

OXIDATIVE STABILITY OF SILVER CARP OIL SUPPLEMENTED WITH POTATO PEELS EXTRACT COMPARED TO SYNTHETIC ANTIOXYDANTS DURING LONG TERM STORAGE

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Abstract

The main goal of the current investigation is to use Potato peel extract (PPE) compared to butylated hydroxyl anisole (BHA) on the oxidative stability of silver carp oil over a period of 70 days at two temperatures (25°C and 45°C). The progress of lipid oxidation was assessed in terms of Peroxide value (PV), Conjugated dienes (CD), Thiobarbituric acid reactive substances (TBARS), free fatty acid (FFA) and Iodine value (IV). Different organic solvent, including ethanol, methanol, hexane and acetone were used to prepare potato peels extracts. The antioxidant capacity of the ethanol and methanol extract was tested in-vitro. This extracts showed strong reducing power and diphenyl picryl-hydrazyl (DPPH) radical scavenging ability. After 70 days storage at 25°C, silver carp oil containing 800, 1600 and 2400 ppm ethanol extract of potato peels showed lower values of PV (30.48, 14.83, 11.7 meq/kg) and CD (11.68, 10.97, 9.41 nmol/g) than the control samples of oil (PV 68.75 meq/kg, CD 34.43 nmol/g). Also the oil containing 200 ppm of BHA showed PV and CD values of 9.61 and 8.32 nmol/g respectively after 70 days. Similarly after 70 days at 25°C, TBARS and FFA of the oil containing 800, 1600, 2400 ppm were 163.56, 156.39, 120.69 and 0.41, 0.31, 0.225 respectively which were lower than the control (TBARS: 346.44 and FFA: 0.486). These results demonstrated that potato peel is a potent source of natural antioxidant that may be used to prevent oxidation of silver carp oil.

Keywords: Potato peel extract, antioxidant, lipid oxidation, silver carp oil

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1. INTRODUCTION

Fish oils contain high content of polyunsaturated fatty acids and are therefore vulnerable to lipid oxidation. Oxidation is a serious concern for the entire food industry, due to the production of off-flavors and odors, discolored, and toxic compounds. Oxidation can also decrease the nutritional content in food products and lead to a huge economic loss. To retard or prevent oxidative deterioration, synthetic antioxidants such as butylated hydroxyl anisole (BHA), butylated hydroxytoluene (BHT), tert-butyl hydroquinone (TBHQ) have been used in many countries (Sébédio et al., 1991). Nowadays the use of synthetic antioxidants is limited because of undesirable effects on human health (Hraš, Hadolin, Knez & Bauman, 2000). Synthetic antioxidants may cause liver swelling and influence liver enzymes activities (Martin & Gilbert, 1968). Therefore, there is a strong need for replacing synthetic antioxidants by natural sources.

Natural antioxidants have been recently used extensively in the food industry. The literature is replete with extracts from natural sources that demonstrated strong antioxidant activity. Considerable attention has been focused on herbs and spices, on which many related studies have been reported.

Potatoes (*Solanum tuberosum* L.) are one of the most important staple crops for human consumption, together with wheat, rice and corn. Potatoes are a source of dietary energy due to their carbohydrate levels and also contain a high value protein (Schieber & Saldaña, 2009). The global consumption of potato is shifted from fresh potato to value added products such as French fries, chips and puree. As a consequence of growing processed potato products, considerable quantities of waste are generated. Peels are the major by-product of potato processing industries which represent a severe disposal problem. Various workers have reported the antioxidant ability of potato peels extract

(Al-Weshahy et al., 2010; Babbar et al., 2012; Mohdaly et al., 2010). Some of the studies revealed that potato peels were efficient in retarding lipid oxidation in vegetable oils (Mohdaly et al., 2010; Rehman et al., 2004). Others findings showed also the effectiveness of potato peels in the oxidative stability of fish muscle and oil (Habeebullah et al., 2010; Sabeena et al., 2012). All the previous studies were focused on the use of potato peels extract during a short term storage. Hence the use of potato peels extract as antioxidant in fish oil during long term storage has not yet been studied. The data presented in this manuscript are intended to complete those previously reported, in order to make the information reported on the antioxidant activity of potato peels extract in oils more complete. The overall objective of this study was to provide more knowledge about the potential of potato peel extracts as source of effective antioxidant in silver carp oil, a fish with high content of n-3 polyunsaturated fatty acids (PUFA). The specific aims were to determine the total phenolic content and the effectiveness of potato peels extract in-vitro assays.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

The chemicals xylenol orange, butylated hydroxyl anisole and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Chemicals (Shanghai, China). All the others chemicals were of analytical grade and obtained from the chemical store of the Jiangnan university (Wuxi, China).

2.2 Potato peels extract samples

Potatoes were purchased from a local market in Wuxi, China. After cleaning potato samples were peeled with mechanical peeler to obtain uniformly thick peels. The peels were dried in a hot air oven at 55°C for 72 h and powdered by a kitchen blender. The material that passed through 80 mesh sieve was retained for use. Five grams of grounded peels were extracted with 50 ml of organic solvents (methanol, ethanol, hexane,

acetone) overnight in shaker at room temperature and centrifuged at 2800rpm for 10 min. the supernatant was collected in a separate bottle and the residue was re-extracted three times under the same conditions as mentioned above.

2.3 Determination of total phenolic content (TPC)

Total phenolic content in the extracts was determined according to method of (Singleton & Rossi, 1965). Folin Ciocalteu reagent 0.75 ml (1:10 diluted) was added to 100 µl of extract and allowed to stand at room temperature for 5 min. contents were mixed thoroughly and 0.75 ml of sodium bicarbonate (6%) was added to the mixture and incubated at room temperature for 90 min. the absorbance was measured at 725 nm using a spectrophotometer (Shimadzu UV 1100). The content of total phenolic content was determined using a standard curve prepared from gallic acid and the results were expressed as mg of gallic acid equivalents per 100g of dried potato peels.

2.4 In -vitro antioxidant assays

The ethanol and methanol extracts were screened for antioxidant activity by employing two in-vitro systems: 1,1 diphenyl-2-picrylhydrazyl (DPPH) and reducing power.

2.4.1 DPPH radical scavenging activity

The DPPH assay of extract was performed according to the methods of (Shimada et al., 1992) with some modifications. DPPH solution (1.5 ml, 0.1 mM in 95% ethanol) was mixed with 1.5 ml of extract at final concentration of 800, 1600, 2400, 3200, and 4000 ppm). The mixture was shaken and left for 30 minutes at room temperature. Absorbance of the resulting solution was measured at 517 nm using a spectrophotometer (Shimadzu UV 1100). For the blank, 1.5 ml distilled water was used instead of the sample and sample control was made for also made for each fraction by mixing 1.5 ml of sample with 1.5 ml of 95% ethanol. Radical scavenging capacity was calculated as follows.

DPPH radical scavenging capacity(%)

$$= 1 - \frac{(\text{Abs. of sample} - \text{Abs. of sample control})}{\text{Abs. Blank}} \times 100$$

2.4.2 Reducing power

The reducing power was measured according to the method of Oyaizu (1986) with some modifications. To 1 ml of extract (800, 1,600, 2,400, 3,200 and 4,000 ppm) was added 1 ml 0.2 M phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min and 1 ml of 10% TCA was added into this reaction mixture. An aliquot of 2 ml from the incubation mixture was mixed with 2 ml of distilled water and 0.4 ml of 0.1% ferric chloride in test tubes. After 10 min the solution was measured at 700 nm using a spectrophotometer (*Shimadzu UV 1100*). Increased absorbance (At 700 nm) of the reaction mixture indicated increased reducing power.

2.5 Fish samples

Silver carp fish (*Hypophthalmichthys molitrix*) were purchased from a local market in Wuxi, China and were transported within 30 minutes in ice boxes to the laboratory of school of food sciences and technology of Jiangnan University, People's Republic of China. The fish were prepared using the handling method, beheaded and skin removed before thoroughly washed with clean water to remove contaminants and unwanted parts. Fish muscle retrieved with care, separating the fillets from the bones. Fish fillets were vacuum packed in 500g packages and rapidly frozen at -46°C until needed.

The method of (*Bligh & Dyer, 1959*) was used for the oil extraction. The recovered oil was flushed with nitrogen and stored without further refining at -46°C until used (usually 2 – 3 days).

2.6 Determination of oxidative stability

2.6.1 Sample preparation

Triplicate samples of 800, 1600, 2400 ppm of Potato peels extract and 200 ppm of butylated hydroxyl anisole were initially prepared in

hexane. After evaporation of solvent under nitrogen, each antioxidant sample was placed in three brown reagent bottles and mixed thoroughly with the fish oil. All the bottles were stored at the final temperature of 25 and 45°C. Control samples in which no antioxidants were used, were set with the test samples for the two experimental temperatures. Analyses were conducted at 7 days intervals during 70 days for PV, CD, TBARS, FFA and IV.

2.6.2 Peroxide value

Peroxides values determinations were performed according the ferrous oxidation/xylenol orange method (Meites, 1963). Sample 0.01-0.3 g was mixed in a disposable glass tube with 9.8ml chloroform-methanol (7+3,v/v) on a vortex mixer for 2-4 s. Xylenol orange solution 10mM (50µl) was added and the sample was mixed on a vortex mixer for 2-4 s. then 50µl of iron (II) solution was added, and the sample was mixed on a vortex mixer for 2-4 s. After a 5 min incubation at room temperature, the absorbance of the sample was determined at 560 nm against a blank of chloroform –methanol (7+3, v/v). The peroxide value expressed as milliequivalents of peroxide value per kilogram of sample was calculated using the following formula:

$$\text{Peroxide value} = \frac{(A_s - A_b) \times m}{55.84 \times m_0 \times 2}$$

Where A_s : Absorbance of the sample; A_b : Absorbance of the blank, m = slope obtained from the calibration curve, m_0 = mass in gram of the sample, 55.84 =atomic weight of iron.

2.6.3 Conjugated diens (CD)

The method proposed by (*Zuta et al., 2007*) was slightly modified and used for the measurement of CD content in the oil. Samples (0.02 g) were diluted with isooctane, and the absorbance of the sample was measured against a blank made of isooctane at 233 nm. The CD value was calculated from the absorbance value and the final concentration of sample (g/100 ml).

The results were expressed as CD values computed as follows:

$$CD = A/C \times P$$

Where **A** is the absorbance of the sample at 233nm; **C** denotes the final dilution concentration of the sample (g/100ml); and **P** represents the length of the measuring cell (cm).

2.6.4 Thiobarbituric acid reactive substances (TBARS)

TBARS were determined according to (Abuzaytoun & Shahidi, 2006). 0.05-0.2 g of oil was mixed with 25 ml of 1-butanol and then sonicated for 5 min. A 5 ml aliquot of the mixture was transferred to a dry test tube, 5 ml of 2-TBA reagent (0.2 g of TBA/100ml of 1-butanol) was added to the same tube, and the solution was well mixed and then heated at 100°C for 2 hours in a water bath. The intensity of the colored complex was measured at 532 nm with spectrophotometer against 1-butanol as blank. The TBARS value was calculated as:

$$TBARS(\mu\text{mol/g}) = 0.415 B$$

where **B** is the absorbance read at 532nm and 0.415 is a constant determined from a standard using 1,1,3,3-tetramethoxypropane as a precursor of malonaldehyde (Abuzaytoun et al., 2006).

2.6.5 Free fatty acid (FFA)

FFA determinations were performed according to the method by (Wang et al., 2011). Samples (3g) were dissolved in a 50 ml mixture of neutral ether-ethanol (1:1,v/v); the mixture was then shaken by hand. After being cooled to room temperature, the mixture was titrated against potassium hydroxide (0.01 M) using phenolphthalein (10 g/l) as an indicator. The FFA value (meq/kg) was calculated according to

$$FFA (\text{mg/g}) = (V \times C \times 56.11) / m$$

Where **V** is the volume of potassium hydroxide exhausted by the samples(ml), **C** represents the concentration of potassium hydroxide (mol/l) and **m** the mass of the fish oil (g) sample.

2.6.6 Iodine value (IV)

The iodine value in oil samples were

determined according to Wij's method, as described by (Horwitz, 1965).

3. Statistical analysis

All experiments were replicated three times. Each replicate is expressed as mean \pm SD, and ANOVA test (using SPSS 17) was used to compare the mean values of each treatment. The significance of difference among the treatment at the end of each time point during the 10 weeks storage was determined by analysis of variance (ANOVA) using the one-way ANOVA with Duncan's test ($P \leq 0.05$).

4. RESULTS AND DISCUSSIONS

4.1 Extraction

The percentage yield of potato peel extract obtained with the four organic solvent (ethanol, methanol, acetone, hexane) was summarized in table 1. About 6.3 -16.2% potato peel extract were obtained with these solvent. The highest yield of potato peels extract was obtained with methanol (16.2%), followed by hexane (12.59%) and ethanol (9.8%). The antioxidant activity of ethanolic extract was tested in the silver carp oil at 25 and 45 °C during 70 days storage.

4.2 Total phenolic content

Table 2 depicts the total phenolic content of PPE for the four organic solvent. The amount of TPC was ranged from 1.07 to 2.84 mgGAEg⁻¹ dry weight. The results showed that methanol and ethanol were better than the others solvent at extracting phenolic content due to their higher polarity and good solubility for phenolic compounds from plant materials (Peschel et al., 2006; Siddhuraju & Becker, 2003). Table 2 shows methanol as the best solvent for extracting phenolic compounds followed by ethanol, acetone and hexane with respective values of 2.84; 2.69; 2.36; and 1.07 mg GAE/g of potato peel. In a study conducted by (Kähkönen et al., 1999), 4.3 mg of gallic acid equivalents of phenolic per g of dry potato peel have been reported. Thus our results agree with this finding by being slightly lower compared to the value 4.3 mgGAEg⁻¹. This is because phenolic content

of potato depend on many parameters such variety , color , geographical origin , season and storage (del Mar Verde et al., 2004; Reyes et al., 2004).

Table1: Percent Yield of potato peels extract with different organic solvent.

Organic solvent	Potato peels extract yield (%) ^a
Ethanol	9.8± 0.81 ^b
Methanol	16.2± 1.07
Acetone	6.3± 1.36
Hexane	12.59± 1.42

^a g PPE/100 g dried peels

^bmean value± standard deviation for triplicate determination.

4.3 Antioxidant activity in-vitro system

4.3.1 DPPH radical scavenging activity

The DPPH method is currently used to assess the capability of antioxidants to scavenge free radicals which are known to be a major threat for biological system by oxidative stress. The scavenging percentages (Fig 1a) on the DPPH radical at different doses (800,1600,2400 , 3200 and 4000 ppm) for methanolic and ethanolic extracts were exhibited . Methanolic extracts showed higher (91-94%) DPPH radical scavenging activity than ethanolic extract in all concentrations tested. Our results are in good agreement with the finding of (Habebullah et al., 2010; Kanatt et al ., 2005) who found approximatively 90% DPPH free radical scavenging by ethanolic extract of potato peel. A linear correlation between phenolic content and radical scavenging activity has been reported by previous study (Robards et al., 1999). The higher DPPH radical scavenging activity of the methanolic extract compared to the ethanolic extract may be due to a high total phenolic acids.

Table 2: Total phenolic content of potato peels extract with different organic solvent

sample	Solvent	Total phenolic (mgGAE/g DW)
Potato peel	Methanol	2.84
	Ethanol	2.69
	Acetone	2.36
	Hexane	1.07

4.3.2 Reducing power

The reducing power can be used to assess the potential antioxidant activity of a compound that may lead to the reduction of Fe(III)/ferric cyanide complex to the ferrous form by donating an electron. The reducing power of methanolic and ethanolic extract (Fig 1b) is dose-dependent. Similarly to the DPPH radical scavenging activity, The methanolic extract showed higher reducing power than the ethanolic extract .

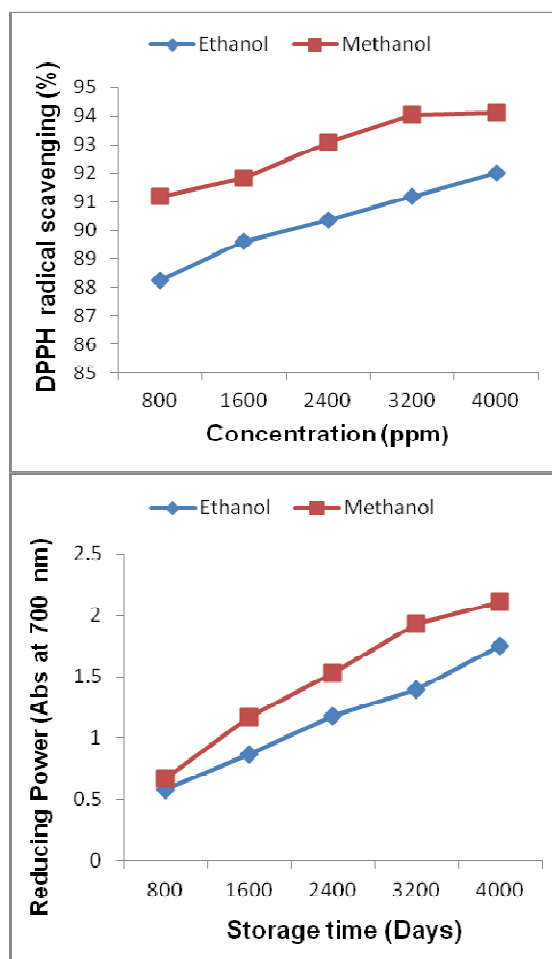


Fig.1 a DPPH radical scavenging activity ; b Reducing power .Results are average of triplicate determination+standard deviation

5. Antioxidant activity of potato peel and synthetic antioxidant in Silver carp Oil

The for control sample with the rest of treated samples are presented in the following sections.

5.1 Effect of potato peel extract and butylated hydroxyl anisole on the silver carp oil peroxide values

Peroxide value (PV) is one of the most widely-used test for the measurement of oxidative rancidity in oils and fats. The PV of silver carp oil containing PPE at different concentrations (800,1600,2400 ppm) and BHA(200ppm) are illustrated in Fig.2. As shown in figure , the PV of control sample increased rapidly than treated samples at both temperatures (25°C and 45°C). At T0 , there is no significant difference between control and treated samples for all temperatures. As the storage progressed, the PV showed a gradual increase in control and the sample containing 800ppm of PPE. The peroxide value of oil with extracts and BHA were found to be lower compared to the control sample after 70 days storage. The PV levels of samples with PPE 800 , PPE1600 were 30.48 and 14.83 meq/kg respectively after 70 days , while the PV levels of samples with PPE 2400 , BHA200 were 11.7 and 9.61 at the same temperature (25°C) for 70 days. These PV levels measured at 25°C were lesser than the PV levels measured at 45°C for the same duration (Fig .2 .A and B). The Duncan test showed significant differences in PV values between control and treated samples at all temperatures from day 14 until the end of the storage. The BHA 200 is the most effective antioxidant against oxidation , followed by PPE 2400 ,PPE 1600 and PPE 800. Nevertheless ,there is no significant differences between BHA 200 and PPE 2400 along the storage except day 56 and day 70 of Storage .Our results are in good agreement with REIHMAN et al.(2004) who studied the use of petroleum ether extract of potato peel in soy bean oil and compared the efficiency of this extract with BHA and BHT. They found that after 60 days of storage , the PV of oil treated with BHA at 25°C was 7 meq/kg oil while the PV of the oil treated with potato peel at different concentrations were 21 , 8.8 ,and

7.7 meq/kg oil for sample containing 800 , 1600 , 2400 ppm respectively .

5.2 Conjugated diens (CD) values

CD is usually used to measure hydroperoxides. CD determination gives a good information about oil oxidation, because most hydroperoxides formed by oxidation of unsaturated fatty acids have a conjugated dienic system. The CD values increased over time for all temperatures and PPE concentrations studied (Fig.3). At 25°C , the CD values of the different concentrations of potato peels extract (800 ,1600 ,2400 ppm) were 11.68 , 10.97 , 9.41% respectively after 70 days storage. At the same temperature ,the CD for BHA and Control were 8.32 and 34.43 % respectively after 70 days. When stored at 45°C , the CD values of control was 54.67 % after 70 days ; while the CD for 800, 1600 , 2400 ppm of PPE and 200 ppm BHA were 49.83 ,36.76 ,28.15 and 25.79 % respectively for 70 days. The results showed that high content of CD were observed for control samples indicating greater intensity of oxidation followed by PPE 800, PPE 1600 ,PPE 2400 and BHA200. The greater the levels of CD ,the lower the antioxidant activity of the oils (Shahid et al., 2006). The CD values of all samples decreased with decreasing temperature. It may be concluded that antioxidant activity of potato peels was suitable at lower temperature compared to higher temperature.

5.3 Thiobarbituric acid reactive substances (TBARS)

TBARS is a test which involves reaction of TBA with malonaldehyde (MDA) from oxidized fat/oils to produce a red chromophore with a Peak absorbance at 532 nm. This colour complex results in the condensation of 2 moles of TBA with 1 mole of MDA (Mlakar & Spitteller, 1996).

TBARS is used to monitor secondary oxidation products . There was an increase in TBARS value in control samples ($P \leq 0.05$) compared with treated samples for both temperatures 25 and 45°C (Fig .4). Samples stored with BHA200 and those PPE2400

were found to be the most effective against oxidation. When stored at 25°C, PPE 800, PPE 1600, PPE 2400 and BHA200 lower TBARS levels compared to the storage at 45°C for 70 days. As it can be seen from Fig.4, Potato peel extract inhibited the formations of TBARS at all concentrations; the inhibitory effect was stronger than that of control but still lower than BHA. As confirmed by the data obtained from the PV and CD, Potato peels, particularly PPE2400 were found to reduce the TBARS significantly compared with control samples and protect oil against oxidation.

5.4 Free fatty acid (FFA)

FFA formation is an important indicator of rancidity in foods. The FFA values of all samples can be seen in Fig.5. At 25°C the FFA levels of all samples gradually increased until the end of the storage. The control exhibited the highest FFA levels after 70 days (0.486% oleic acid). BHA200 samples recorded the lowest FFA content followed by PPE2400, PPE1600, PPE800. There was marginal differences between PPE2400 and BHA 200 samples over the whole storage period

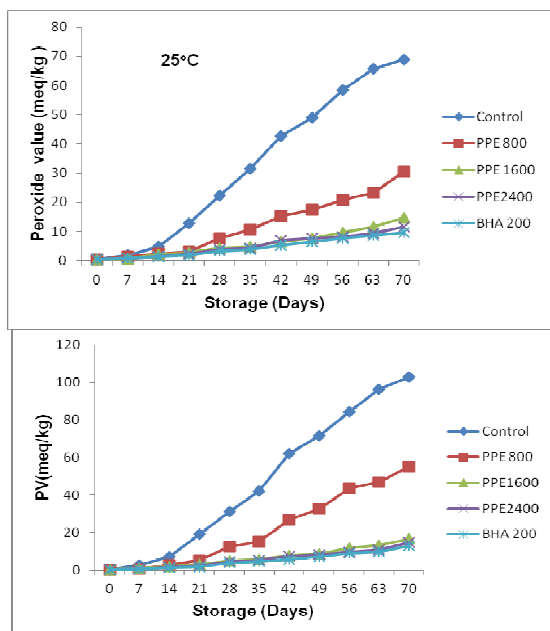


Fig 2. Effect of potato peels extracts and Butylated hydroxyanisole on the peroxide value of silver carp oil at 25°C and 45°C. Results are average of triplicate ± standard deviation

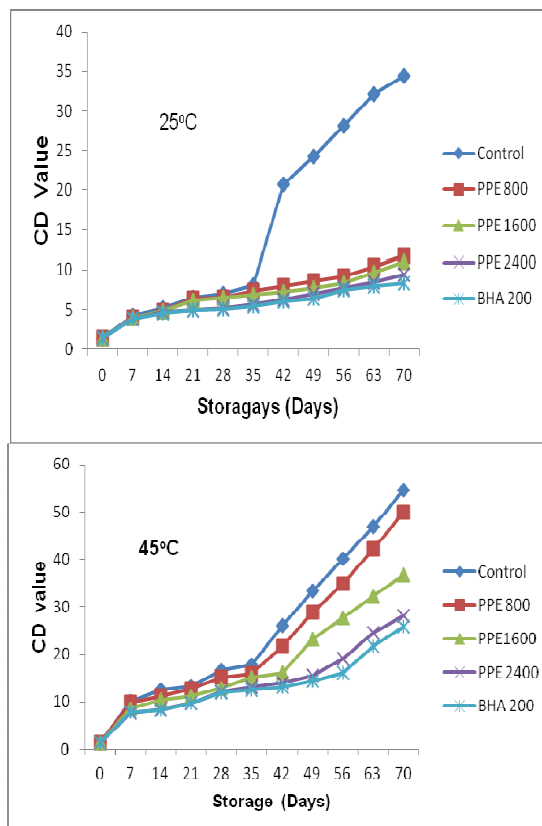


Fig 3. Effect of potato peels extracts and Butylated hydroxyanisole on the Conjugated diens of silver carp oil at 25°C and 45°C. Results are average of triplicate ± standard deviation

At 45°C, the FFA level of the control sample noticeably ascended; after 70 days it reached (0.588% oleic acid), a value which is significantly different ($P \leq 0.05$) compared with the treated samples. During the first two weeks, the FFA levels of potato extracts showed no significant differences. The PPE 800 sample distinctly increased after 42 days to reach a value of 0.4763%; the FFA levels of PPE 1600, PPE 2400 were 0.372 and 0.271% after 70 days. The BHA200 sample reached 0.1886%. A comparison of the data between the groups from the two temperature shows that the FFA levels of samples at 25°C were lower than those at 45°C. these results are in agreement with (Wu & Bechtel, 2008) who observed increasing FFA content with increasing temperature and time.

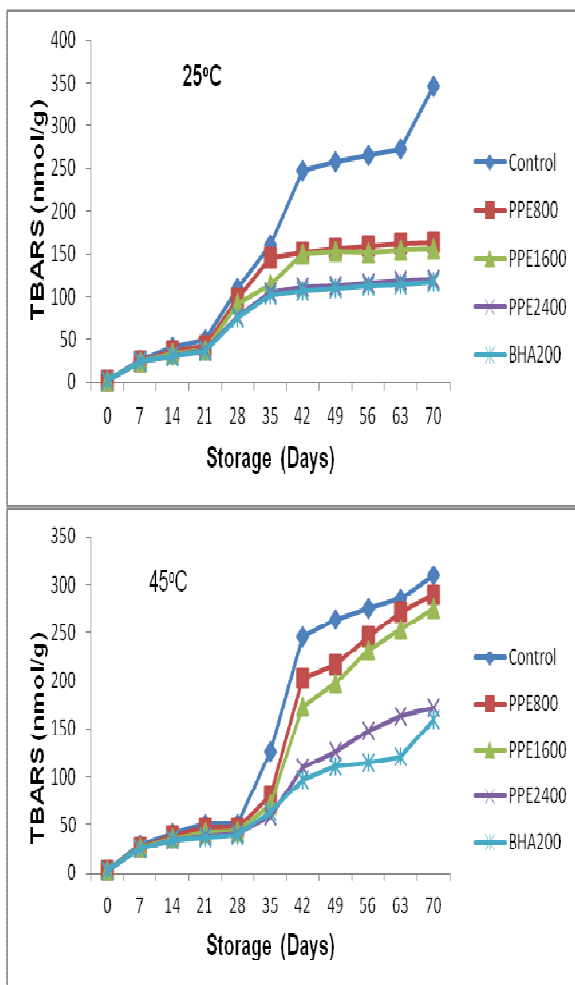


Fig.4 effect of potato peels and butylated hydroxyanisole on thiobarbituric acid reactive substances at 25 and 45°C

5.5 Iodine value (IV)

The IV is used to determine the level of unsaturation in a fat/oil system. It is expressed as the number of grams of iodine that react with 100g of sample. The IV of all samples decreased with increasing storage time (Fig 6). The decreasing iodine value is due to oxidation and because of this reaction, the double bonds of unsaturated fatty acid in the oil are broken during storage and the iodine value decreases (Noor & Augustin, 1984). The results of Fig. 6 showed that butylated hydroxyl anisole and potato peel extract addition retarded the decreasing trend of iodine value in silver carp oil during storage. Addition of PPE 800, PPE 1600, and

PPE2400 in the oil showed iodine values of 60.5, 65.66 and 69.52 respectively, after 70 days storage at 25°C (Fig 6). Similarly iodine value of oil treated with BHA 200 was 78.06 after 70 days storage at 25°C. On the other hand, the IV of the control sample was 55.11 at 25°C after 70 days. It is obvious from these results that Potato peels extract and BHA lower the IV than the control sample. Similar results were reported by (Rehman et al., 2004). They found that the IV of control samples was 62 at 25°C while the IV of samples containing BHA 200, PPE 800, PPE 1600 and PPE 2400 were respectively 82, 69, 71 and 77 at 25°C after 60 days storage.

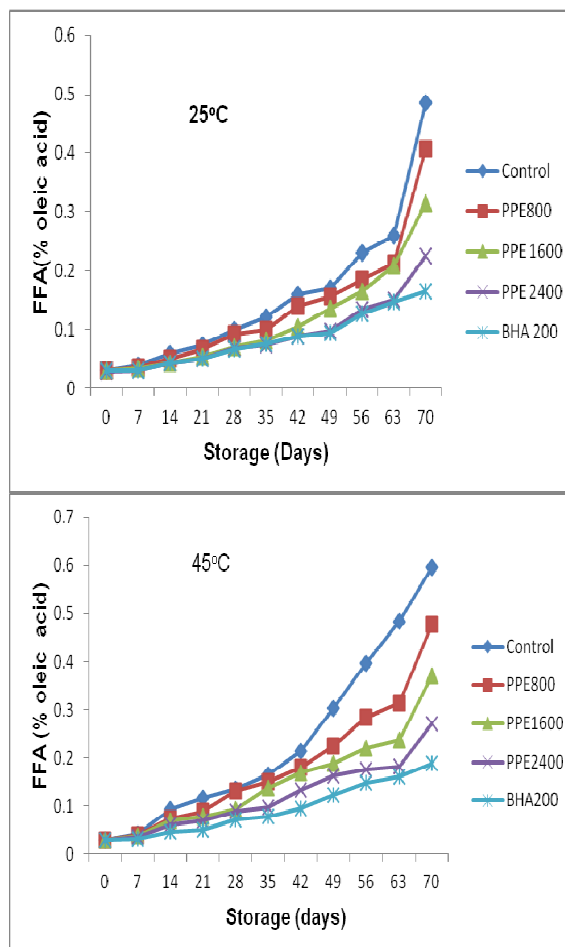


Fig.5 effect of Potato peels extract and butylated hydroxyanisole on the free fatty acid of silver carp oil at 25 and 45 °C

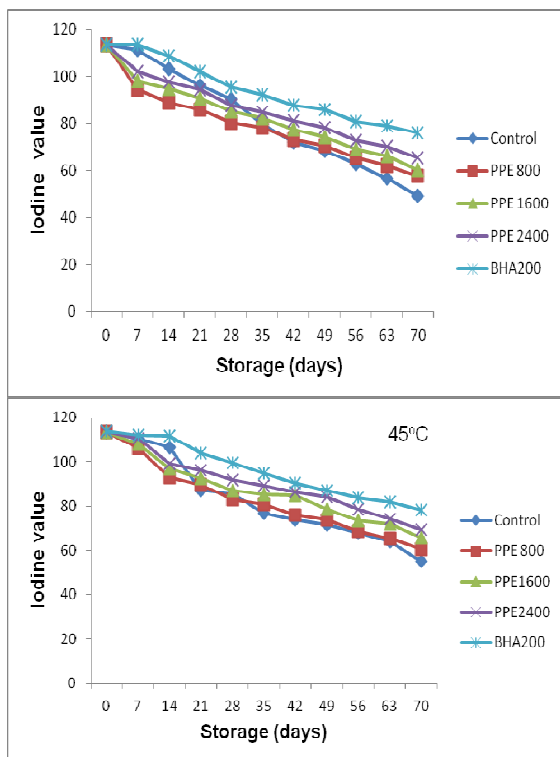


Fig.6 Effect of potato peels extract and butylated hydroxyanisole on the iodine value of silver carp oil at 25 and 45°C

6. CONCLUSION

As a continuation of the study on potato peel extracts of these by-products were prepared using different organic solvent. The in-vitro antioxidant activity of the ethanolic and methanolic extracts was investigated. The antioxidant activity of the ethanolic extract was also evaluated using silver carp oil. As observed methanolic extract showed slightly better characteristics than ethanol as solvent for phenolic compounds and in-vitro antioxidant activity but the differences were not huge. From the present study, it can be said that potato peels showed strong antioxidant activity during the long term storage of silver carp oil. When compared to the potato peels extract, the synthetic antioxidant (BHA) showed better antioxidant activity. But the 2400 ppm of potato peels show similar antioxidant activity as the butylate hydroxy anisole. Potato peels which otherwise would be discharged as waste in the environment can

safely used instead of synthetic antioxidant during long term storage of fish oil

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