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Research article

Efficiency of potato peel extract in the preservation of cow butter

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ABSTRACT

This study investigates that the phenolic compound extracted from the potato peels using ethanol by maceration as a natural preservation agent for cow butter, yielding 10.42 ± 0.03 % phenolic compound. A quantitative and qualitative analysis of potato peels extract (PPE) was conducted to examine the phenolic compounds. The major preliminary phytochemical screenings (Alkaline Reagent Test, Ferric Chloride Test, Chloride Test) were performed to detect the presence of phenols, flavonoids, and tannins. The total phenolic content was measured using the Folin–Ciocalteu method with UV spectrophotometry, which produced 2.9468 ± 0.03 mg GAE/g of dry extract. The total flavonoid content was determined using the aluminum chloride colorimetric method, resulting in 3.6885 ± 0.02 mg equivalent Quercetin/g of dry extract. During 21 days, butter samples treated with PPE at various concentrations (0.2 % and 0.3 %) and storage temperatures (20 °C and 45 °C) were examined for chemical parameters (peroxide value and free fatty acid value) and microbiological parameters (aerobic total bacterial count and yeast mould count). The findings showed that the samples preserved at 20 °C with a concentration of 0.3 % extract had better preservation than samples stored at other temperatures. It also showed lower values of peroxide and free fatty acids, as well as less microbial growth. On the other hand, samples without extract that were kept at 45 °C demonstrated more oxidation and microbial growth. The oxidative stability of cow butter was assessed using the Rancimat method. Results indicate that PPE significantly enhances both oxidative stability and shelf life, offering preservative benefits for up to six months. Specifically, the induction period (IP) at room temperature reached 3960 h (165 days) with PPE, compared to only 120 h (5 days) without it. These findings suggest that the phenolic compounds in the potato peels could serve as natural preservatives for cow butter, particularly when stored at lower temperatures.

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1. Introduction

Potatoes (Solanum tuberosum, a staple crop worldwide, possess significant potential for cultivation, particularly in regions such as Ethiopia [1,2]. Nearly 83 % of potato farmers in the nation are found in the Central, Eastern, North-Western, and Southern regions. Potatoes can be produced on most of the agricultural land that is currently available [3]. As a number of literatures suggest that potatoes possess many health benefits such as antihypertensive [4], anticancer [5], and antimicrobial [6]. Both the pulp and peel parts of potatoes have beneficial properties. However, the peel is often discarded as a byproduct during potato food processing. It was indicated that phenolic was found to be low in the tube and concentrated in peel and adhesive tissues [7]. Potato peels, which are abundant in phenolic compounds, particularly chlorogenic acid, provide a natural substitute for synthetic antioxidants often used in food preservation [8]. The aqueous extract of potato peels (PP) is rich in different phenolic acids, such as hydroxycinnamic acids and flavonoids, which have strong antioxidant capacity and offer a therapeutic effect, including protection of erythrocytes, without having mutagenic effects [9,10]. The use of PPE as natural antioxidants for foods containing oil and lipids would have a promising solution, other than environmentally friendly. Foods containing oil or lipids deteriorate due to a chain reaction (i.e. oil or lipids) [11]. These reactions in food can result in rancidity, the formation of potentially toxic compounds, colored products, and loss of nutritional value due to the destruction of essential fatty acids and vitamins (A, D, and E) [12]. This results in the degradation of food composition and this disintegration is the main factor for cancer in the human body.

The process of oxidation can be mitigated through the incorporation of specific synthetic or natural antioxidants into lipids and oils [13]. Synthetic antioxidants such as tert-butyl hydroquinone (TBHQ), butylated hydroxy anisole (BHA) and butylated hydroxytoluene (BHT) have been used as food additives to combat the stability challenges associated with oils and fats [14]. However, recent research has indicated that chronic utilization of these substances can be correlated with various health hazards, including carcinogenicity and the development of cancer [15,16]. In light of the potential health concerns related to the use of synthetic antioxidants in food preservation, there is increasing interest in exploring natural antioxidant substitutes. The primary class of natural antioxidants consists of phenolic compounds [17]. Consequently, plants and their products are gaining attention as sources of natural antioxidants, leading to a significant surge of interest, particularly in plant-derived antioxidants [18]. For example, potato peels contain phenolic acids, predominantly chlorogenic acid (CGA), along with smaller amounts of other phenolics such as gallic acid (GAC), caffeic acid (CFA), and protocatechuic acid (PCA) [19]. Therefore, the objective of this work was to investigate the antioxidant capacity of phenolic compounds obtained from potato peels and their effectiveness in preserving cow butter in comparison to synthetic antioxidants. The study uses the inherent qualities of potato peels to tackle food preservation issues while reducing waste and encouraging environmentally friendly methods.

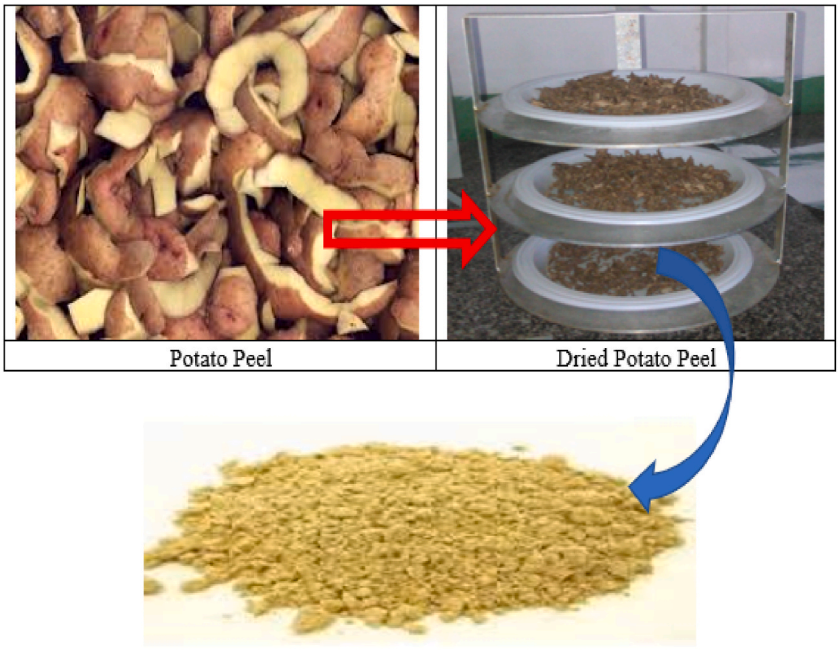

Fig. 1. Potato peel sample preparation.

2. Materials and methods

2.1. Materials and reagents

The main raw materials used in this study were potato peel (*red type potato-solanium tuberosom*) collected from the local chipper and cow butter purchased from the local cow milk processer. Chemicals such as DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2-azino-bis (3-ethylbenzo thiazoline-6-sulfonic acid) diammonium salt), TPTZ (2,4,6-tri(2-pyridyl)-s-triazine), synthetic antioxidants (butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ)), sodium tungstate and sodium molybdate (99 %), were purchased from chemical supply companies, Addis Ababa, Ethiopia. Ethanol (97 %), plate count agar (PCA) and Potato dextrose agar (PDA) obtained from the BiT laboratory were also used for the experimentation and characterization processes. All chemicals and reagents were analytical grades and used without further purifications.

2.2. Methods

2.2.1. Material preparation

The collected red-type fresh potato peels were thoroughly washed with tap water and remove all foreign matter. The washed raw potato peels were then dried for three days using a freeze drier (*Lyo Capsule 7-vial Freeze dryer*) to avoid degradation of the phenolic compound by heating at high temperature. The freeze-dried sample was then ground with mortar and milled and sieved with a size of 0.5 μm sieve size. The ground potato peels were sealed in a plastic bag and stored in a refrigerator at 4 °C until used for the next analysis. The preparation of potato peel sample is presented by in Fig. 1.

2.2.2. Phenol extraction process

The preparation of the extract was done by using the method described by Ma et al. except pp dried by freeze dryer [20]. Compared to the higher-polarity solvents, the lower-polarity solvents, namely hexane, petroleum ether, and diethyl ether showed a significantly reduced capacity to extract phenolic compounds [21]. For use in the food industry, ethanol would be a more appropriate solvent as it is more polar than water. Therefore, at the end of the extraction, as the antioxidant was applied to the food product, ethanol was used as a solvent for the extraction. Thus 10 mL of solvent was mixed with 1 g of powder in the volumetric flask, the mixture was then stirred with a magnetic stirrer and allowed to shake on a shaker (*Heidolph unimax 2010*) for 3 days at room temperature, and then the extract filtered for removal of peel particles followed by evaporation in a rotary evaporator for a temperature of 38 °C to minimize the phenolic compound with high temperature and finally the remaining solvent was removed through evaporation by using a drying oven (*M4*0-VF*, MPM instruments*) with 20 °C. Finally, the extracted compound was sealed in an airtight plastic jar and stored in a refrigerator until the parameters were characterized and applied to the cow butter.

***Percentage of Extract Yield*:** The potato peel extract in percentage of yield was obtained by using the method described by El-Hadary et al. which enables determining the quantity of sample for extraction purposes as given by equation (1) [22].

$$Percent\ Yield = [EPP / PPP] \times 100 \quad (1)$$

Where EPP is the weight of the dry extract of the potato peel after extraction, PPP is the weight of the dry potato peel powder before extraction.

***Phytochemical Screening Test*:** As the name indicates, phytochemical is the name for plant chemicals. These chemicals may be either primary or secondary metabolites, where the first indicates plant components that are essential for growth and reproduction. Their absence in the plant negatively affects the plant. Secondary metabolites are not as essential as primary metabolites which benefit the plant by imparting color, flavor, aroma, etc. A qualitative phytochemical test of the sample was performed according to the method described by Rotimi et al. [23]. The standard solution of the plant extract was used for the screening of the major phytochemicals such as flavonoids, phenols, and Tannins. A stock solution was prepared by dissolving 1g of ethanolic PP extract in 100 mL of ethanol.

***Alkaline Reagent Test*:** It was carried out as a 1 mL stock solution was taken in a test tube and a few drops of dilute sodium hydroxide (NaOH) solution was added. A few drops of 10 % hydrochloric acid were added. There was an intense yellow color indicating the presence of flavonoid [24].

Ferric chloride test: the ferric test was conduct as 1 mL stock solution was prepared and taken in a test tube and few drops of 10 % ferric chloride ($FeCl_3$) were added. A dark green color was observed, which indicates the presence of phenols in the sample [25].

Chloride test: The chloride test was performed as 1 mL of the stock solution was taken into the test tube with 10 % addition of ferric chloride ($FeCl_3$) in the test tube containing the sample and stirred well. An intense greenish black color is formed which indicates the presence of tannins [25].

2.2.3. Quantification of the total phenol compound in the PP extract

The Folin–Ciocalteu reagent (FCR) method was used to measure the total phenolic content. 0.5 mL of the Folin–Ciocalteu reagent prepared and added, then 0.1 mL of the sample was combined with 7.9 mL of distilled water and left to stand for 5 min. Next, 1.5 mL of 20 % w/v sodium carbonate was added to the mixture. The mixture was shaken and then allowed to sit for 90 min. Because the Folin–Ciocalteu reagent is sensitive to reducing substances, such as polyphenols, the reaction results in a blue color. Next, using a UV spectrophotometer, the absorbance was measured at 765 nm. Various concentrations of gallic acid (GAC) were utilized for the standard curve measurement. Lastly, the findings are presented as milligrams of Gallic acid equivalents for each grams of powdered potato peel

[26].

Preparation of Folin–Ciocalteu Reagent: 10g of sodium tungstate ($Na_2WO_4.2H_2O$) and 25 g of sodium molybdate ($Na_2MoO_4.2H_2O$) were dissolved in 70 mL of distilled water in a flask. 0.5 mL of concentrated phosphoric acid and 10 mL of concentrated hydrochloric acid were added consecutively and then refluxed for 10 h. 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 h. The mixture was then boiled for 15 min to expel excess bromine and cooled. Then it was filtered and diluted with 100 mL of distilled water [27].

2.2.4. Determination of the total phenolic content

For the determination of total phenol, 0.1 g of dry extract was dissolved in 100 mL of methanol to prepare stock solutions of 1000 mg/L. Taking 0.1 mL of the stock solution, a 5 mL sample with 20 mg/L was prepared in triplicate, a series of 20–100 mg/L with a difference of 20 mg/L concentrations of methanolic solutions with gallic acids was also prepared and used as a standard for the calibration curve. Total phenol content was done as 1 mL Standard Gallic acid solution was taken from each sample in a test tube and 5 mL of FCR (10 times diluted) was added to each test tube. Then 4 mL of sodium carbonate (7.5 %) was also added and allowed to stand for 30 min. The absorbency of the sample was then read from a UV–vis spectrophotometer (*PerkinElmer UV–Vis spectrometer, Lambda 35*) at 765 nm. Similarly, sample absorbance was also measured in triplicate, and equation (2) provided the formula to express the total phenol value, which was derived from the regression equation and expressed as mg of Gallic acid equivalent per grams of sample.

$$Cp = CV/m \tag{2}$$

Where c is the gallic acid concentration (mg/L) determined from the curve, V is the extract volume (0.1 mL), m is the weight of the pure plant ethanolic extract (0.1 g), and Cp is the total content of phenolic compounds in the GAE/g sample mg.

2.2.5. Determination of total flavonoid content

The total flavonoid content (TFC) of the extract was determined using the aluminum chloride colorimetric method adapted from Vieira et al. [28]. Briefly, 1 mL of extract was mixed with 300 μL of 5 % $NaNO_2$ solution in a 20 mL volumetric flask. After 5 min, 300 μL of 10 % $AlCl_3$ solution was added. The mixture was incubated for another 6 min, followed by the addition of 2 ml of 1 M NaOH solution and 2.4 mL of distilled water. The absorbance of the final solution was measured at 510 nm using a UV–Vis spectrophotometer (*PerkinElmer Lambda 35*). A standard quercetin curve (0–250 μg/mL) was prepared and used to quantify the TFC, expressed as quercetin equivalents (QE) per gram of dry weight powder.

2.3. Application of PPE on cow butter

Cow butter, a high-fat dairy product that spoils quickly if left untreated or refrigerated, was treated with a PPE phenolic compound to extend its shelf life. The factors that ensure the stability of butter in storage conditions were studied to determine the efficiency of PPE preservative applications. Therefore, the use of natural plant extract can solve these problems and extend shelf life by some time and one of these sources of phenolic compound is PPE. The characterization in terms of antioxidant activities (ABTS assay, DPPH Assay, and FRAP test) and oxidation stability of PPE in comparison with synthetic antioxidants (BHT, TBHQ, and BHT/TBHQ) was investigated.

2.3.1. ABTS assay (2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid))

The antioxidant activity of the phenolic compounds of potato peel extract (PPE) for preserving cow butter was analyzed with the ABTS assay method. The ABTS reagent was prepared by mixing 7 mM ABTS with 2.45 mM potassium persulfate and allowed for 14 h of reaction at room temperature to form the radical cation. The ABTS solution was diluted with ethanol to obtain an approximate absorbance of 0.7 ± 0.02 at 734 nm. A 1 mL diluted ABTS radical solution was then mixed with each of the cow butter incorporated with synthetic antioxidants (BHT, TBHQ and BHT/TBHQ)-incorporated cow butter (200 μL). The absorbance was measured after incubation of 6 min. A control sample without PPE was also measured for comparison [29]. The antioxidant activity is expressed as a percentage reduction in the ABTS and DPPH radicals according to Equation (3).

$$ABTS\ and\ DPPH\ \%\ of\ inhibition = \frac{A_{Control} - A_{sample}}{A_{Control}} \times 100 \tag{3}$$

2.3.2. DPPH assay (2,2-diphenyl-1-picrylhydrazyl)

The free DPPH radical scavenging ability was assessed following the method outlined by Bierncka et al. [30]. A 0.1 mM DPPH was dissolved in ethanol in a dark environment and allowed to show an absorbance of approximately 0.900 ± 0.02 at 517 nm. A 1 mL DPPH solution was added to different antioxidants PPE, BHT, TBHQ and BHT/TBHQ within butter samples (200 μL each). With a 15-min time difference of incubation in a dark place, the absorbance was measured at 517 nm and the result was compared to the control one (DPPH without PPE). The antioxidant activity was then calculated as the percentage inhibition of DPPH radicals using Equation (3) in the same fashion as ABTS methods.

2.3.3. FRAP assay (ferric reducing antioxidant power)

To measure an antioxidant's capacity to reduce, the ferric-reducing antioxidant power (FRAP) assay reduces the Fe^{3+}-2,4,6-tri-pyridyl-s-triazine (TPTZ) complex while measuring absorbance at 593 nm [31]. The FRAP reagent was prepared by mixing acetate

buffer (300 mM, pH 3.6), TPTZ solution (10 mM in HCl) and $FeCl_3$ (20 mM) in a 10:1:1 ratio. 900 μL of FRAP reagent was added to 100 μL of the PPE-incorporated butter sample and the BHT, TBHQ and then BHT/TBHQ butter samples and the absorbance was measured at 593 nm after incubation at 37 °C for 30 min. A standard curve is created using known concentrations of $FeSO_4$ to quantify antioxidant activity. FRAP values are expressed in mmol Fe^{2+} equivalents per gram of sample [28,32].

2.4. Microbiological and chemical analysis of cow butter with the PPE preservative

Butter samples collected from the local producer in Bahir Dar City were treated as follows: control (without PPE) and with 0.2 % and 0.3 % PPE (by weight). These samples were stored at two temperatures, 20 °C (T1) and 45 °C (T2), for 21 days. Microbiological (total aerobic bacteria and yeast mould count) and chemical analyses (peroxide value and free fatty acid value) were performed at 4-day intervals. Duplicate measurements were taken on days 1, 5, 9, 13, 17, and 21 to assess the impact as procedure was reported by Sepelevs et al. [33]. PPE was extracted under optimal conditions: sample size 0.5 μm, ethanol as solvent and a sample-to-solvent ratio of 1g:10 ml. The quantity and quality of the main phenolic compounds were verified before application. The extract was dissolved in a 46 °C water bath for uniform mixing and then stored at the designated temperatures. Factors considered during application included extract concentration, storage temperature, and time, all of which influence butter storage conditions. Storage temperature and time will affect the occurrence of various reactions and the growth of bacteria and will yeast mould and also be a major factor in the perishability of butter without treatment [5].

2.4.1. Microbiological analysis

In this study, two microbial quality parameters, the total aerobic plate count and yeast-mould counts, were performed according to the Bacteriological Analytical Manual (BAM). The reason for choosing these parameters is that dairy products, including butter, are mainly affected by these microbes [34].

***Total Aerobic Bacteria*:** The potato peel extract was incorporated into a butter sample with a concentration of 0.2 % PPE and 0.3 % PPE, and a control without any additive was prepared on a base of 5 g butter for all treatments with two storage temperatures (20 °C and 45 °C). During the analysis, the samples were transferred aseptically to a stomacher bag and homogenized with 40 mL of peptone water. To ensure an even distribution, plate count agar (PCA) was cooled to 46 °C after autoclaving and before pouring into Petri dishes. A milliliter of homogenized sample was added to a sterile test tube containing 9 mL of peptone water. Serial dilutions were performed up to 10^{-5}, ensuring thorough mixing at each step. A 1 mL of the appropriate dilutions was then pipetted onto separate Petri dishes. Molten PCA (10–15 mL) was poured onto each dish, and the plates were gently swirled to distribute the sample uniformly (pour plate technique). After solidification, plates were incubated for 48 h at 35 °C. Colony-forming units (cfus) were then counted using a colony counter. The final cfus count per milliliter of the original sample was calculated by multiplying the colony count on a plate by the dilution factor (10^n), where n represents the corresponding dilution used.

Yeast-Mould Count: Butter samples with a potato peel powder concentration of 0.2 %; 0.3 % and a control without extract were prepared for storage temperatures of 20 °C and 45 °C. The potato dextrose agar (PDA) medium was tempered in a water bath at 45 °C after being autoclaved for 15 min at 121 °C. 0.1 mL volumes of the suitable dilutions of the sample were placed on Petri dishes after it had been serially diluted in peptone water. Using potato dextrose agar, serial dilutions up to 10^{-4} were also prepared by spreading technique. The sample was uniformly distributed on a plate using a spreader with the help of a flame to minimize contamination from sample to sample. The Petri dish plates were then incubated at 25 °C for three to five days after solidification. The yeast and mould were identified by their blue-green colonies, which were counted using a colony counter measuring colony formation units per gram of butter (cfu/g).

2.4.2. Chemical analysis of cow butter with preservative

Chemical parameters (free fatty acid and peroxide values) were examined following the Official Analytical Chemists method [35].

Peroxide value: The peroxide value of the butter samples was determined according to the method described by Anconi et al. with slight modifications [36]. 5 g of the butter sample was weighed and dissolved in 10 mL of chloroform. The solution was then mixed with 15 mL of glacial acetic acid and 1 mL of saturated potassium iodide (KI) solution. The mixture was kept in the dark for 5 min at room temperature. After vigorous shaking, 75 mL of distilled water was added, followed by 1 mL of 1 % starch solution. The resulting solution was titrated with a solution of sodium thiosulfate ($Na_2S_2O_3$) of 0.001 N until the endpoint, indicated by a clear color change. A blank sample was prepared and titrated identically. Finally, the peroxide value was calculated according to Equation (4).

$$PV = \left[\left(V_f - V_i\right) N / M\right] \tag{4}$$

Where V_f is the volume difference of $Na_2S_2O_3$ used for the titration (mL), V_i is the volume of $Na_2S_2O_3$ used for the blank titration (mL), N is the normality of $Na_2S_2O_3$ titrated against 0.001 N sodium thiosulfate solution until the yellow color almost disappeared, and M is the amount of sample (g). *PV* is the peroxide value, expressed as a gram equivalent of oxygen per kilograms of sample.

Free Fatty Acid Value Determination: The free fatty acid (FFA) content of the butter samples was determined using a titrimetric method. Five grams of butter sample were weighed and mixed with 30 mL of ethanol. Several drops of the phenolphthalein indicator were added to the solution, which was then warmed to promote dissolution. The mixture was titrated with a 0.001 N potassium hydroxide (KOH) solution until a stable pink endpoint was reached for at least 15 sec. A blank sample was prepared and titrated identically. The FFA is determined using equation (5).

$$\%\ FFA = \left(\left(V_f - V_i\right) \times N \times 56\right) / \left(weight\ of\ butter(g)\right) \quad (5)$$

Where FFA (%) is the percentage of free fatty acids, N is the normality of KOH used for the titration and V_f and V_i are the volume of KOH used for sample titration, and blank titration respectively.

2.5. Oxidative stability test using rancimat method

PPE was dissolved with butter with 500 ppm and then heated at 90, 100, and 110 °C from room temperature (20 °C) to assess the oxidative stability using Rancimat method using a professional instrument 892 (*Metrohm, 892 Herisau, Switzerland*) instrument. Synthetic antioxidants BHT (200 ppm) and TBHQ (200 ppm) were added to the butter, giving a final concentration of 500 ppm, and examined under the same conditions (concentration, storage conditions, cow butter). A combination of BHT and TBHQ was also added to examine and compare the synergetic effect with the three antioxidant activities [13]. The volatile compounds were absorbed in a conductivity cell by allowing a continuous air stream (20 L/h) to pass through a heated sample under ambient conditions. Conductivity was continuously monitored until a sudden rise signified the end of the induction period [37,38]. The oxidation stability oxidation index was then analyzed at various temperatures including room temperature (20 °C) and would be expressed as induction time (h). A fat or oil, such as butter, resists oxidation during what is known as the induction time before it begins to break down and become rancid.

3. Results and discussions

3.1. Preservative extract/phenolic compound and analysis

Percentage Yield of PPE: The result for the percentage yield of the extract sample obtained by using the maceration extraction with ethanol solvent was 10.42 ± 0.03 %. This result was better compared from the previous study by Sepelevs et al. (that is 10.15 ± 0.33% where it was mean plus or minus standard deviation) with the same solvent extraction [33]. The reason may be the potato peel drying system where in this thesis freeze drying was performed and the PPP was soaked for more time than this one and enables the extraction of more solute. The study by Samarin et al. reported that although the yields were higher during water extraction than other alcoholic solvents (methanol, ethanol, hexane and acetone), the total phenolic compounds obtained were lower, which was not preferred for high antioxidant activity [39]. The researcher also showed the extraction ability in the order of high to low, such as methanol, water, ethanol, acetone, and hexane. Ethanol is frequently used in the food and pharmaceutical sectors for extraction purposes due to its classification as generally recognized as safe (GRAS) by regulatory bodies such as the FDA [40]. However a recent report by El. Beltagy and other studies have shown that methanol is not the preferred solvent for extracting antioxidants used in food products due to its toxicity [41,42]. Variation in percentage yield of extract will come from different extraction methods, extraction solvent type, plant variety, geographic location of the plant, time of ripening, temperature, etc. All these factors play an important role.

Phytochemical screening test: In this study, many secondary metabolites (alkaloids, terpenes, flavonoids, lignin, steroids, curcumins, saponins, phenolic, tannins) were tested to determine the presence of basic components that play a role in preservative activity (phenol, flavonoid, and tannin). The test was performed to identify a color change and confirm the presence of these antioxidants in the

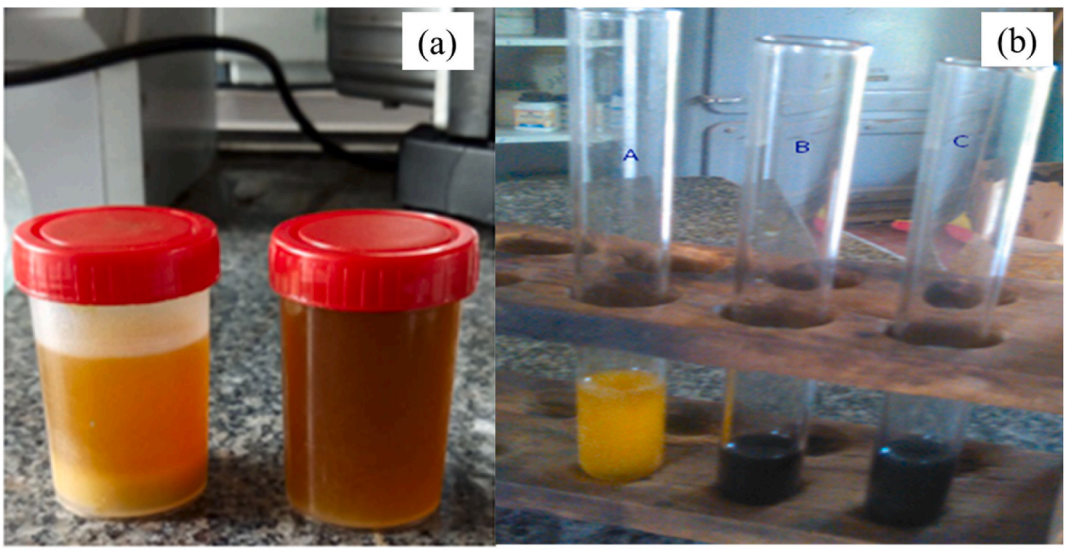

Fig. 2. a) Potato peel extract, b) Phytochemical Screening Test Result (i.e. (A) tests of the phytochemical screening test (i.e., (A) Flavonoids tests, (B) Phenol test, and (C) tannin test).

extract. For this qualitative test, the result showed its presence in the potato peel extract, making it a better preservative for fat- or lipid-containing food products. The presence of these phenolic compounds has the properties of anti-allergenic, anti-atherogenic, anti-inflammatory, antimicrobial, antioxidant, cardioprotective and vasodilatory effects [43]. The color change observed here indicates the availability of the three phytochemicals (flavonoid, phenol, and tannin) as described in Fig. 2 (b).

Total Phenol Content: The total phenolic content was determined in terms of equivalent gallic acid using a standard curve (Fig. 3) with the fitting equation (6), which was obtained from the concentration versus absorbance data with R^2 of 0.9475.

$$y = 0.0079x - 0.0586 \tag{6}$$

The total phenol content of equation (5) was found to be 2.9468 ± 0.03 mg GAE/g. In a previous study, the ethanolic pp extract, using an ultrasonic extraction technique, potato peel has reported to contain a total phenolic content of 0.280 ± 0.00521 mg GAE/g of dry extract [26]. Another study also reported that with a similar extraction method (maceration) and ethanol solvent, the total phenol was 2.74 ± 0.03 mg GAE/g of dray extract [44,45]. The variation in total phenolic may come from the color and variety of the potato, the selection of the solvent, and the extraction method that affected the concentration. The other study also suggested that the variation may come from geographical location, season, and storage condition [46,47]. In this research, the potato peel is from Ethiopia, so the variation may come from geographical location and also soil type.

***Total Flavonoid Content*:** In addition to the broad spectrum of chemical and biological activities, flavonoids possess radical-scavenging properties that are important for food oxidation stabilization. This implies that the higher the quantity of flavonoid, the better the preservation of the product [22]. The total flavonoid content was determined in terms of the Quercetin standard from the standard curve equation y = 0.0007x + 0.0259, the total flavonoid content was found to be 3.6885 ± 0.02 mg equivalent Quercetin/g of dry extract which is mean plus or minus the standard deviation as presented in Fig. 4. In the previous study, the total flavonoid content was found to be 0.81 ± 0.04 mg equivalent Quercetin/g dry extract [21]. This PPE derived from potato peels exhibited a higher total flavonoid concentration compared to sugar beet pulp and sesame cake, which recorded values of 0.91 ± 0.02 and 0.29 ± 0.01 mg of equivalent Quercetin/g of dry extract, respectively, using an analogous extraction method and ethanol solvent as used in this study [48].

3.2. Antioxidant activity and analysis of butter with preservative effect

3.2.1. Antioxidant activities

Cow butter with PPE phenolic compound preservative radical scavenging activity of PPE phenolic compound was evaluated using the FRAP, ABTS, and DPPH scavenging assay, and the results are presented in Table 1. An effective colorimetric technique for determining the antioxidant activity of a substance, especially in biological and food samples, is the ABTS assay [49]. The ABTS scavenging assay in PPE showed 76.2 % (μL/mL) which indicates that PPE has a more comparable efficacy with the synthetic antioxidant, BHT (71.7 %), TBHQ (79.6 %), and hybrid antioxidant BHT/TBHQ (82.3 %). As a recent study reported, antioxidant efficiency would show variations with pH and time fluctuations [50].

The DPPH radical elimination assay is the most widely used technique for investigating antioxidant activity [51]. The ability to quench DPPH radicals is measured by the DPPH test. The result showed that BHT/TBHQ had a higher DPPH (84.6 %), PPE (79.8 %), BHT (72.3 %) and TBHQ (80.6 %). It was reported to have a DPPH scavenging activity of 22–55 % at different concentrations of PPE [6]. In this study, the phenolic compound showed a higher DPPH scavenging activity, which could be due to the concentration or extraction method (solvent used) [52].

When applying PPE and synthetic antioxidants (BHT, TBHQ, and BHT/TBHQ), the DPPH and FRAP tests give us a guarantee by determining the antioxidant activity of cow butter. The antioxidant capacity of the phenolic compounds found in PPE is evaluated by their ability to convert ferric iron to ferrous iron using the FRAP technique [6]. In the FRAP assay, the BHT/TBHQ and TBHQ also showed a greater FRAP efficacy (80.54 and 78.61 mmol Fe_2SO_4/100g respectively) than the other antioxidants. The PPE antioxidant ability (75.4 mmol Fe_2SO_4/100g) is also high as compared to BHT antioxidant efficiency (52.3 mmol Fe_2SO_4/100g). The high FRAP

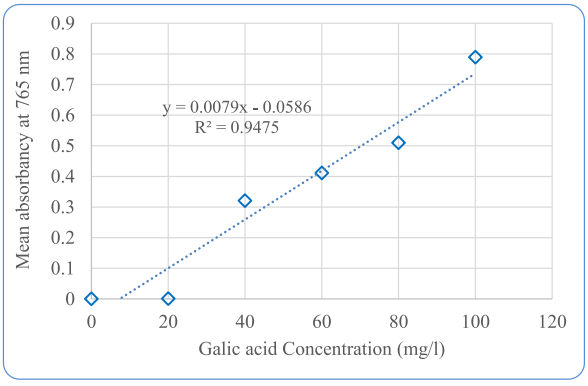

Fig. 3. Calibration curve in Total Phenol content determination.

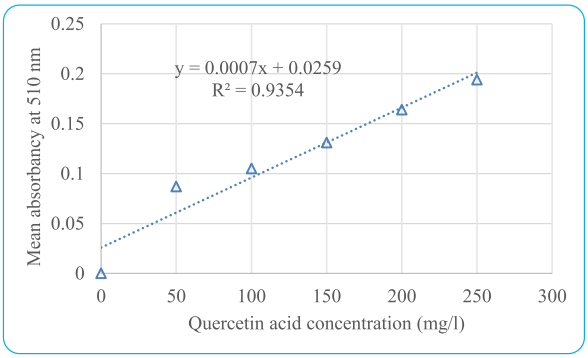

Fig. 4. Calibration curve for Total Flavonoid Determination.

Table 1
Antioxidant activity of PPE and synthetic antioxidants.

Antioxidants	ABTS Assay (%)	DPPH Assay (%)	FRAP (mmol Fe_2SO_4/100g)
PPE	76.2 ± 1.13	79.8 ± 0.72	75.4 ± 0.69
BHT	71.7 ± 1.04	72.3 ± 0.46	52.3 ± 0.61
TBHQ	79.6 ± 1.28	80.6 ± 0.81	78.61 ± 0.83
BHT/TBHQ	82.3 ± 1.63	84.6 ± 0.98	80.54 ± 0.92

value in TBHQ and hybrid (BHT/TBHQ) would be Its ability to donate electrons and stabilize free radicals making it a more effective reducing agent than other antioxidants [15,53]. Whereas the natural extract, PPE would have a high phenolic content that can reduce Fe^{3+} effectively [10]. In the previous study, it was reported that the potato peel extract has the FRAP test showing 18.6 mmol Fe_2SO_4/100g in the preservation of wheat flour biscuits [54]. However, as can be observed in the scavenging assay, the result obtained in this study showed a higher FRAP efficiency of the PPE phenolic compounds more than three times. The reason might be the same as those mentioned in the DPPH assay due to the concentration and solvent used in the extraction. Although what happened in, the use of PPE has a multipurpose preservative effect compared to other synthetic preservatives, such as health benefits due to its phenolic content, which is linked to anti-inflammatory, anticancer, and cardiovascular health benefits [1].

3.2.2. Microbiological analysis

The microbiological test of cow butter by natural potato peel extract was carried out for 21 storage days and the test was carried out over a 4-day interval for a total of 6 times. The microbiological tests performed in this study were total aerobic bacteria count and yeast mould count.

***Total Aerobic Bacterial Count*:** The average logarithmic total aerobic bacterial count of the sample is shown in Fig. 5. The result of the first day suggest that variations in concentrations and temperatures did not lead to significant differences in bacterial counts.

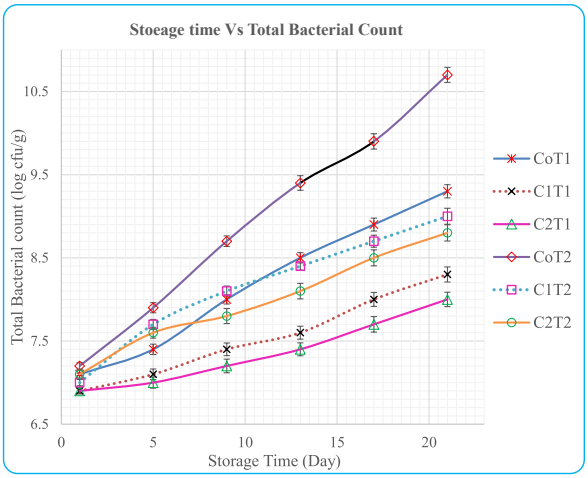

Fig. 5. Average bacterial count of butter stored at different temperatures and preservative concentration
N.B: [Co: control; C1:0.2 % PPE; C2: 0.3 % PPE; T1: 20 °C; T2: 45 °C].

However, an increase in time correlates with a rise in bacterial populations. As illustrated in Fig. 5, overall six-time intervals, the total aerobic bacterial counts for the control sample (butter without preservative) at the elevated temperature of 45 °C surpassed those of the other samples. The reason for this may be the higher storage temperature and the fact that it is free of extract. In the first two cycles (up to the first day 9), the next higher logarithmic bacterial count value was 0.2 % antioxidant concentration and 45 °C (C1T2), but as the time increased after the ninth day the logarithmic value of the control at T1 (C0T1) becomes higher than C1T2. The reason is that as time passes, even though the temperature T1 is lower than T2, C0T1 was the control without preservative, and its total bacterial load increased over time. This indicates that the extract at T2 (45 °C) also shows some antioxidant effect in comparison to the control at T1 (20 °C). This implies that both concentration versus temperature and time have a significant effect. The order of the total aerobic bacterial count after the 11th day is arranged as C0T2 > C0T1 > C1T2 > C2T2 > C1T1 > C2T1 from the highest logarithmic aerobic total bacterial count to the lowest. After the 11th day, the level of total bacterial count is greater than the standard level (i.e. 9.2 cfu/g) [55].

Yeast and mould count: Mould and yeast count for control butter sample were higher than the PPE-treated butter samples in the three weeks and at two storage temperatures, as described in Fig. 6. The number of yeasts mould increased significantly during each week. With 0.2 % and 0.3 % PPE treated butter, samples showed lower value of aerobic mould and yeast count for all storage time than the control at both temperatures. A 0.3 % potato peel extract with a storage temperature was better than others. However, the control that is free of preservatives showed a higher yeast mould count for both temperatures throughout the storage time. This indicates that time versus concentration with temperature had a significant effect on the butter sample throughout the storage time. Therefore, the result validated that the potato peel extract had an antimicrobial effect against the growth of yeast and mould in butter at both storage temperatures.

According to the report by Tola et al., the mean yeast and mould count for the untreated butter sample at the initial preservation time was 6.70 log cfu/g, which was greater than the value obtained in this study result, where initially it was 6.02 log cfu/g. The author also suggested the maximum tolerable limit of yeast and mould regulated by the Ethiopian Standard Authority (ESA), which is 1 log cfu/g and by Tola A. et al. [56]. But both results are beyond the acceptable limit. Many reasons may be for this as poor hygiene of milk utensils, unclean water used for cleaning purposes, cow dung, lack of knowledge in milk processor, storage conditions (temperature, humidity), etc. may cause low quality of butter produced [57]. Here in the study, it is proved that the extract limits the growth of yeasts and mould compared to that of the control throughout the storage time (21 days), shows the preservation effect, and can be used as a preservative.

3.2.3. *Chemical analysis*

Peroxide value: Peroxide value is an indicator of primary oxidation products quantitatively 1 mg of active oxygen within 1 g of lipid which can be measured by its ability to liberate iodine from potassium iodide. As shown in Fig. 7, the treatment creates variation in the preservation of the butter. Individually, the blank sample also shows a significant effect on both temperatures with time. However, the blank sample with a higher temperature (45 °C) showed a higher peroxide value throughout the time than all other treatments, which indicates a higher formation of the primary oxidation product than other treated samples and the control at 20 °C. The increase in photovoltaic for the blank sample with a lower temperature (20 °C) was lower than the blank with a higher temperature (45 °C). This is because of the storage temperature, as the two temperatures showed significant differences. During the 21 storage days, the butter sample treated with 0.3 % PPE and a storage temperature of 20 °C followed by a sample treated with 0.2 % PPE and a storage temperature was more effective. At 45 °C, the butter sample treated with 0.3 % potato peel extract exhibited a greater inhibitory action than 0.2 % potato peel extract. Generally, the resulting data shows that treating butter with a concentration of 0.3 % natural potato peel extract and a storage temperature of 20 °C was better than the rest treatments tested in this work. One reason for

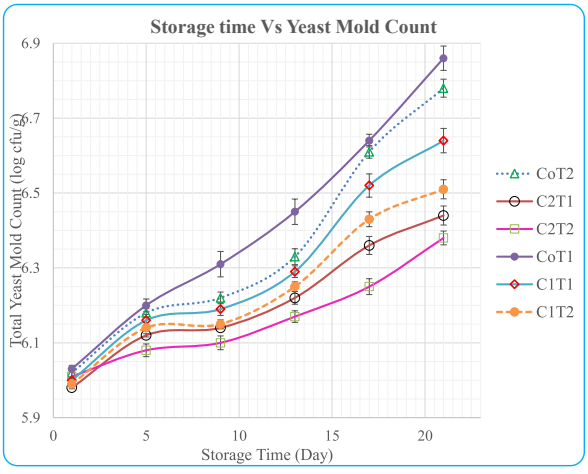

Fig. 6. Average yeast-mould count of Butter Stored at different temperatures and preservative concentrations
N.B: [CO: control; C1:0.2 % PPE; C2: 0.3%PPE; T1: 20 °C; T2 45 °C].

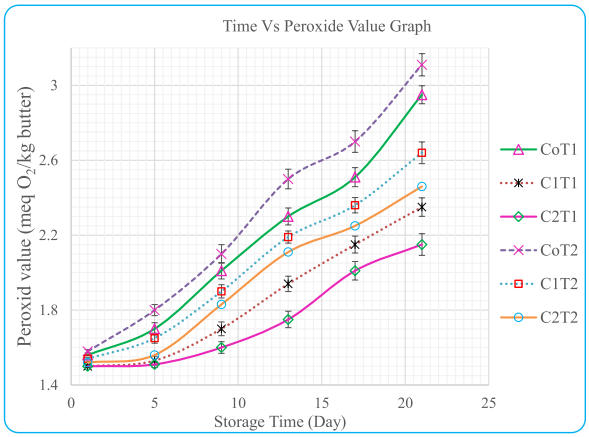

Fig. 7. Average peroxide value of Butter Stored at different temperatures with preservative effect ***N.B***: [CO: control; C1:0.2 % PPE; C2: 0.3%PPE; T1: 20 °C; T2: 45 °C].

this may be that at higher temperatures, there is higher oxidation reaction formation and also lower distraction of phenolic compound which is used to preserve foods. Some phenolics can be taken into account as antioxidants in butter during storage. At all time (up to 3rd week), the peroxide values in this work(1.5–3.4 meq O_2/kg) are within the standard limit values (10 meq O_2/kg) for food stuffs [58].

Free fatty acids: Free fatty acids are formed by hydrolysis or oxidation (cleavage) of double bonds. As shown in Fig. 8, there was a significant increase in free fatty acid in all butter samples, and the least increase in FFA was observed for the sample treated with 0.3 % concentration of potato peel extract throughout storage time. There was knowingly higher formation of FFA for a control at a storage temperature of 20 °C and 45 °C than in the butter incorporated with potato peel extract at both temperatures. So, all treatments had more significant effect than temperature for the change in free fatty acid. This is due to the presence of phenolic compounds [25]. Even though, the recommended FFA values for oily foodstuffs should be below 0.3 %, according to WHO guidelines [59]. Higher FFA levels can indicate hydrolytic rancidity, leading to potential off-flavors and spoilage.

3.3. Oxidative stability analysis

Oxidation stability refers to the tendency of oils, fats, and fat-containing foods to resist oxidation. It is a fundamental criterion for quality control in the production of oils and fats by the food sector or for inspecting the arriving products in processing facilities. The measurement of oxidative stability using the Rancimat method is given in Table 2. At room temperature (20 °C), high induction periods of 4984.1, 4230.6, 3872.3, and 3960.7 h were found when BHT/TBHQ, BHT, and PPE were applied to cow butter respectively. The non-preservative showed an induction of 120.4 h at 20 °C, which means that the butter can wait for five days before it oxidizes. TBHQ exhibits exceptional thermal and storage stability, making it well-suited for all frying applications and other high-temperature procedures [14]. The preservatives synthesized from potato peels are also guaranteed to be promising preservatives for cow butter for up to six months (i.e. 3960.7 h = 165.1 days) at room temperature (20 °C). On the other hand, the cow butter without PPE would have a maximum of 5 days (i.e. 120.4 h = 5 days) shelf life at room temperature. As a result, as the temperature increases, the induction period decreases and the shelf life. The longer induction period indicates the longer shelf life expectation and an inverse linear relationship between temperature and induction time [60]. Therefore, the use of PPE, BHT, TBHQ, and BHT/TBHQ significantly extends the induction period by slowing the oxidative process, mainly the synergetic effect of the two synthetic preservatives extends the shelf life of fat and oil-containing foods such as butter.

4. Conclusions

Potato peel extract, rich in flavonoids, tannins, and phenolic compounds, demonstrates notable antioxidant properties when obtained by maceration of ethanol. The extract effectively preserves cow butter during storage, evidenced by reduced oxidation and microbial proliferation. The phenolic compounds in PPE also demonstrated strong antioxidant activity, comparable to synthetic antioxidants such as BHT and TBHQ, as shown through ABTS (76.2 %), DPPH (79.8 %), and FRAP (75.4 mmol Fe_2SO_4/100g) evaluations, indicating its potential as an effective preservative for cow butter. Compared to untreated samples (butter without PPE) and samples stored at higher temperatures, butter samples treated with 0.3 % PPE extract concentration and stored at 20 °C demonstrated the best preservation results, with significantly lower total aerobic bacteria count, yeast mould count, peroxide value, and free fatty acid value. According to these results, PPE may be able to naturally preserve cow butter, especially when stored at lower temperatures. The application of PPE also significantly extends the oxidative stability and shelf life of cow butter, with PPE showing promising preservative potential for up to six months (i.e. 3960.7 h of IP) at room temperature. Utilizing this readily accessible natural extract can reduce microbiological proliferation and oxidative reactions in lipid- and oil-rich food, thereby mitigating the associated toxicological

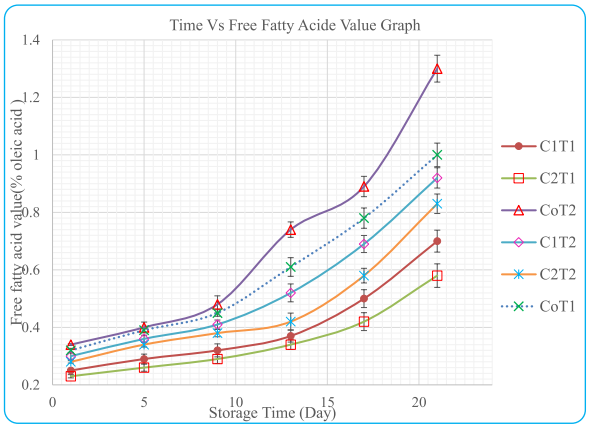

Fig. 8. Average Free Fatty Acid Value of Butter Stored at Different Temperatures with preservative effect
N.B: [CO: control; C1:0.2 % PPE; C2: 0.3%PPE; T1: 20 °C; T2:45 °C].

Table 2
Oxidative stabilities of cow butter with PPE preservatives and without PPE preservatives.

Temperatures (°C)	IP (h) without PPE	IP (h) with PPE	IP (h) with BHT	IP (h) with TBHQ	IP (h) with BHT/TBHQ
20 (Room temp.)	120.3 ± 2.4	3960.7 ± 3.8	3872.3 ± 5.2	4230.6 ± 6.3	4984.1 ± 5.4
90	10.4 ± 0.6	55.5 ± 2.6	50.6 ± 4.6	60.52 ± 4.2	76.24 ± 3.7
100	8.5 ± 0. 2	46.3 ± 2.1	42.3 ± 3.9	52.35 ± 2.2	60.13 ± 2.5
110	5.6 ± 0.4	34.9 ± 2.7	32.4 ± 1.7	38.78 ± 2.1	43. 39 ± 1.6

risks to human health. The optimization of parameters (preservative concentration, storage temperature, butter size) will be the subject of further study.

CRediT authorship contribution statement

Lemlem Tadesse Teklehaymanot: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Talbachew Tadesse Nadew:** Writing – review & editing, Visualization, Supervision. **Abebe Teshome Ayele:** Writing – review & editing, Data curation.

Disclosure statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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