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ORIGINAL ARTICLE

Anti-oxidative effect of turmeric on frying characteristics of soybean oil

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Abstract Curcumin, the active principle of turmeric, is known to act as an anti-oxidant, anti-mutagen and anti-carcinogen. This study aimed to find out the thermal and oxidative stability of soybean oil when potatoes marinated with turmeric were deep fried in the oil. Two sets of experiment were carried out. In one set, 1 L of oil was heated for 24 h (8 h daily for 3 consecutive days) and 200 g of potato chips without any marination were fried each time twice daily. Foods were fried in batches to replicate the commercial practice of the food industries. The temperature maintained during the whole experiment was at 180–190 °C i.e. at the frying temperature. About 50 ml of the oil sample was collected after every 4 h. In the second set, another 1 L of soybean oil was heated for 24 h in the similar manner and potato chips marinated with turmeric was fried twice daily. Oil samples were collected as before and comparative studies were done. The chemical parameters like acid value, peroxide value, content of 4-hydroxy-2-trans-nonenal (HNE) and fatty acid composition for all the oil samples of each set were determined. The comparative studies on peroxide value and content of HNE revealed that the antioxidant property of curcumin in turmeric helped in reducing the oxidation of the oil initially, but with increase in duration of time, the antioxidant potency got gradually reduced. The loss of unsaturated fatty acids were calculated from the fatty acid composition and it was found that loss of unsaturation in soybean oil where turmeric marinated potatoes were fried was 6.37 % while the controlled one showed 7.76 % loss after 24 h of heating. These results indicated higher thermal and oxidative stability of the soybean oil in presence of turmeric. However, the antioxidant effect gradually decreased with increase in duration of heating.

Keywords Turmeric · Curcumin · Soybean oil · Deep frying · Antioxidant

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Abbreviations

PUFA	Polyunsaturated fatty acids
HNE	4-hydroxy-2-trans-nonenal
DNPH	2 4-dinitrophenylhydrazine
FA	Fatty acids
Con	Control soybean oil
Exp	Experimental soybean oil

Introduction

Turmeric (*Curcuma longa*) is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae (Chan et al. 2009). Apart from colouring and flavouring food, turmeric is used for several purposes. The active component of turmeric is the curcumin, which is a polyphenol and may constitute up to 2–8 % of the spice (Wickenberg et al. 2010). Curcumin is known as C.I. 75300, or Natural Yellow 3. It is an oil soluble pigment, practically insoluble in water at acidic and neutral pH. The keto-enol tautomerism of curcumin structure imparts antioxidative property to it. The antioxidant activity of curcumin was reported (Sharma 1976) as early as 1975. It acts as a scavenger of oxygen free radicals (Ruby et al. 1995; Subramanian et al. 1994) and can protect haemoglobin from oxidation (Unnikrishnan and Rao 1995). In vitro, curcumin can significantly inhibit the generation of reactive oxygen species (ROS) like superoxide anions, H_2O_2 and nitrite radical

generation by activated macrophages, which play an important role in inflammation (Joe and Lokesh 1994). Curcumin also lowers the production of ROS in vivo (Joe and Lokesh 1994). Its derivatives, demethoxycurcumin and bis-demethoxycurcumin also have antioxidant effect (Unnikrishnan and Rao 1995; Song 2001). Curcumin exerts powerful inhibitory effect against H_2O_2-induced damage in human keratinocytes and fibroblasts (Phan et al. 2001) and in NG 108–15 cells (Mahakunakorn et al. 2003). Curcumin reduces oxidized proteins in amyloid pathology in Alzheimer transgenic mice (Lim et al. 2001). It also decreases lipid peroxidation in rat liver microsomes, erythrocyte membranes and brain homogenates (Pulla Reddy and Lokesh 1992). Studies have revealed (Chattopadhyay et al. 2004) that curcumin prevents oxidative damage during indomethacin-induced gastric lesion not only by blocking inactivation of gastric peroxidase, but also by direct scavenging of H_2O_2 and ·OH. Several studies have shown that curcumin has antioxidant and anti-inflammatory properties (Hsu and Cheng 2007). Human clinical trials also indicate that curcumin has no toxicity when administered at doses of 1–8 g/day (Cheng et al. 2001) and 10 g/day (Aggarwal et al. 2003).

Diabetic rats, given curcumin, showed a significant reduction in renal dysfunction and oxidative stress (Sharma et al. 2006) indicating that curcumin has a protective role against diabetic nephropathy. It has been reported that turmeric may have an effect on insulin secretion (Wickenberg et al. 2010). Studies have also revealed that curcumin could also serve in cancer therapy as a drug or as an adjuvant to traditional chemotherapy (Duvoix et al. 2005).

Deep frying is the most popular cooking process followed worldwide for both industrial and domestic food preparation. Fried foods have unique organoleptic and sensorial properties. Investigations have indicated diminishing availability of spice active principles in cooked foods, when the food ingredients have been subjected to either boiling or pressure cooking for few minutes (Suresh et al. 2007). Therefore, the antioxidant potency of the curcumin during deep frying of food items, marinated with turmeric, needs to be investigated.

For the present study, soybean oil has been chosen as the frying medium as it is the most important vegetable oil used worldwide as frying oil. Soybean oil, because of its high content PUFA is considered to be superior to many vegetable oils and hydrogenated fats from a nutritional standpoint, but for the same reason it possesses inferior thermal stability (Tyagi and Vasishtha 1996). The percentage of natural antioxidant, tocopherols, present in soybean oil is known to get reduced on heating (Rubalya Valantina and Neelamegam 2012).

Antioxidants like butylated hydroxyanisole (BHA) and tertiary butylhydroquinone (TBHQ), alone or in combination, are commonly added to fats and oils to retard oxidative deterioration due to storage and heat. But studies have found lack of agreement on the effectiveness of these antioxidants in retarding deterioration of oils during frying (Tyagi and Vasishtha 1996). Studies have also confirmed that antioxidant butylated hydroxytoluene (BHT) did not prevent deterioration of used oils during heating (Tsaknis et al. 2002), which has also been observed by other workers in different oils (Augustin and Berry 1983; Boskou 1988). Therefore, the effectiveness of the natural ingredient, turmeric, in preventing the thermal and oxidative degradation of the soybean oil during deep frying of potatoes marinated with turmeric, was examined.

Materials and methods

Materials

Refined soybean oil of 'Fortune' brand manufactured by Adani Wilmar Ltd., Haldia, India, was purchased from local market. Turmeric powder was prepared in the laboratory by grinding the dry turmeric bought from market. The mesh size of the powder was −60/+80. Potatoes were purchased from local market. The heating of the oil was carried out in a non-stick frying pan to prevent leaching from utensils by heating on a temperature controlled hot plate [Make: Schott, DURAN, Germany].

All the chemicals used were of analytical grade and procured from E-Merck India Ltd.

Methods

The oil (1 L/910 g) was heated for 24 h (8 h daily for three consecutive days). Potatoes were peeled and washed 1 h before use and were sliced into 0.5 cm thick and 2.5 cm wide discs using a mechanical slicer. 200 g of potato chips were fried twice for 5 min, once at the beginning of the experiment and other at the end. For 1 L of oil, the total amount of potato chips fried was 1,200 g (200 g×6 times). The temperature maintained during the whole experiment, including the frying operation, was within the range of 180º–190 °C. Neither salt nor any other spices were added during frying of the potato chips. Oil samples of about 50 ml were collected after every 4 h and kept in refrigerator (−20 °C) for further analysis after cooling under nitrogen atmosphere. At the end of the day, the oil was cooled and stored in refrigerator and on the next day, the same process was repeated. Fresh oil was never added to the frying vessel.

In another set of experiment, another batch of 200 g of sliced potatoes marinated with 0.5 g of turmeric powder for just before frying was fried each time in another 1 L of soybean oil following the same method as above. The turmeric being yellow in colour, imparted a yellow hue to the fried potatoes. Oil samples of about 50 ml were collected after every 4 h and stored in refrigerator (4 °C) for comparative

study with the oil sample collected after frying potatoes without marinating with turmeric.

Three sets of replicate studies were performed for each set of experiment.

Quantification of curcumin in the turmeric sample The quantification of curcumin was done by isocratic high performance liquid chromatography (HPLC), using ultraviolet detection at a wavelength of 262 nm. An aliquot (20 μL), was injected onto a reversed-phase column. Chromatographic separation was done using Zorbax SC-C 18 (4.6 mm I.D. x 250 mm), type 5 μm C_{18} column. The flow rate was 1.0 mL/min. The content of curcumin was determined by peak area and is based on a standard curve in a methanol matrix, generated by using pure external standard (Tayyem et al. 2006). The analysis was performed in triplicate.

Oil analysis

The following oil quality parameters were determined for the oil samples collected at different times:

Acid Value Acid value of the oil samples was determined following the AOCS Official Method Te 1a-64.

Peroxide value Peroxide value was determined by standard method of AOCS (Official Method Ja 8–87).

Estimation of 4-hydroxy-2-trans-nonenal (HNE) by DNPH derivatization (Tatsuhiro et al. 2002). Standard HNE-DNPH derivative was prepared by the reaction of HNE and DNPH. An HNE–water solution was mixed with an equal volume of DNPH solution (3.5 mg of DNPH dissolved in 10 ml of 1 M HCl) and stored in the dark at room temperature (32 °C) for 2 h. HNE-DNPH was extracted twice from the mixture with CH_2Cl_2 and concentrated under nitrogen. HNE-DNPH was separated from the reaction mixture by thin layer chromatography (TLC) using CH_2Cl_2 as developing solvent. The HNE-DNPH band was scraped off and extracted with methanol. The extract was filtered, dried with anhyd. sodium sulphate followed by stream of nitrogen, and redissolved in methanol. The concentration of HNE-DNPH was measured by spectrophotometer (UV–VIS Shimadzu 1,700) at 370 nm and calculated by using the molar absorptivity $\varepsilon 370\ nm = 28{,}000\ M^{-1}\ cm^{-1}$.

Fatty acid composition by gas–liquid chromatography Methyl esters of the corresponding fatty acids were prepared by BF_3-Methanol (Metcalfe et al. 1966) and subjected to GC instrument (Agitent 6890 Series Gas Chromatograph) equipped with a flame ionization detector (FID). The DB-WAX capillary column (J&W Scientific Columns from Agilent Technologies, USA) employed for the analysis was 30 m (length) × 0.25 mm (i.d), 0.25 mm (film thickness), packed with polyethylene glycol. Two ANALAB GC Generator; HG 300 (Hydrogen generator) and N-A-300 (air and nitrogen generator) was employed for the GC gas supply. The GC inlet temperature and FID detector temperature was maintained at 250 °C. 30 ml/min hydrogen flow, 300 ml/min air flow and 29 ml/min nitrogen flow was maintained in the FID detector. 1 ml/min carrier gas (Nitrogen) flow was programmed as follows in the capillary column. Oven temperature was maintained at 150 °C for 2 min, then 15 °C/min upto 190 °C, then 5 min hold, 4 °C/min upto 230 °C, 10 min hold. The percentage of each component was calculated by the software provided by the Agilent Technologies Chemstation Family, based on the method of dividing the area of each peak by the total area of the peaks. The peaks were identified by the standard fatty acid methyl esters (FAME) purchased from Sigma Chemical Co., St. Louis, MO, USA.

Statistical analysis

All experiments were done in triplicate and all the parameters were doubly checked and average was taken. The results were expressed as the mean value±standard error mean (SEM) of three measurements. One way ANOVA was computed using Origin software.

Results and discussion

The thermal deterioration of the frying oil samples was determined by measuring the acid value, peroxide value, content of HNE and fatty acid composition of the samples.

The curcumin content in the turmeric was determined by the HPLC method and it was found that the average value was 3 % of dry weight of turmeric.

The acid values of control and experimental soybean oil are presented in Fig. 1 which showed linear increment with duration of frying from 0 to 24 h. An increase in acid value has been known to result from hydrolysis of triglycerides, triggered by infusion of moisture from food into the oil (Hwang et al. 2013) However, the increase was almost in the same rate for both of them and at the end of 24 h of heating, the acid value for the control oil was 0.291±0.01 while the experimental oil showed a value of 0.290±0.01, indicating no virtual difference.

In order to assess the oxidative damage of oil samples during potato chips frying, peroxide value was determined and presented in Fig. 2. Detection of peroxides gives the initial evidence of development of rancidity in unsaturated fats and oils. At 4 h, the peroxide value for control soybean oil was 2.9±0.6 meq/kg and that of the experimental oil was 1.3±

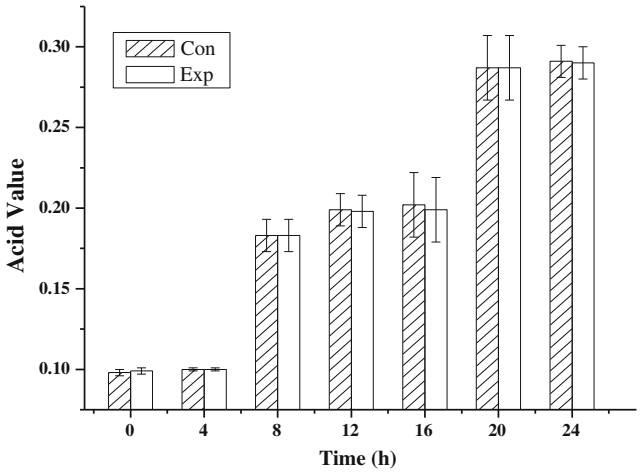

Fig. 1 Acid value of control (Con) and experimental (Exp) soybean oil in course of frying at different time intervals

0.4 meq/kg and at 8 h, the control oil showed a value of 5.6± 0.5 meq/kg while the peroxide value for the experimental one was only 2.4±0.8 meq/kg. Thus there were considerable differences between the peroxide values of the oil samples, having a lower value in case of the sample containing turmeric. This may be attributed to the antioxidant property of curcumin present in turmeric. However, the differences in values between the control and experimental oil gradually decreased with further increase in duration of time, indicating reduction in the effect of curcumin in long duration of frying.

The process of lipid peroxidation is a free radical-mediated deterioration of FA in the presence of air. During lipid peroxidation, hydroperoxides are formed, which then undergo decomposition and yield peroxy (FA) free radicals and hydroxy radicals via β cleavage. Subsequently, this chain cleavage leads to the formation of a wide variety of secondary lipid peroxidation products, including aldehydes, ketones, and other carbonyl-containing compounds. The groups of aldehydes known as 4-hydroxyalkenals, are of particular interest as some of them have been recognized as cytotoxic and mutagenic. The toxic aldehyde 4-hydroxy-2-*trans*-nonenal (HNE) is an oxidation product of linoleic acid and is formed during the thermal oxidation of linoleic rich oil like sunflower oil, soybean oil etc. at frying temperature. It has been seen that toxic HNE is readily incorporated into food fried in thermally oxidized oil and therefore extensive consumption of such fried foods could be a health concern (Seppanen and Csallany 2004). Therefore, HNE can be considered as an important parameter to determine lipid peroxidation. It can be seen from Fig. 3 that the content of HNE in experimental soybean oil is much lower than that of control soybean oil till 16 h of heating. The antioxidant property of curcumin in turmeric along with the natural antioxidant present in soybean oil i.e. the tocopherols might have helped in reducing the oxidation of the oil initially, but with continuous heating for long duration, this property might have become ineffective. The oil sample collected after 24 h of heating showed reverse trend. At 24 h, the control oil had 4.40±0.20 μM/gm of HNE while the experimental soybean oil had 4.48±0.10 μM/gm of HNE which is a bit higher than the control oil. This may be because of increase in oxidative deterioration of the oil after the loss of antioxidant property of curcumin.

The changes in fatty acid composition of both the control and the experimental oil samples were determined by GLC and presented in Table 1 and Table 2 respectively. From the results of both the tables it can be noticed that there was a significant and gradual increase in total saturated fatty acids (SFAs) with

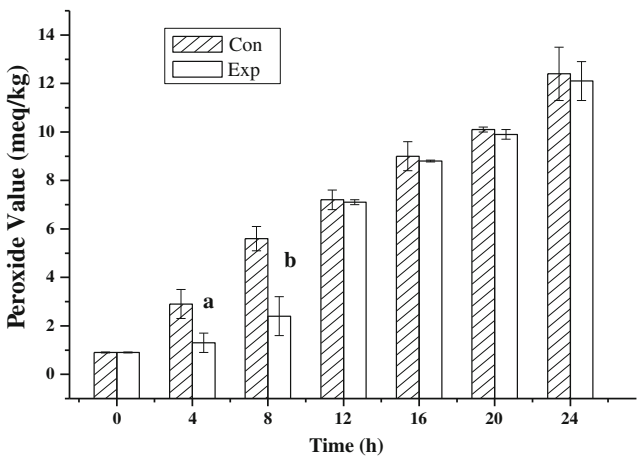

Fig. 2 Peroxide value of control (Con) and experimental (Exp) soybean oil in course of frying at different time intervals. The superscript letters represent statistical significance at $p<0.05$. *a* Comparison between control soybean oil and experimental soybean oil at 4 h. *b* Comparison between control soybean oil and experimental soybean oil at 8 h

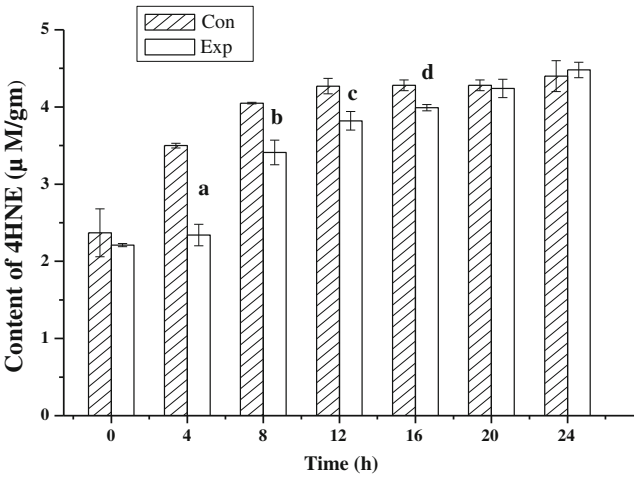

Fig. 3 Content of 4HNE (μM/gm) in control (Con) and experimental (Exp) soybean oil in course of frying at different time intervals. The superscript letters represent statistical significance at $p<0.05$. *a* Comparison between control soybean oil and experimental soybean oil at 4 h. *b* Comparison between control soybean oil and experimental soybean oil at 8 h. *c* Comparison between control soybean oil and experimental soybean oil at 12 h. *d* Comparison between control soybean oil and experimental soybean oil at 16 h

Table 1 Fatty acid composition (% w/w) of the control soybean oil in course of frying at different time intervals

Fatty acids (%w/w)	Time (h)						
	0	4	8	12	16	20	24
$C_{14:0}$	0.34±0.02	0.35±0.01	0.38±0.02	0.40±0.01	0.44±0.03[d]	0.53±0.02[e]	0.58±0.02[f]
$C_{16:0}$	12.49±0.8	13.82±1.0[a]	14.46±1.3[b]	15.64±2.1[c]	16.41±1.8[d]	17.16±1.0[e]	18.38±1.7[f]
$C_{18:0}$	3.74±0.3	3.58±0.8[a]	4.45±0.9[b]	4.48±0.6[c]	4.64±0.9[d]	4.66±0.9[e]	5.37±1.2[f]
$C_{18:1}$	25.21±1.0	26.17±1.1[a]	26.32±1.2[b]	28.37±1.0[c]	28.83±2.1[d]	29.25±1.4[e]	29.84±2.4[f]
$C_{18:2}$	51.92±2.1	50.23±3.1[a]	48.78±2.3[b]	46.62±2.9[c]	45.31±2.1[d]	44.24±2.2[e]	42.48±2.3[f]
$C_{18:3}$	6.30±0.6	5.85±0.4[a]	5.61±0.4[b]	4.49±0.5[c]	4.37±0.2[d]	4.16±0.2[e]	3.35±0.3[f]
TSFAs	16.57±1.1	17.75±1.4[a]	19.29±1.4[b]	20.52±1.0[c]	21.49±1.2[d]	22.35±1.3[e]	24.33±1.6[f]
TUFAs	83.43±3.7	82.25±3.2[a]	80.71±3.1[b]	79.48±2.4[c]	78.51±2.2[d]	77.65±2.8[e]	75.67±1.2

Values are expressed as mean ± SEM ($n=3$) of three different set of experiments

The superscript letters represent statistical significance at $p<0.05$

[a] Comparison between 0 h and 4 h

[b] Comparison between 0 h and 8 h

[c] Comparison between 0 h and 12 h

[d] Comparison between 0 h and 16 h

[e] Comparison between 0 h and 20 h

[f] Comparison between 0 h and 24 h

TSFAs Total saturated fatty acids, *TUFAs* Total unsaturated fatty acids

increase in time of heating. The total SFAs percentage in control soybean oil increased from 16.57±1.1 % to 24.33± 1.6 % while in case of experimental soybean oil, it increased from 16.57±1.1 % to 22.94±1.5 % after 24 h of heating. On the other hand, the sum of the unsaturated fatty acids (UFAs) for the control oil decreased gradually from 83.43±3.7 % for fresh unheated oil to 75.67±1.8 % for the oil sample collected after 24 h of heating, indicating a loss of unsaturation of 7.76 %. Whereas, in case of experimental oil, the sum of UFAs gradually decreased from 83.43±3.7 % for unheated oil to only

Table 2 Fatty acid composition (% w/w) of the experimental soybean oil in course of frying at different time intervals

Fatty acids (%w/w)	Time (h)						
	0	4	8	12	16	20	24
$C_{14:0}$	0.34±0.02	0.38±0.02	0.39±0.01	0.40±0.04	0.46±0.03[d]	0.49±0.01[e]	0.51±0.01[f]
$C_{16:0}$	12.49±0.8	13.14±0.6[a]	13.72±1.0[b]	14.21±0.9	14.43±0.3[d]	14.98±0.2[e]	17.14±1.1[f]
$C_{18:0}$	3.74±0.3	3.86±0.2[a]	3.91±0.1[b]	4.63±0.1[c]	4.70±0.2[d]	4.84±0.6[e]	5.29±0.4[f]
$C_{18:1}$	25.21±1.0	26.55±1.1[a]	28.0±1.3[b]	29.07±1.4[c]	29.48±0.6[d]	30.02±1.4[e]	30.25±0.8[f]
$C_{18:2}$	51.92±2.1	50.17±0.8[a]	48.31±1.1[b]	47.36±0.6[c]	46.72±0.8[d]	45.78±0.8[e]	43.48±1.2[f]
$C_{18:3}$	6.30±0.6	5.90±0.40[a]	5.67±0.3[b]	4.33±0.6[c]	4.21±0.1[d]	3.89±0.5[e]	3.33±0.4[f]
TSFAs	16.57±1.1	17.38±0.8[a]	18.02±1.1[b]	19.24±1.0[c]	19.59±0.5[d]	20.31±0.8[e]	22.94±1.5[f]
TUFAs	83.43±3.7	82.62±2.1[a]	81.98±2.4[b]	80.76±2.2[c]	80.41±1.5[d]	79.69±1.6[e]	77.06±1.4[f]

Values are expressed as mean ± SEM ($n=3$) of three different set of experiments

The superscript letters represent statistical significance at $p<0.05$

[a] Comparison between 0 h and 4 h

[b] Comparison between 0 h and 8 h

[c] Comparison between 0 h and 12 h

[d] Comparison between 0 h and 16 h

[e] Comparison between 0 h and 20 h

[f] Comparison between 0 h and 24 h

TSFAs Total saturated fatty acids, *TUFAs* Total unsaturated fatty acids

77.06±1.4 % for the oil sample collected after 24 h of heating, indicating a loss of 6.37 % unsaturation. The loss of fatty acids thus calculated gave an indication of degree of oxidative polymerization, scission and other side reactions taking place during deep frying (Tyagi et al. 1998; Yoon et al. 1987). The lesser loss of unsaturated fatty acids in presence of turmeric confirms the higher oxidative stability of the oil.

Conclusion

Analysis of quality parameters revealed that the quality of frying oil started deteriorating with time, the rate of deterioration being more in soybean oil than the oil in which food marinated with turmeric is fried. The amount of HNE present in oil could be taken as an important marker to assess the quality characteristics of frying medium. However, the antioxidant potency of curcumin present in turmeric is seen to get reduced with increase in duration of heating of the oil.

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