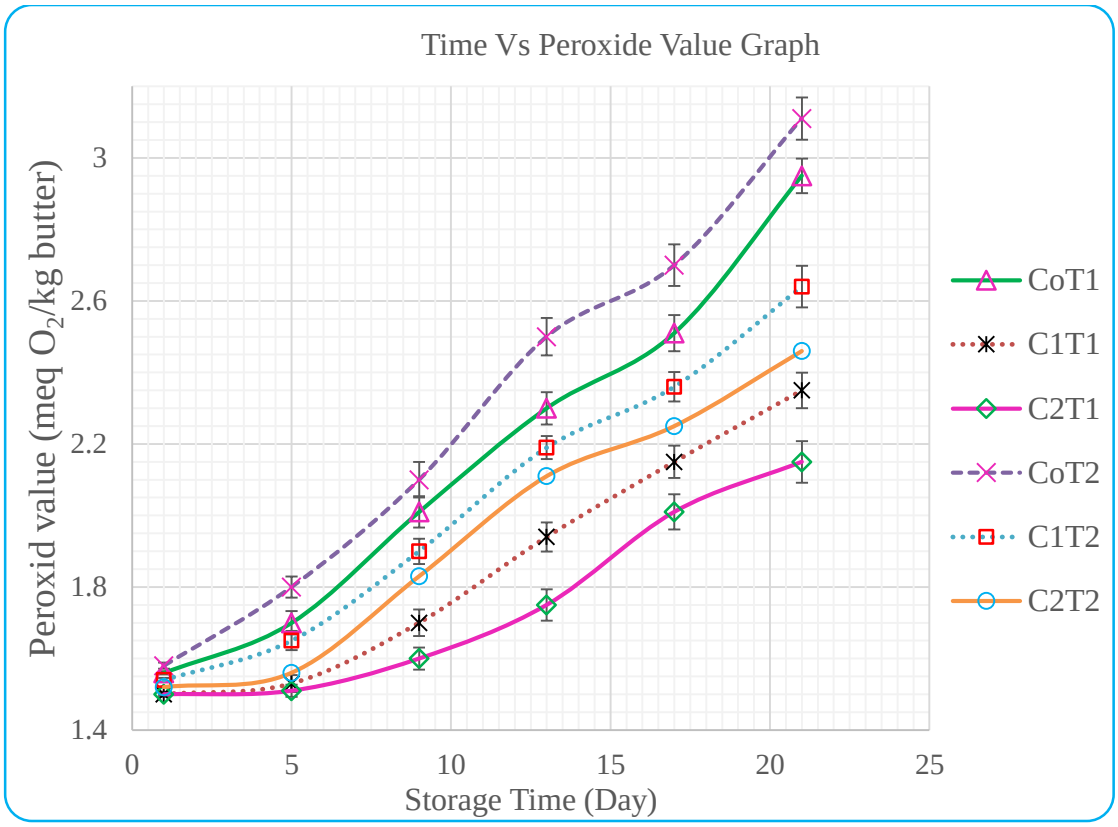


Figure 4. 7 Average Peroxide Value ± Standard Deviation of Butter Stored at Different Temperature and Extract Concentration

[CO: control; C1:0.2% PPE; C2: 0.3%PPE; T1: 20 ºC; T2: 45 ºC]

B. Free Fatty Acids

Free fatty acids are formed due to hydrolysis or oxidation (cleavage) of double bonds (Asha et al., 2015). As shown in appendix E 2, there was a significant increase of free fatty acid ($P< 0.05$) in all butter samples. As shown in Fig 4.8, least increase in FFA was observed for sample treated by 0.3% concentration potato peel extract throughout the storage time. There was significantly higher formation of FFA for a control at a storage temperature of both 20ºC and 45ºC than in the butter incorporated with potato peel extract at both

temperatures. As it was seen from ANOVA table appendix: E3, appendix: E4 and appendix: E5 all the treatments had more significant effect than temperature for the change in free fatty acid. This is because of the presence of phenolic compounds as it was reported by (Soulti and Roussis 2007).

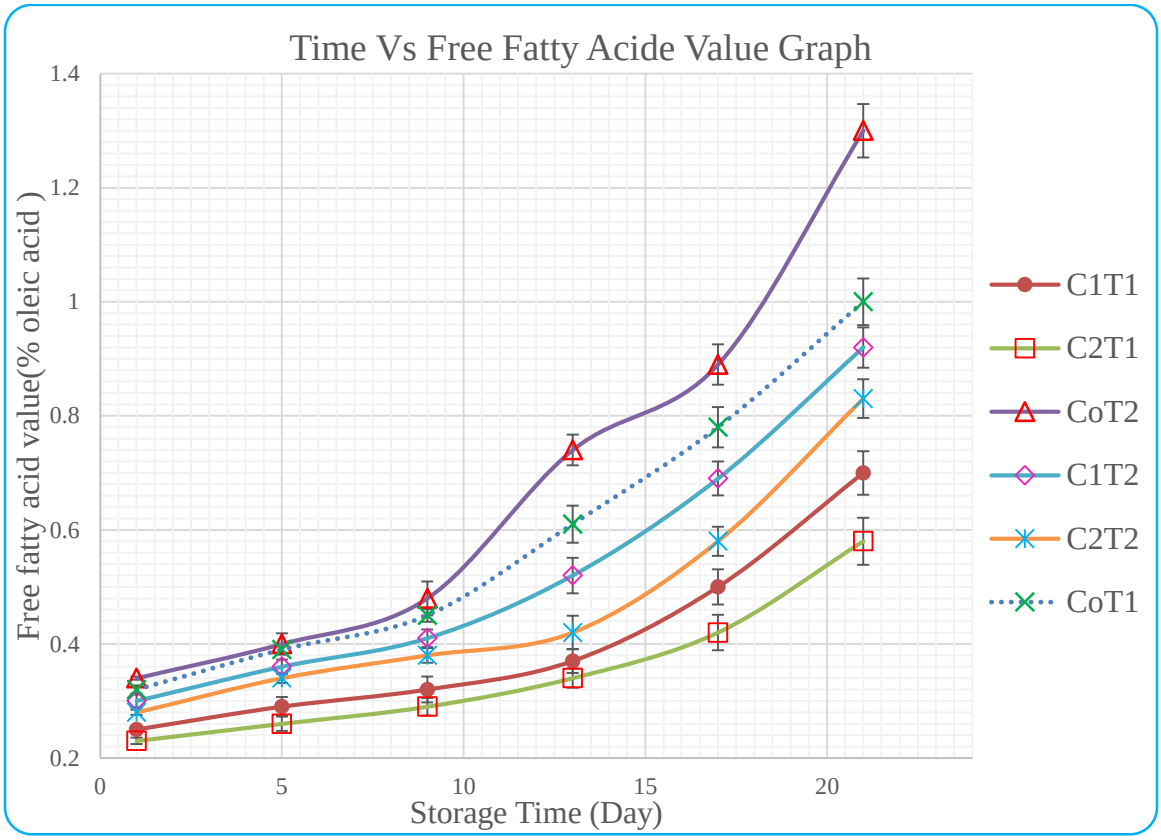


Figure 4. 8 Average Free Fatty Acids Value ± Standard Deviation of Butter Stored at Different Temperature and Extract Concentration

[CO: control; C1:0.2% PPE; C2: 0.3%PPE; T1: 20 ºC; T2:45 ºC]

CHAPTER FIVE

5 Conclusion and recommendation

5.1 Conclusion

It is possible to extract an antioxidant from potato peel by the method of maceration extraction using ethanol as a solvent and a sample to solvent ratio of 1g: 10ml. By this method a total percentage yield of 10.42 ± 0.03 % (which is mean plus or minus standard deviation) potato peel extracts were obtained.

The extracted antioxidant was checked for its qualitative property (phytochemical test) and the result indicated the presence of the main antioxidant components namely phenol, flavonoid and tannin which play a role by their food preservation property.

In this research the total quantity of phenol obtained from the potato peel extract as indicated by the method of Folin-Ciocalteau reagent (FCR) was found to be 2.9468± 0.03 mg GAE/g of dry extract which is mean plus or minus standard deviation. Different authors got different result for total phenol content and the variation may come from color or variety of potato, extraction method or solvent type, geographic location and ripening time.

Flavonoid is also the other main component of the antioxidant extract that play a critical role for preserving food. The quantity of total flavonoid obtained from this potato peel extract was 3.6885±0.02 mg Quercetin equivalent /g of dry extract which is mean plus or minus standard deviation. When compared with other plants like sugar beet pulp, the resulting total flavonoid was better and enables the potato peel extract act as an antioxidant.

According to the results obtained the potato peel extract shows a preserving effect for all microbial and chemical parameter analysis when compared with the control and the extract concentration. Both time and concentration with storage temperature shows a significant effect during analysis for total aerobic bacteria count. From the selected temperatures storing a sample at 20^{o}C by an extract concentration of 0.3% was better in preserving cow butter than the rest and a control with a storage temperature of 45^{o}C showed a higher average total bacterial count than the other treatment and storage temperature throughout a storage time.

Average yeast-mould count shows some different effect with that of bacterial count. Where the extract of 0.3% concentration with 45^{o}C storage temperature showed lower average yeast-mould count than the rest treatment and storage temperature. But a control without potato peel extract at 20 ^{o}C storage temperature showed the higher number of yeast-mould count than the rest treatment. The reason may because of high temperature instability of yeast-mould. Therefore from the treatments listed here, treating butter by 0.3% concentration with 45 ^{o}C storage temperature was better in preserving cow butter from yeast-mould.

The peroxide value tested shows a significant effect for the storage time and extract concentration with temperature. The butter sample without extract at higher temperature (45 ^{o}C) showed higher number of peroxide value (primary oxidation product) throughout the storage time and the extract with higher concentration (0.3%) at a lower storage temperature (20 ^{o}C) showed minimum peroxide value. The result from ANOVA analysis also showed the presence of significant effect.

The free fatty acid analysis showed that there were formation of higher FFA in the control than the rest treatment. But a butter sample with 0.3% extract concentration showed lower free fatty acid formation. The ANOVA analysis also showed the presence of significant effect in concentration, time and temperature.

Generally storing the cow butter sample at 20^{o}C and treating it with 0.3% extract concentration was better for total bacterial growth, peroxide value and free fatty acid analysis than 45 ^{o}C and lower extract concentration. But for yeast-mould count from the two temperatures and concentration storing at 45 ^{o}C and higher extract concentration (0.3%) showed lower number of yeast and mould than the rest treatment done here. The reason for this may be most yeast and mould species grow at 20^{o}C and not stable at very high temperature 45 ^{o}C.

5.2 Recommendation

Deeply identification of phenolic compound components and study there nature will help to understand how effectively use them for fat and oil product preservation.

Study additional more PPE parameters and after applying study additional parameters like sensory analysis, thiobarbitheric acid (secondary oxidation products) and health effect of potato peel extract need to be analyzed.

Comparison of this antioxidant with the synthetic and see its effectiveness will give better preference of it.

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APPENDIXES

Appendix A: Some Laboratory photo

Appendix B:

Appendix B. 1 Concentration (mg/L) Vs Absorbance of total Phenol @ 765 nm data

Concentration	Absorbance (mean)
0	0
20	0.001
40	0.321
60	0.411
80	0.51
100	0.789

Appendix B.2 Concentration (mg/L) Versus mean Absorbance of flavonoid

Concentration	Mean Absorbance
0	0
50	0.087
100	0.105
150	0.131
200	0.164
250	0.194

Appendix C:- Microbiological Analysis

Appendix C.1 Average total plate count of butter stored at 20^{o}C & 45^{o}C (log cfu/g)

	Average Total bacterial Count					
Day	**COT1**	**C1T1**	**C2T1**	**COT2**	**C1T2**	**C2T2**
1	7.1	6.9	6.9	7.2	7	7.1
5	7.4	7.1	7	7.9	7.7	7.6
9	8	7.4	7.2	8.7	8.1	7.8
13	8.5	7.6	7.4	9.4	8.4	8.1
17	8.9	8	7.7	9.9	8.7	8.5
21	9.3	8.3	8	10.7	9	8.8
Day	Standard Deviation					
1	0.01414	0.03536	0.03394	0.03818	0.03677	0.05091
5	0.06223	0.06364	0.06647	0.06081	0.0594	0.06505
9	0.05515	0.07637	0.08202	0.06364	0.06081	0.09051
13	0.06223	0.07778	0.07778	0.08768	0.04808	0.09334
17	0.07778	0.08344	0.09334	0.09192	0.07212	0.09617
21	0.0792	0.09051	0.08627	0.09051	0.09617	0.09758

Appendix C.2 ANOVA table analysis for total plate count

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Time	16.2914	5	3.25828	31.1664	5.6589E-10	2.60299
Concentration Vs. Temperature	9.55806	5	1.91161	18.2852	1.2244E-07	2.60299
Error	2.61361	25	0.10454			
Total	28.4631	35				

Appendix C.3 Average yeast and Mold count of butter treated by crude extract & control stored at temperatures 20 ºC & 45 ºC (log cfu/g)

Day	Average Yeast-Mold Count					
	COT2	**C2T1**	**C2T2**	**COT1**	**C1T1**	**C1T2**
1	6.02	5.98	6.01	6.03	6	5.99
5	6.18	6.12	6.08	6.2	6.16	6.14
9	6.22	6.14	6.1	6.31	6.19	6.15
13	6.33	6.22	6.17	6.45	6.29	6.25
17	6.61	6.36	6.25	6.64	6.52	6.43
21	6.78	6.44	6.38	6.86	6.64	6.51
Day	Standard Deviation					
1	0.0198	0.00283	0.01131	0.00141	0.00424	0.00283
5	0.01131	0.00424	0.01697	0.01697	0.01556	0.00849
9	0.01556	0.00283	0.01838	0.03394	0.01697	0.01131
13	0.02121	0.01697	0.01556	0.03394	0.01556	0.01273
17	0.01697	0.02404	0.02121	0.01697	0.03111	0.0198
21	0.02404	0.02546	0.01838	0.03253	0.03253	0.02546

Appendix C.4 ANOVA table analysis for yeast and Mold count of butter treated by crude extract & control stored at temperatures 20 ºC & 45 ºC

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Time (day)	1.44821	5	0.28964	60.2572	3.89026E-13	2.60299
conc vs. temp	0.26298	5	0.0526	10.9421	1.16311E-05	2.60299
Error	0.12017	25	0.00481			
Total	1.83136	35				

Appendix D: - Chemical Analysis

Appendix D.1 Average free fatty acid value of butter treated by crude extract

Time	Sample code	Sample weight (g)	Initial volume (ml)	Final volume (ml)	Difference volume (ml)	Difference volume(ml) (sample-blank)	FFA
	Blank		0.0	0.5	0.5		
1st Day	C_0T_1	2.013	0.5	12.5	12.0	11.5	0.32
	C_1T_1	2.0164	12.5	22.0	9.5	9.0	0.25
	C_2T_1	2.006	22.0	30.8	8.8	8.3	0.23
	C_0T_2	2.017	30.8	43.5	12.7	12.2	0.34
	C_1T_2	2.0142	43.5	54.8	11.3	10.8	0.30
	C_2T_2	2.003	54.8	65.3	10.5	10.0	0.28
5tht Day	C_0T_1	2.0074	3.5	18.0	14.5	14.0	0.39
	C_1T_1	2.0023	18.0	28.8	10.9	10.4	0.29
	C_2T_1	2.0056	28.9	40.1	11.2	10.7	0.30
	C_0T_2	2.0024	40.1	54.9	14.8	14.3	0.40
	C_1T_2	2.0035	54.9	68.3	13.4	12.9	0.36
	C_2T_2	2.0094	68.3	81.0	12.7	12.2	0.34
9th Day	C_0T_1	2.0172	0.0	16.7	16.7	16.2	0.45
	C_1T_1	2.0048	16.7	28.7	12.0	11.5	0.32
	C_2T_1	2.0067	28.7	39.6	10.9	10.4	0.29
	C_0T_2	2.0086	39.6	57.2	17.6	17.1	0.48
	C_1T_2	2.0287	57.2	72.6	15.4	14.9	0.41
	C_2T_2	2.0532	72.5	86.9	14.4	13.9	0.38
13th Day	C_0T_1	2.044	0.0	22.7	22.7	22.2	0.61
	C_1T_1	2.0346	22.6	36.5	13.9	13.4	0.37
	C_2T_1	2.0473	36.5	49.4	12.9	12.4	0.34
	C_0T_2	2.0689	49.4	77.1	27.7	27.2	0.74
	C_1T_2	2.0275	77.1	96.4	19.3	18.8	0.52
	C_2T_2	2.0297	96.4	112.1	15.7	15.2	0.42
17th Day	C_0T_1	2.003	0.0	28.3	28.3	27.8	0.78
	C_1T_1	2.0076	28.3	46.7	18.4	17.9	0.50
	C_2T_1	1.2046	46.7	56.3	9.6	9.1	0.42
	C_0T_2	1.4075	56.3	79.1	22.8	22.3	0.89
	C_1T_2	2.0671	79.1	105.0	25.9	25.4	0.69
	C_2T_2	2.044	105.0	126.5	21.5	21.0	0.58
21th Day	C_0T_1	2.054	0	37.2	37.2	36.7	1
	C_1T_1	2.0365	37.2	63.2	26.0	25.5	0.70
	C_2T_1	2.0483	63.1	84.8	21.7	21.2	0.58
	C_0T_2	2.0592	84.8	133.1	48.3	47.8	1.3
	C_1T_2	2.0575	133	167.3	34.3	33.9	0.92
	C_2T_2	2.0497	167.3	198.2	30.9	30.4	0.83

For 1st day C_0T_1 → $\%FFA = [(V_f - V_i)*N*56/M] = [(12 - 0.5)*0.001*56/2.013] = 0.32$

Appendix D.2 Free Fatty Acid Value of Butter Treated by potato peel Extract

Day	Average Free Fatty Acid value					
	COT1	**C1T1**	**C2T1**	**COT2**	**C1T2**	**C2T2**
1	0.32	0.25	0.23	0.34	0.3	0.28
5	0.39	0.29	0.26	0.4	0.36	0.34
9	0.45	0.32	0.29	0.48	0.41	0.38
13	0.61	0.37	0.34	0.74	0.52	0.42
17	0.78	0.5	0.42	0.89	0.69	0.58
21	1	0.7	0.58	1.3	0.92	0.83
Day	Standard Deviation					
1	0.00707	0.00141	0.00566	0.00283	0.01131	0.00424
5	0.00849	0.01697	0.01273	0.01838	0.01273	0.00849
9	0.01131	0.02263	0.01556	0.0297	0.01556	0.01273
13	0.03253	0.02121	0.01697	0.02687	0.03111	0.0297
17	0.03536	0.03111	0.03111	0.03536	0.0297	0.02546
21	0.04101	0.03818	0.04101	0.04667	0.03536	0.03394

Appendix D.3 average Peroxide Value of Butter Treated by Potato Peel Extract

Time	Sample code	Sample weight butter (g)	Initial volume (ml)	Final volume (ml)	Difference volume (ml)	Difference volume(ml) (sample-blank)	POV
	blank		0	1.3	1.3		
1st Day	C_0T_1	2.0025	7.2	11.6	4.4	3.1	1.56
	C_1T_1	2.008	11.6	15.9	4.3	3.0	1.5
	C_2T_1	2.0055	15.9	20.2	4.3	3.0	1.5
	C_0T_2	2.004	20.2	24.7	4.5	3.2	1.58
	C_1T_2	2.0034	24.7	29.1	4.4	3.1	1.54
	C_2T_2	2.0065	29.1	33.4	4.3	3.0	1.52
5th Day	C_0T_1	2.015	1.3	6.0	4.7	3.4	1.7
	C_1T_1	2.07	6	10.5	4.5	3.2	1.53
	C_2T_1	2.0182	10.5	14.8	4.3	3.0	1.51
	C_0T_2	1.915	14.8	19.5	4.7	3.4	1.8
	C_1T_2	1.1926	19.5	22.8	3.3	2.0	1.65
	C_2T_2	1.1111	22.8	25.8	3.0	1.7	1.56
9th Day	C_0T_1	2.039	13.7	19.1	5.4	4.1	2.01
	C_1T_1	2.0179	19.1	23.8	4.7	3.4	1.7
	C_2T_1	2.0212	23.8	28.3	4.5	3.2	1.6
	C_0T_2	2.0224	28.3	33.8	5.5	4.2	2.1
	C_1T_2	2.055	33.8	39.0	5.2	3.9	1.9
	C_2T_2	2.0466	39	44.0	5.0	3.7	1.83
13th Day	C_0T_1	2.0306	0	6.0	6.0	4.7	2.3
	C_1T_1	2.0326	6	11.2	5.2	3.9	1.94
	C_2T_1	2.0364	11.2	16.1	4.9	3.6	1.75
	C_0T_2	2.0433	16.1	22.5	6.4	5.1	2.5
	C_1T_2	2.0464	22.5	28.3	5.8	4.5	2.19
	C_2T_2	2.074	28.3	34.0	5.7	4.4	2.11
17th Day	C_0T_1	2.06	0	6.5	6.5	5.2	2.51
	C_1T_1	2.0456	6.5	12.2	5.7	4.4	2.15
	C_2T_1	2.0532	12.2	17.6	5.4	4.1	2.01
	C_0T_2	2.0624	17.6	24.5	6.9	5.6	2.7
	C_1T_2	2.0823	24.5	30.7	6.2	4.9	2.36
	C_2T_2	2.0451	30.7	36.6	5.9	4.6	2.25
21th Day	C_0T_1	2.0581	0	7.4	7.4	6.1	2.95
	C_1T_1	2.046	7.4	13.5	6.1	4.8	2.35
	C_2T_1	2.0632	13.5	19.2	5.7	4.4	2.15
	C_0T_2	2.0822	19.2	27.0	7.8	6.5	3.11
	C_1T_2	2.0743	27	33.8	6.8	5.5	2.64
	C_2T_2	2.0561	33.8	40.2	6.4	5.1	2.46

For 1st day C_0T_1 → PV= $[(V_f - V_i)\ N/M] = [(4.4 - 1.3)*0.001*1000/2.0025g] = 1.56$

Appendix D.4 Peroxide Value of Butter Treated by Potato Peel Extract

Day	Average Peroxide Value					
	COT1	**C1T1**	**C2T1**	**COT2**	**C1T2**	**C2T2**
1	1.56	1.5	1.5	1.58	1.54	1.52
5	1.7	1.53	1.51	1.8	1.65	1.56
9	2.01	1.7	1.6	2.1	1.9	1.83
13	2.3	1.94	1.75	2.5	2.19	2.11
17	2.51	2.15	2.01	2.7	2.36	2.25
21	2.95	2.35	2.15	3.11	2.64	2.46
Day	Standard Deviation					
1	0.00424	0.00283	0.00707	0.00849	0.00566	0.0099
5	0.03253	0.02404	0.01697	0.0297	0.02687	0.03111
9	0.04384	0.03677	0.03111	0.0495	0.03536	0.04384
13	0.04525	0.04101	0.04384	0.05233	0.03253	0.04101
17	0.05091	0.04525	0.0495	0.05798	0.04101	0.0495
21	0.04808	0.0495	0.05798	0.0594	0.05798	0.0495

Appendix E:-ANOVA table for chemical analysis

Appendix E1:- ANOVA table for peroxide value determination

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Time(day)	5.25619	5	1.05124	71.8298	5.14228E-14	2.60299
Temperature vs Concn.	1.20842	5	0.24168	16.514	3.19123E-07	2.60299
Error	0.36588	25	0.01464			

Appendix E 2:- ANOVA table for free fatty acid value determination

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Time(day)	1.52739	5	0.30548	42.4563	1.99633E-11	2.60299
Concentration Vs. temperature	0.46336	5	0.09267	12.8797	2.95901E-06	2.60299
Error	0.17988	25	0.0072			
Total	2.17062	35				

Appendix E 3:- ANOVA table for free fatty acid value determination (effect of temperature on CO Vs. time).

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Temperature	0.03	1	0.03	4.96689	0.07629	6.60789
CO Vs. Time	0.97217	5	0.19443	32.1909	0.00083	5.05033
Error	0.0302	5	0.00604			
Total	1.03237	11				

Appendix E 4:- ANOVA table for free fatty acid value determination (effect of temperature on C1 Vs. time).

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Temperature	0.04941	1	0.04941	20.8621	0.00602	6.60789
C1 Vs. Time	0.40384	5	0.08077	34.1034	0.00072	5.05033
Error	0.01184	5	0.00237			
Total	0.46509	11				

Appendix E 5:- ANOVA table for free fatty acid value determination (effect of temperature on C2 Vs. time).

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Temperature	0.04201	1	0.04201	15.285	0.0113	6.60789
C2 Vs. Time	0.27548	5	0.0551	20.0467	0.00254	5.05033
Error	0.01374	5	0.00275			
Total	0.33123	11				