QUALITY EVALUATION OF EDIBLE OILS/FATS IN INDIAN PROCESSED FOOD AND ROLE OF NATURAL ANTIOXIDANTS

VIKAS KARDAM

CENTER FOR RURAL DEVELOPMENT AND TECHNOLOGY

INDIAN INSTITUTE OF TECHNOLOGY DELHI

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by

VIKAS KARDAM

Center for Rural Development and Technology

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DEDICATED

TO

MY PARENTS

CERTIFICATE

This is to certify that the thesis entitled "**Quality evaluation of edible oils/fats in Indian processed food and role of natural antioxidants"** being submitted by **Mr. Vikas Kardam** to the **Indian Institute of Technology Delhi** for the award of **'Doctor of Philosophy'** is a record of bonafide research work carried out by him under our guidance and supervision in conformity with the rules and regulations of Indian Institute of Technology Delhi. The research report and results presented in this thesis have not been submitted, in part or in full, to any other university or institute for the award of any degree or diploma.

(Dr. Santosh Satya)

Professor

Center for Rural Development and Technology

Indian Institute of Technology Delhi

(Dr. S.N. Naik)

Professor

Center for Rural Development and Technology

Indian Institute of Technology Delhi

(Dr. K.K. Pant)

Professor

Dept. of Chemical Engineering

Indian Institute of Technology Delhi

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VIkas Kardam

ABSTRACT

Deep fat frying (DFF) is a very common, complex and dynamic process in food processing sector. The major chemical reactions during DFF include hydrolysis, oxidation, polymerization and isomerization in frying oil and produce volatile and nonvolatile compounds. The volatile compounds evaporate into the atmosphere, whereas the nonvolatile compounds participate in in-situ chemical reactions, thereby generating chemical compounds responsible for deteriorating quality of oil and fried food items. Present study focused on the current status of the Indian frying food industry, especially considering the unorganized food sector. Through a field study in Delhi, the important parameters responsible for deteriorating quality changes in oils/fats during DFF were identified. These include frying cycles, operational temperature, intermittent cooling along with changes in *trans* fat, free fatty acids, polar compounds, peroxides and polyunsaturated fatty acids. Also, an innovative method to improve the performance of frying oil using natural antioxidants was developed and tested.

To begin with, in order to understand the existing frying food scenario, a field study in various food outlets related to frying practices, quality of frying medium and quantity of fat in fried food items was conducted and samples collected were analyzed for various parameters. Data reveal that the amount of *trans* fat in oils used ranged from 5.1-23.0 g/100g of oil, which is 5 to 11 times higher as compared to maximum limit set for TFAs in Denmark and approx. 2 to 5 times higher if compared to recently established maximum limit set for TFA in India. The analysis of the TFA levels in the food products obtained from *halwais*, street food, etc. in South Delhi as well as in hostels and canteens located in IIT Delhi campus were found in the range 1.61-2.79, 2.27-2.72, 0.69-1.20-and 1.38-1.72 g/100g of food serving respectively. TFA have adverse effects on health and are strongly associated with many clinical conditions, so such knowledge regarding TFA content in food would enable people to make proper food choices.

During the DFF process, various oils show their performance in different ways which mainly depend on their fatty acid composition and frying protocols. Also, in the Indian subcontinent, reusing the same oil repeatedly for successive frying cycles is a common traditional frying practice due to economical aspect especially in the unorganized food sector. Therefore, the behavior pattern of individual oil during discontinuous DFF of *vanaspati* (VO), palmolein oil (PO) and soybean oil (SBO) was studied. The highly positive and linear relationship between frying time and frying oil quality based on the generation of TFA, peroxides, FFA, TPC and loss of unsaturated fatty acids were noted in each oil. Surprisingly, SBO fresh oil in spite of having the best initial composition as compared to other selected oils, its stability was found inferior to VO and PO under the same frying conditions. The maximum increase in TFA was found in SBO (1.8 fold) followed by PO (1.4 fold) and VO (1.2 fold). The content of TPC after 6 days frying cycles increased from 8% to 34.5%, 7.5 to 33.5% and 8.5 to 39% in PO, VO and SBO respectively.

The process of DFF involves the oil-food interaction due to which nutritional quality of food gets deteriorated. Moreover, the extent of deteriorating changes occurring in frying medium can be determined by the composition of fried food. To understand both these concepts, two different types of commonly consumed food products [protein rich *mung dal pakoda* (MDP) and carbohydrate rich potato chips (PC)] were fried at every 4 hours frying cycle up to 24 hours in *vanaspati* oil. The quality changes in frying medium as well as nutritional changes in fried food were determined. The results revealed that successive frying cycles of MDP causes early and major deteriorating changes in the oil than frying cycles of PC. The TFA content increases from

its initial value (9.02%) to 11.5% and 10.9% in the frying medium used for MDP and PC respectively. Total polar compounds were increased from 7.5 % to 39.5%, 37.5% and 35.5% in the frying medium used for MDP and PC respectively. The protein content of fried MDP decreased significantly ($P<0.05$) in the successive frying cycles. The first batch of MDP constituted about 20.93% protein, which is decreased to 20.21, 20.06,19.51, 19.25 and 19.01% in MDPs batches of 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} frying cycles respectively. The MDP prepared during first frying cycle absorbs 15.1g of fat, whereas MDP prepared at 6^{th} frying cycle absorb 22.5g of fat. The increase in oil absorption of the food products also increases the energy intake from 135 kcal to 202 kcal per 100g serving of MDP. Similar increase in energy intake was noted for PC. No significant loss was observed in mineral content in fried products in both the cases.

Efficacy of natural antioxidants as substitute of harmful synthetic antioxidant (BHT) to improve the frying performance of selected oils was also studied. Ten different plant leafy biomass procured from IIT Delhi campus as natural antioxidants were investigated. In addition to determining phenolic and flavonoid content, DPPH assay, reducing assay, rancimat assay (antioxidant activity index at 110^0C), FTIR, thermo-gravimetric analysis was performed to know their antioxidant potential and thermal stability. Thermo-gravimetric analysis confirmed the higher thermal stability of natural leafy extracts than BHT. Finally, on the basis of the best characteristics of guava leafy biomass as antioxidant, it was evaluated under frying conditions in sunflower oil and palmolein oil. In sunflower oil (without extract), the TFA significantly ($p<0.05$) increased from its initial value 2.49 to 3.37% within 6 hours and then finally to 4.1% after 12 hours of the frying process. In comparison, sunflower oil with added leafy extract (1000 ppm) inhibits the generation of TFA. Similarly, addition of guava leaf extract successfully increased and improved the frying performance of palmolein oil at frying temperature for 24 hours. Therefore, application of such natural antioxidants obtained from the plant kingdom in oil and fat industry may prove a novel way of improving the thermo-oxidation stability of commonly used oils in the unorganized food sector.

In order to bridge the gap between the food and health, the milk fat, especially *ghee* (butter oil) as per *Ayurveda* health care system is reported to possess both nutritional and therapeutic value. Preparation of *ghee* particularly in rural areas are closely associated with the local knowledge and devices. The change in the chemical profile of *ghee* owing to the different milk sources and methods of preparation was attempted. To investigate this important aspect, cow and buffalo *ghee* was prepared using traditional knowledge system (TKS) with two different churning processes involved, i.e. manual churning with local wooden device and a modern electric churner. Significant differences were found in the overall fatty acid profile of cow *ghee* owing to the method of preparation (including churning process). However, the method of preparation did not affect the value of FFA, PV, color etc. Overall, cow *ghee* prepared using the manual churning method was found to have the best nutritional quality as compared to other *ghee* samples prepared.

The research findings of the present study demonstrated the complexity of the lipid thermo-oxidation stability phenomenon affected by frying process and oil composition. The frying operations above 180^0C not only shortened the frying shelf life of the oil but also impact the nutritional quality of fried items. Furthermore, natural plant based antioxidants can effectively increase the usage time and better performance of the oil during frying. Overall, the research findings would help in evolving guidelines regarding the selection of oil vis-à-vis raw food to be fried, food quality vs frying cycles, frying performance, oil quality parameters, optimization of frying process and application of natural antioxidants etc.

सार

फ्राइंग खाद्य प्रसंस्करण क्षेत्र में एक बहुत ही आम, जटिल और गतिशील प्रक्रिया है। फ्राइंग के दौरान प्रमुख रासायनिक प्रतिक्रियाओं में हाइडोलाइज़िस, ऑक्सीकरण, पोलीमराइजेशन और फ्राइंग ऑयल में आइसोमेराइजेशन शामिल होता है और वाष्पशील और नॉनवोलाटाइल यौगिकों का उत्पादन होता है। वाष्पशील यौगिक वायुमंडल में लुप्त हो जाते हैं, जबकि गैर-व्हॉलैटिल यौगिक इन-सिटू रासायनिक प्रतिक्रियाओं में भाग लेते हैं, जिससे तेल और तले हुए खाद्य पदार्थों की बिगड़ती गुणवत्ता के लिए जिम्मेदार रासायनिक यौगिकों को पैदा किया जाता है। वर्तमान अध्ययन में भारतीय फ्राइंग खाद्य उद्योग की वर्तमान स्थिति पर ध्यान केंद्रित किया गया है, विशेषकर असंगठित खाद्य क्षेत्र को देखते हुए। दिल्ली में एक क्षेत्रीय अध्ययन के माध्यम से, फ्राइंग के दौरान तेल / वसा में गुणवत्ता में बिगड़ती गुणवत्ता के लिए जिम्मेदार महत्वपूर्ण मापदंडों की पहचान की गई। इनमें फ्राइंग चक्र, परिचालन तापमान, आंतरायिक शीतलन शामिल है जिसमें ट्रांस वसा, फ्री फैटी एसिड, ध्रुवीय यौगिकों, पेरोक्साइड और पॉलीअनसैचुरेटेड फैटी एसिड में परिवर्तन शामिल हैं। इसके अलावा, प्राकृतिक एंटीऑक्सीडेंट का उपयोग करके फ्राइंग ऑयल के प्रदर्शन में सुधार के लिए एक अभिनव विधि विकसित और जांच की गई थी।

मौजूदा फ्राइंग खाद्य परिदृश्य को समझने के लिए, फ्राइंग प्रथाओं से संबंधित विभिन्न खाद्य आउटलेटों में एक क्षेत्रीय अध्ययन, फ्राइंग माध्यम की गुणवत्ता और तले हुए खाद्य पदार्थों में वसा की मात्रा का आयोजन किया गया और विभिन्न मापदंडों के लिए इकट्ठे किए गए नमूने का विश्लेषण किया गया। डेटा से पता चलता है कि इस्तेमाल किए जाने वाले तेलों में ट्रांस वसा की मात्रा 5.1-23.0 ग्रा / 100 ग्राम तेल से है, जो डेनमार्क में टीएफए के लिए निर्धारित अधिकतम सीमा के मुकाबले 5 से 11 गुना अधिक है। भारत में टीएफए के लिए निर्धारित हाल ही में स्थापित अधिकतम सीमा की तुलना में अगर 2 से 5 गुना ज्यादा होता है । दक्षिण दिल्ली में स्ट्रीट फूड के साथ-साथ आईआईटी दिल्ली परिसर में स्थित छात्रावासों और कैंटीन से प्राप्त खाद्य उत्पादों में टीएएफए 1.61-2.79, 2.27-2.72, 0.69-1.20 और 1.38-1.72 ग्राम / 100 ग्राम भोजन पया गया । टीएफए का स्वास्थ्य पर प्रतिकूल असर हुआ है और कई क्लीनिकल परिस्थितियों से दृढ़ता से जुड़ा हुआ है, इसलिए भोजन में टीएफए सामग्री के बारे में इस तरह के ज्ञान से लोगों को उचित भोजन विकल्प बनाने में मदद मिलेगी।

फ्राइंग प्रक्रिया के दौरान, विभिन्न तेल अलग-अलग तरीकों से अपना प्रदर्शन दिखाते हैं, जो मुख्य रूप से अपने फैटी एसिड संरचना और फ्राइंग प्रोटोकॉल पर निर्भर करते हैं। इसके अलावा, भारतीय उपमहाद्वीप में, लगातार फ्राइंग चक्रों के लिए बार-बार वही तेल का पुन: उपयोग करते हुए, आम तौर पर असंगठित खाद्य क्षेत्र में आर्थिक पहलू के कारण एक आम पारंपरिक फ्राइंग प्रथा है। एस.बी.ओ के ताजे तेल में अन्य चयनित तेलों की तुलना में सबसे अच्छा प्रारंभिक संरचना होने के बावजूद, फ्राइंग परिस्थितियों में वी.ओ और पी.ओ से नीचा पाया गया। टीएफए में अधिकतम वृद्धि एस.बी.ओ (1.8 गुना) में हुई, इसके बाद पी.ओ (1.4 गुना) और वी.ओ (1.2 गुना) में मिला। टी.पी.सी की सामग्री 6 दिनों के फ्राइंग चक्र के बाद 8% से बढ़कर 34.5%, 7.5 से 33.5% और पी.ओ, वी.ओ और एस.बी.ओ में 8.5 से 3 9% की वृद्धि हुई।

प्राकृतिक एंटीऑक्सिडेंट की क्षमता के रूप में चयनित तेलों के फ्राइंग प्रदर्शन में सुधार करने के लिए हानिकारक सिंथेटिक एंटीऑक्सीडेंट (बीएचटी) के विकल्प का भी अध्ययन किया गया। आईआईटी दिल्ली परिसर से दस अलग-अलग वनस्पति पत्तेदार बायोमास की प्राकृतिक एंटीऑक्सिडेंट की जांच की गई। फीनोलॉलिक और फ्लेवनॉयड सामग्री, डीपीपीएच, थर्मा-ग्रेमीमैट्रिक विश्लेषण को उनकी एंटीऑक्सीडेंट क्षमता और थर्मल स्थिरता को जानने के लिए किया गया था। थर्मो-ग्रेमीमैट्रिक विश्लेषण ने बीएचटी की तुलना में प्राकृतिक पत्तेदार अर्क के उच्च तापीय स्थिरता की पुष्टि की। अंत में, पत्तेदार बायोमास की एंटीऑक्सिडेंट की सर्वोत्तम विशेषताओं के आधार पर, यह सूरजमुखी तेल और

पामोलिन तेल में तलने की स्थिति के तहत मूल्यांकन किया गया था। तेल और वसा उद्योग में पौधों के साम्राज्य से प्राप्त ऐसे प्राकृतिक एंटीऑक्सिडेंट के आवेदन असंगठित खाद्य क्षेत्र में आमतौर पर इस्तेमाल किए जाने वाले तेलों की थर्मो-ऑक्सीकरण स्थिरता में सुधार का एक नया तरीका साबित हो सकता है।

भोजन और स्वास्थ्य के बीच के अंतर को कम करने के लिए, दूध वसा, विशेष रूप से आयुर्वेद स्वास्थ्य देखभाल प्रणाली के अनुसार घी (मक्खन तेल) को पोषण और चिकित्सीय मूल्य दोनों के पास बताया जाता है। ग्रामीण क्षेत्रों में घी की तैयारी विशेष रूप से स्थानीय ज्ञान और उपकरणों से जुड़ी हुई है। विभिन्न दूध स्रोतों और तैयारी के तरीकों के कारण घी के रासायनिक प्रोफाइल में बदलाव का प्रयास किया गया। इस महत्वपूर्ण पहलू की जांच के लिए, दो अलग मंथन प्रक्रियाओं के साथ पारंपरिक ज्ञान प्रणाली का उपयोग करके गाय और भैंस घी तैयार किया गया था, अर्थात स्थानीय लकड़ी के उपकरण और एक आधुनिक इलेक्ट्रिक के साथ मंथन। तैयारी की विधि (मंथन प्रक्रिया सहित) के कारण गाय घी के समग्र फैटी एसिड प्रोफ़ाइल में उल्लेखनीय अंतर पाए गए थे। हालांकि, तैयारी की विधि ने एफएफए, पी.वी, रंग इत्यादि के मूल्य को प्रभावित नहीं किया। कुल मिलाकर, मैनुअल मंथन पद्धति का उपयोग करके गाय घी तैयार की गई थी, जो तैयार किए गए अन्य घी नमूनों की तुलना में सर्वोत्तम पोषक तत्वों की गुणवत्ता में पाया गया था। वर्तमान अध्ययन के शोध निष्कर्षों ने दिखाया कि लिपिड थर्मो-ऑक्सीकरण स्थिरता फ्राईंग प्रक्रिया और तेल संरचना से प्रभावित घटना है। 180^0C से ऊपर फ्राइंग ऑपरेशन न केवल तेल के फ्राइंग शैल्फ लाइफ को छोटा किया बल्कि फ्राइड मदों की पोषण गुणवत्ता को भी प्रभावित किया। इसके अलावा, प्राकृतिक संयंत्र आधारित एंटीऑक्सीडेंट फ्राइंग के दौरान तेल के उपयोग के समय और बेहतर प्रदर्शन को प्रभावी ढंग से बढ़ा सकते हैं। कुल मिलाकर, शोध निष्कर्ष कच्चे भोजन के साथ-साथ, फ्राइंग चक्र, फ्राइंग प्रदर्शन, तेल की गुणवत्ता के मापदंडों, फ्राइंग प्रक्रिया का अनुकूलन और प्राकृतिक एंटीऑक्सीडेंट आदि के इस्तेमाल के बारे में तेल के चयन के बारे में दिशानिर्देश तैयार करने में मदद करेंगे।

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LIST OF ABBRIVIATIONS

Abbreviation	Full form
AAI	Antioxidant activity index
ADI	Acceptable daily intake
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemist's Society
ATR-FTIR	Attenuated total reflectance Fourier transform infrared spectrometry
BEC	Buffalo ghee prepared by the electric churning method
BHA	Butylated hydroxyanisol
BHT	Butylated hydroxytoluene
BMC	Buffalo ghee prepared by the manual churning method
CAO	Canola oil
CCO	Coconut oil
CEC	Cow ghee prepared by the electric churning method
CFAM	Cyclic fatty acid monomers
CHD	Coronary heart disease
CMC	Cow ghee prepared by the manual churning method
CN	Chicken nugget
CO	Corn oil
COL	Coconut olein
CRC	Colorectal cancer
CRP	C-reactive protein
CSE	Centre for Science and Environment
CSO	Cotton seed oil
DFF	Deep fat frying
DPPH	2,2-Diphenyl -1-picrylhydrazyl
EDTA	Ethylene diamine tetra acetic acid
EFA	Essential fatty acids
EFSA	European Food Safety Authority

EVOO	Extra virgin olive oil
FAME	Fatty acid methyl esters
FAO	Food and Agriculture Organization of the United Nation
FC	Frying cycle
FDA	Food and Drug Administration
FF	French fries
FFA	Free fatty acids
FG-SESBO	First-grade solvent extracted soybean oil
FID	Flame ionization detector
FOM	Food oil monitor
FSSAI	Food Safety and Standards Authority of India
FTIR	Fourier transform infrared spectroscopy
GMP	Good manufacturing practices
GNO	Groundnut oil.
GRAS	Generally recognized as safe
GSO	Guava enriched sunflower oil
HDL	High density lipoprotein
HOSO	High oleic sunflower oil
IV	Iodine value
LCSFA	Long chain saturated fatty acids
LDL	Low density lipoprotein
MCSFA	Medium chain saturated fatty acids
MDP	*Mung dal pakoda*
MO	*Moringaoleifera* seed oil
MOFPI	Ministry of Food Processing Industries
MRO	Mustard rapeseed oil
MTFA	Monounsaturated *trans* fatty acids
MUFA	Monounsaturated fatty acids
NSSO	National Sample Survey Office
PHVO	Partially hydrogenated vegetable oil

PO	Palmolien oil
PSBO	Pressed soybean oil
PTFA	Polyunsaturated trans fatty acids
PUFA	Polyunsaturated fatty acids
PV	Peroxide value
QE	Quercetin equivalent
RBD	Refined, bleached, and deodorized
RBO	Rice bran oil
RO	Rapeseed oil
RTFA	Ruminant trans fatty acids
SBO	Soybean oil
SCFA	Short chain fatty acids
SCSFA	Short chain saturated fatty acids
SFA	Saturated fatty acids
SO	Sunflower oil
SSO	Sesame oil
TAE	Tannic acid equivalent
TAG	Triacylglycerol
TBHQ	Tert-butylated hydroquinone
TFA	Trans fatty acids
TG-SESBO	Third-grade solvent extracted soybean oil
TPC	Total polar compounds
TSFA	Total saturated fatty acids
TTFA	Total *trans* fatty acid
TUFA	Total unsaturated fatty acid
UFA	Unsaturated fatty acid

USFDA	US Food and Drug Administration
VLDL	Very low density lipoprotein
VOC	*Vanaspati* oil used for frying potato chips
VOP	*Vanaspati* oil used for MDP
W/R	Without replenishment of fresh oil
WFF	Western fast food joints
WHO	World Health Organization