REDUCTION OF OXIDATIVE STRESS BY ADDING CRUDE GREEN TEA EXTRACT AND IT'S FRACTION TO CORN OIL DURING OVEN HEATING AND DEEP FRYING

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ABSTRACT

Purpose: An imbalance between antioxidants and reactive oxygen species causes oxidative stress, leading to cellular damage. Oxidative stress has been allied to cancer, aging, atherosclerosis, ischemic injury, inflammation and neurodegenerative diseases. Heating of oil either deep frying or oven heating causes production of oxidative product.

Methods: Preparation of Polyphenolic Extract from Green Tea. Separation of polyphenolic extract into neutral and acidic fraction. Addition of three extracts in corn oil and treatment with oven heating and deep frying. Result: Primary oxidative products PV and CD increased with 24 hr. treatment by oven heating and deep frying. Secondary oxidative products p - AV and CV also increased by 24 hrs. heating treatment.

Natural antioxidant is more effective for human body. Crude green tea (C. sinensis: Camellia sinensis) extract exhibits the antioxidative activity. The order of decrease in values of PV, CD, p - AV and CV of corn oil is crude green tea extract > neutral phenolic fractions of green tea > acidic phenolic fraction of green tea. The crude tea extract added oil has the lowest CD, p - AV and CV. While CD, p - AV and CV of deep fried corn oil is higher than oven heated corn oil. The order of the highest CD, p - AV and CV of corn oil is deep fried oil > oven heated oil. Conclusion: Oxidation of oil is increased with heating time. Deep frying has highest oxidation than oven heating of oil. Crude green tea extract has highest antioxidative activity. From this study it is concluded that we can control the oxidative stress by controlling the oil quality which is one of the major source of reactive oxygen species in the body.
KEY WORDS: Camellia sinensis, Oxidative stress, PV: Peroxide value, CD: Conjugated Diene, $p$ - AV: Para Anisidine value, CV: Carbonyl value.

INTRODUCTION

The high fat content in diet causes number of diseases (obesity, heart, high cholesterol, etc.) in human body. Especially deep fat fried food has more adverse effect.\cite{1} Deep fat fried food absorbs oil during frying (temperature 180° C). Oil undergoes oxidative and hydrolytic degradation during heating. Exposure to heat\cite{2,3,4,5,6} and moisture\cite{8} enhances their chemical reactivity. Heating techniques (oven heated, microwAVe or deep frying) have different intensity effects on oil quality. Oil quality can be determined by peroxide value, $p$ - anisidine value, carbonyl value and conjugated diene.

Antioxidants are the substances that interfere the process of oxidation by accepting free radical or chelating metals. Antioxidants protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species causes oxidative stress, leading to cellular damage. Oxidative stress has been allied to cancer, aging, atherosclerosis, ischemic injury, inflammation and neurodegenerative diseases (Parkinson's and Alzheimer's).

There are four general groups of major antioxidative plant phenolics: phenolic acids (gallic, protochatechuic, caffeic, and rosmarinic acids), phenolic diterpenes (carnosol and carnosic acid), flavonoids (quercetin and catechin), and volatile oils (eugenol, carvacrol, thymol, and menthol). Trapping free radicals is the mode of action of phenolic acids antioxidants; flavonoids can scavenge free radicals and chelate metals as well. Microsomal lipid peroxidation is effectively inhibited by a variety of flavonoids (rutin, dihydroquercetin, quercetin, gallate, epigallocatechin and epicatechin gallate), the catechins.\cite{9} All were able to chelate Fe$^{2+}$, Fe$^{3+}$, and Cu$^{2+}$ and were effective O$^{-2}$ scavengers to varying degrees. Authors speculate that the relative ability to scavenge O$^{-2}$ may be responsible for the relative antioxidative difference among these compounds.

Different plants extract are identified as antibacterial\cite{10} and antioxidant.\cite{11,12,13,14,15} The three primary types of tea, green, black, and oolong, are produced by different processing procedures. Of these types, green tea extracts have the highest total phenolics content, 94% of which are flavonoids – catechins.\cite{16} Oolong tea contains about 18% total phenolics and
4.4% flavonoids. Theaflavins and thearubigins predominate in black tea. It also contains chlorogenic, caffeic, p-coumaric, and quinic acids.\[17] Much of the antioxidative activity of green tea (C. sinensis) appears to be due to natural flavonoids, tannins, and some vitamins.\[18] The antioxidant activity is linearly related to the phenol content\[19] that has been reported to be about 450 mg/g. Catechins in green tea consist primarily of gallic acid derivatives.\[20] Catechin flavanols appear to account for more than 80% of the total antioxidant activity of green tea but less than 60% of that of black tea.\[21] The radical-quenching ability of green tea has shown to be more than 20% more effective than that of black tea in both aqueous and lipophilic systems.

In tea extracts, the strongest antioxidant and \( \text{H}_2\text{O}_2 \) - scavening activity is due to phenols, with 3 – OH groups bonded to the aromatic ring, adjacent to each other.\[22] Epigallocatechin, which has three adjacent – OH substitutions in the B ring, has the highest antioxidant activity.

The primary catechin polyphenol [epigallocatechin-3-gallate] is also the primary peroxyl radical scavening compound in tea extracts.\[23,24] In terms of free radical-scavening ability, epicatechin gallate > epigallocatechin > epicatechin.\[25] The first two compounds have 3,4,5-trihydroxy (galloyl) groups while the last does not. Both their iron-chelating and free radical-scavening activities appear to be responsible for the ability of these compounds to protect membranes from \( \text{Fe}^{2+}/\text{Fe}^{3+} \) initiated lipid oxidation. Grey and Adlercreutz\[26] concluded that catechin’s chelating ability, rather than its radical-scavening mechanism alone, is responsible for the observed antioxidative activity.\[27]

Different studies conducted in more complex food systems for tea catechins as an antioxidative activity. Tea catechins to raw beef inhibited lipid oxidation.\[28] Huang and Frankel\[29] found that epigallocatechin (140 M), epigallocatechin gallate, and epicatechin gallate were better antioxidants than either epicatechin or catechin in maize (corn) oil triglycerides. Both gallic acid and propyl gallate were more effective than epicatechin and catechin.

Yanagimoto and others\[30] found that Alkyl compounds with double bond(s), such as 3,7-dimethyl-1,6-octadien-3-ol in green tea extracts and heterocyclic compounds (furfural) in roasted green tea extracts (major volatile constituents) also exhibit some antioxidative activity.
2. MATERIALS AND METHODS

2.1. Samples
Refined Corn oil was purchased from the local market in 2.5 L pack manufactured by Rafhan Foods. Green tea (Lipton) was purchased from local market in 500g pack. Samples were stored in accordance with the experiment conditions.

2.2 Preparation of Polyphenolic Extract from Green Tea
1kg of green tea leaves purchased from local market were sorted manually for extraneous materials and then 500 g of leaves were soaked in 2.5L of 80% methanol in 5L conical flasks for 48 hours along with constant and gentle stirring using a magnetic stirrer. At the end of soaking period the methanolic extract in flask was filtered through glass wool and the tea leaves residue was again soaked in 1L of 80% methanol for further 24 hours along with gentle stirring and filtered as before. The filtrates from both the extractions were concentrated to remove methanol by rotary drier and stored at 5°C.

2.3 Separation of polyphenolic extract into neutral and acidic fraction
The crude extract of green tea leaves was separated into acidic and neutral fractions following the method of Oszmianski and Lee.[31] For separation, two polypropylene columns (82 mm x 20 mm) were set and filled with 5 g of Lichroprep RP 18 column (25–40 μm). The crude extract was first checked for its pH value using a well calibrated pH Metre and then carefully neutralized to 7.0 using 5N NaOH. The neutralized extract was then passed through one of the two columns previously preconditioned with 10ml methanol and then 10 ml water. The column absorbed the neutral polyphenols while the acidic polyphenols were collected as effluent from the column. The pH of the effluent was set to 2.0 using 1N NaOH and it was introduced into the second column preconditioned with 10ml methanol and 10 ml 0.01 N HCl. Both the neutral polyphenols (absorbed on to first column) and acidic polyphenols (absorbed on to second) were then eluted with 10 ml of absolute methanol. Both the fractions were concentrated on rotary evaporator (Julabo, FE500) to remove methanol, lyophilized and stored at 5°C till further use.

2.4 Determination of Total Phenolics
The crude polyphenolic extract, acidic and neutral fractions were analyzed for the total phenolics using Folin-Ciocalteu reagent, method by Jayaprakash et al.[32] 10 mg of the crude extract and fractions were dissolved in 10 ml of methanol:water (6:4 v/v). Likewise, gallic acid used as standard was dissolved in the same solvent to prepare an appropriate range of
working standards. To an aliquot of the sample fraction and each of the standards 1 ml of One-tenth diluted Folin-Ciocalteu reagent and 3 ml of 7.5% sodium carbonate solutions were added. All the samples and standards were hold at room temperature for 30 minutes and then the absorbance was measured at 765 nm using UV-visible spectrophotometer (Perkin Elmer, Shelton CT06484 USA, Lambda 25). The estimation of phenolics was carried out in all the samples in triplicate. The calibration curve was linear ($r^2 = 0.998$) and the total phenolic contents ranged between as below.

2.5 Oil oxidation by different methods

2.5.1 Sample preparation and composition

The corn oil test samples were prepared by adding 100mg extract or fraction to 1L of oil; corn oil alone was used as control. Since there were three fractions (crude, acidic and neutral fractions of green tea) and one control, so there were overall 4 samples and 12 replicates (each sample in triplicate) in case of oven heating while there were only 8 replicates in case of deep fryer heating (each sample was taken in duplicate).

2.5.2 Oven heating

One hundred milliliters of each of the oil samples and control were taken in 250 mL beakers and kept in an oven (Binder B 34, 7200 Tuttlingen/Germany) at 180°C for over 24 hours. The exposure of oil to heat was intermittent; 8 hours of heating was followed by 12 hours of storage at 4°C. Portions of each sample were taken out after every four hours of heating and were stored at 4°C until further analysis.

2.5.3 Deep frying

Potatoes were peeled, cut into 10 x10 x 90 mm cubical bars and then fried in 2.5 L of each of the oil samples at 180°C in a deep-fryer. The frying was conducted in 50-g batches at constant frying temperature at 10-minutes intervals for 8 hours per day for three consecutive days. At the end of each 4 hours, a portion of the frying oil was taken from fryer, filtered and then stored in the dark at 4°C until analyzed.

2.6 Analyses of the oil samples

2.6.1 Peroxide value (PV)

Peroxide value (PV) was determined using the official ferric thiocyanate spectrophotometric method of the International Dairy Federation (IDF) with modifications according to Ueda et al. (1986) and Undeland et al. (1998).
2.6.2 p-anisidine value (p-AV)
AOCS Official Method Cd 18-90\textsuperscript{[36]} was used to determine p-AV.

2.6.3 Carbonyl value (CV)
One gram of frying oil was taken into a 10-mL volumetric flask, and the volume was made up to the mark with 2-propyl alcohol (2-PrOH) containing 0.4 mg/mL triphenylphosphine. 50 mg of 2,4-Dinitrophenylhydrazine (DNPH) reagent was prepared by dissolving its 50 mg in 100 mL of 2-PrOH containing 3.5 mL concentrated HCl. Standard solutions of aldehydes and ketones were in 2-PrOH at concentrations of 50 to 500 μM. Standard and/or oil solutions (1 mL) were then mixed with 1 mL 2,4-DNP solution in test tubes, heated for 20 minutes at 40°C, cooled in water, and then 8 mL of 2% KOH in 2-PrOH was added. The test tubes were centrifuged at 5000 rpm for 5 minutes at room temperature and the absorbance of the upper layer was measured at 420nm using (Perkin Elmer, Shelton CT06484 USA, Lambda 25). spectrophotometer) (Farhoosh and MoosAVi 2006).\textsuperscript{[37]}

2.6.4 Conjugated dienes (CD)
Method - D2.1.1 in Handbook of Food Analytical Chemistry was used to determine the CD value.\textsuperscript{[38]}

RESULT AND DISCUSSION
Table 1: Total phenolics in the crude extract and fractions of C.sinensis (green tea leAVes)

<table>
<thead>
<tr>
<th>Extract / fractions</th>
<th>Total phenolics (mg GAE/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>14500±330</td>
</tr>
<tr>
<td>Acidic</td>
<td>1538±49.0</td>
</tr>
<tr>
<td>Neutral</td>
<td>11800±158.0</td>
</tr>
</tbody>
</table>

(n=3, p<0.05)

PV of corn oil increased with time (24 hrs. data collected) during oven heating and deep frying. The order of highest PV is 24 hrs. > 20 hrs > 16 hrs. >12 hrs. > 8 hrs. > 4 hrs. (Fig.1,2).

Effect of adding crude tea extract neutral and acidic fractions of green tea extract were analyzed for inhibition of oil oxidation. All fractions show the less PV but with continuous heating and frying, it increases. That indicates we can decrease the rate of oxidation but not completely suppress. The highest PV value is of acidic phenolic fraction added oil and least
PV of Crude green tea extract added oil is observed. The PV of neutral phenolic fraction added oil is intermediate. The order of highest PV is acidic fraction of green tea extract > neutral fraction of green tea extract > Crude green tea extract (Fig. 1, 2).

Deep fried corn oil has high PV w.r.t. oven heated oil. In blank oil the rate of increase of PV of oven heated and deep fried oil is negligible up to 12 hours (Fig. 9). PV of crude tea Extract added oil of deep fried oil is significantly increased w.r.t. oven heated oil after 12 hours of frying (fig. 10). Similarly difference between PV of of neutral and acidic fraction of green tea extract of oven heated and deep fried oil is negligible up to 12 hours treatment (fig. 11, 12).

CD, $p$ - AV and CV of corn oil is directly proportional to time of oven heating and deep frying. The order of highest CD, $p$ - AV and CV is 24 hrs. > 20 hrs > 16 hrs. > 12 hrs. > 8 hrs. > 4 hrs. (Fig.3to8).

Crude tea extract phenols, neutral and acidic fractions of green tea extract has decreased the CD, $p$ - AV and CV of corn oil. The order of decrease in values of CD, $p$ - AV and CV of corn oil is crude tea extract > Neutral fraction of green tea extract > Acidic fraction of green tea extract. That is Crude tea extract added oil has the lowest CD, $p$ - AV and CV (Fig. 3 to 8). CD, $p$ - AV and CV of deep fried corn oil is higher than oven heated corn oil. The order of highest CD, $p$ - AV and CV of corn oil is deep fried oil > oven heated oil (Fig. 13 - 24).

The numeric value of $p$ - AV of deep fried corn oil is closure to oven heated corn oil up to 8 hours. The continuous increase in numeric value of $p$ - AV of deep fried corn oil is significantly observed after and above 8 hours in all samples Control, crude tea extract, neutral and acidic fractions added oil (Fig. 13 to 16).

CV of deep fried corn oil is approximately three times higher than oven heated corn oil at 4 hours (Fig. 17). CV of deep fried corn oil hAVing crude tea extract is jumped significantly after 4 hours (approximately twice) w. r.t. oven heated corn oil (Fig. 18).

CD value of deep fried corn oil has significant difference to oven heated corn oil and the oil added with crude tea extract, neutral fraction of green tea leAVes and acidic fraction of green tea leAVes (Fig. 21 - 24).
Rise in PV and $p$- AV is due to initiation of autooxidation, which starts with the abstraction of hydrogen adjacent to a double bond in a fatty acid (RH) is catalyzed by light, heat or metal ion to form a free radical (R*).

Potatoes are fried in corn oil. The moisture content of potatoes is 76.7%. As food is placed in oil at frying temperature, air and water initiates a series of interrelated reactions. Water hydrolyzes triacylglycerol producing monoacetylglcerol, diacylglycerol and eventually free fatty acids and glycerol. Glycerol volatilizes above 150°C and the equilibrium is shifted in favor of hydrolysis. This is the reason deep frying has highest oxidation rate than oven heated oil.

In the oxidation of oils, monitoring the amount of hydroperoxides as a function of time indicates whether a lipid in the Growth or decay portion of the hydroperoxide concentration curve. Lipids containing methylene interrupted dienes and polyenes show a shift in double bond position during oxidation that is due to isomerization and conjugate formation.

Catechin

The order of highest antioxidative activity is crude tea extract > neutral fraction > acidic fraction. Gallic acid has 3 -OH groups; is the one reason of the lesser antioxidative ability while catechin has more -OH groups 3 - 4. Crude extract has the both antioxidants (neutral and acidic) that's why highest antioxidative activity.

Deep frying has the highest rate of oxidation than oil heated in oven. This is due to moisture content released from the food in oil during frying that accelerates the process of oxidation.

**Fig. 1.** Effect of adding crude extract, neutral and acidic fractions of green tea leaves on PV of corn oil after oven heating at 180°C.
Fig. 2. Effect of adding crude extract, neutral and acidic fractions of green tea LeAVes on PV of corn oil after deep frying.

Fig. 3. Effect of adding crude extract, neutral and acidic fractions of green tea leAVes on p-AV of corn oil after oven heating at 180°C.

Fig. 4. Effect of adding crude extract, neutral and acidic Fractions of green tea leAVes on p-AV of corn oil after deep frying.
Fig. 5. Effect of adding crude extract, neutral and acidic fractions of green tea leaves on CV of corn oil after oven heating at 180°C.

Fig. 6. Effect of adding crude extract, neutral and acidic fractions of green tea leaves on CV of corn oil after deep frying.

Fig. 7. Effect of adding crude extract, neutral and acidic fractions of green tea leaves on CD of corn oil after oven heating at 180°C.
Fig. 8. Effect of adding crude extract, neutral and acidic fractions of green tea leaves on CD of corn oil after deep frying.

Fig. 9: Comparison of PV of corn oil oven heated vs deep fried without any additive.

Fig. 10: Comparison of PV of corn oil oven heated vs deep fried with crude extract.
Fig. 11: Comparison of PV of corn oil oven heated vs deep fried with neutral fraction.

Fig. 12: Comparison of PV of corn oil oven heated vs deep fried with acidic fraction.

Fig. 13: Comparison of p-AV of corn oil oven heated vs deep fried without any additive.
Fig. 14: Comparison of p-AV of corn oil oven heated vs deep fried with neutral fraction.

Fig. 15: Comparison of p-AV of corn oil oven heated vs deep fried with acidic fraction.

Fig. 16: Comparison of p-AV of corn oil oven heated vs deep fried with neutral fraction.
Fig. 17: Comparison of CV of corn oil oven heated vs deep fried without any additive.

Fig. 18: Comparison of CV of corn oil oven heated vs deep fried with crude extract.

Fig. 19: Comparison of CV of corn oil oven heated vs deep fried with acidic fraction.
Fig. 20: Comparison of CV of corn oil oven heated vs deep fried with neutral fraction.

Fig. 21: Comparison of CD of corn oil oven heated vs deep fried without any additive.

Fig. 22: Comparison of CD of corn oil oven heated Vs deep fried with crude extract.
4. CONCLUSION OF THE STUDY

Deterioration of oil is directly proportional to heating time. As mentioned by primary oxidation product (indicated by PV and CD) and secondary oxidation product (indicated by p-AV and CV). In AVerage microwAVe heating has less deteriorative effect on oil than deep frying.

Addition of antioxidants can decrease the oxidation of oil in oven heated oil as well as deep fried oil. Crude tea extract has maximum antioxidative activity than neutral and acidic fraction of green tea extract.
5. REFERENCES


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