Annals. Food Science and Technology
2015

THERMAL PROCESSING EFFECTS ON QUALITY AND ANTIOXIDANT POTENTIAL OF FRESH AND PACKAGED MUSTARD (*Brassica nigra*) OIL

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Abstract
Mustard oil is one of the most common cooking medium. It is prepared from seeds of Brassica nigra by grinding or pressing. The present study addressed the change in the quality and antioxidant capacity of fresh and commercially packaged mustard oil after heating of oil at different conditions. These types of treatments are usually followed by the road-side food joints in West Bengal, India. The thermal treatment included heating of the oils for 30 minutes, and after keeping overnight, again boiling for 30 minutes. The assays performed included determinations of acid value, saponification value and iodine value, as well as DPPH radical scavenging abilities. The results indicated that there were increases in the acid value and decreases in saponification value and iodine number, all of which indicated deterioration of the oils. It was also observed that the deterioration of the packaged commercial samples were lesser than the fresh samples as were reflected in lesser deterioration of their acid values. However, there were improvements in the radical scavenging abilities of the oils after thermal treatment, probably due to dissociation of the phenolic esters of the oils to release the antioxidative biomolecules. The study indicated that repeated use of the same mustard oil should not be practiced as the quality falls for their reuse.

Keywords: antioxidant, mustard oil, brassica nigra, acid value, iodine value, saponification value

Submitted: 15.03.2015 Reviewed: 11.05.2015 Accepted: 08.06.2015

1. INTRODUCTION

The spice oilseeds like mustard, fenugreek, poppy, black cumin and coriander are not only effective antioxidants *in vivo* and *vitro* against oxidative stresses, but also active in stabilizing foods against rancidity and oxidative deterioration (Mariod *et al.*, 2009). That is why, almost all human populace utilize seed oils in their cuisines. Oils are usually extracted from the oilseeds by pressing followed by a processing step. Normally, in rural areas, the oils are extracted in local mills by cold-pressing only without the processing step and are utilized in cooking purposes. The difference in the extraction procedures thus could impart large differences regarding the contents of unsaturated fatty acid as well as their antioxidant capacities.

Brassica nigra Linn. (family – Brassicaceae) is a very important oilseed producing plant, commonly known as 'Black Mustard' in English and 'Sarson' in Hindi (Upwar *et al.*, 2011). The plant is cultivated throughout India and central Europe and traditionally used as simple rubefacient, diuretic, emetic, pneumonia, bronchitis, nerve stimulant and vesicant (Vinyas *et al.*, 2012). The seed oil of mustard or mustard oil is consumed in large quantities in India and its production ranks second among all the oil seeds produced in India (Kumar, 2013). Mustard oil is the most common medium of cooking in the eastern part of India, especially West Bengal. Being rich in ω-3 and ω-6 fatty acid and polyunsaturated and monounsaturated fatty acids, it increases the good cholesterol or HDL in the body and helps decrease the bad cholesterol if consumed regularly. In rural West Bengal, the oil is extracted by cold-pressing, which is known as 'Kachi Ghani' mustard oil. Sometimes, for economic reasons, the oil is heated in rural food joints repeatedly using same sample for several days for deep frying. Packaged

commercial mustard oil is also used for the same purpose, although such samples are less utilized due to cost prohibition.

For the past few years, extensive works have been done to determine the antioxidant potentials of mustard oils (Wojdylo *et al.*, 2007; Bajpai *et al.*, 2005; Obi *et al.*, 2009). In such studies, the extraction procedures were mainly percolative and rigorous thermal treatments were avoided, probably in order to retain the integrity of the bioactive principals. However, the oil undergoes frequent thermal treatments during cooking procedures. The present study deals with the *in vitro* antioxidant profile of fresh and commercially packaged mustard oils before and after thermal processing. The design resembled closely with common cooking procedures used in Indian rural food joints. To our knowledge, it was one of the very few studies that dealt with a thermally treated food grade cooking oil for their quality and radical scavenging abilities, and probably the first with mustard oils undergoing thermal treatment procedures that are followed in the rural food joints in India. In this way, we would be able to know whether the quality of the oil could be retained after rigorous thermal treatments during frying. The present study reports the achievement of the aim through some common *in vitro* chemical and radical scavenging assays.

2. MATERIAL AND METHODS

Two samples of packaged mustard oils (designated PMO-1 and PMO-2) were procured from Barasat, West Bengal whereas two samples of 'Kachi Ghani' mustard oil (viz. KG-1 and KG-2) were procured locally from oil mills of Howrah district of West Bengal. Procured oil samples were stored in the dark amber colored, screw capped glass bottles and were kept away from light to avoid physicochemical changes in their compositions. These bottles were closed tightly to check the loss of volatiles and were opened only for a short while, whenever required. All reagents and chemicals used were of analytical grade procured from Merck (India) or SRL (India). 2,2′- Diphenyl- 1- picryl hydrazyl (DPPH) were obtained from Himedia, India.

2.1 Preparation of Samples

500ml each of the four mustard oil samples were taken and poured in four different iron containers which were heated for 30 minutes at 180±5^{o}C on hot plates. During heating the temperature was monitored throughout the process. After the heating, the samples were cooled and kept overnight in tightly closed bottles. Next day, at same time, the samples were again heated for 30 minutes at 180±5^{o}C on hot plates. The samples were allowed to cool and analyzed further.

2.2 Determination of acid value

The acid value (AV) is determined by direct titration according to a published procedure (Das, 2010). The acid value was calculated with the equation:

$$AV = (56.1 \times V \times N)/m$$

Where,
m = Weight (gm) of the oil taken,
N = Normality of the KOH solution
V = Volume of KOH (ml) consumed by m gm oil; Equivalent weight of KOH = 56.1

2.3 Determination of saponification value

The saponification value (SV) is determined by refluxing of the oil with excess alcoholic KOH solution according to a published method (Das, 2010). The saponification value was calculated with the equation:

$$SV = [56.1 \times (V_1 - V_2) \times N]/m$$

Where,

m = Weight (gm) of the oil taken
N = Normality of the hydrochloric acid solution
V_1 = Volume of standard hydrochloric acid (ml) required for the blank
V_2 = Volume of standard hydrochloric acid (ml) required for the sample
Equivalent weight of KOH = 56.1

2.4 Determination of Iodine value

The Iodine value (IV) is determined by treatment of the sample with Wijs solution. According to a published method (Das, 2010). The iodine value was calculated with the equation:

$$IV = [12.69 \times (V_1 - V_2) \times S]/m$$

Where,

m = Weight (gm) of the oil taken,
S = Normality of the thiosulfate solution
V_1 = Volume of standard 0.1N sodium thiosulfate (ml) required for the blank
V_2 = Volume of standard 0.1N sodium thiosulfate (ml) required for the sample

2.5 DPPH radical decolorization assay

The DPPH assay was performed using a previously described procedure (Chakraborty and Bhattacharyya, 2014) with minor modifications. Oils were diluted 1:5 (v/v) with methanol to prepare the working solution. Decolorization of DPPH solution by decrease in absorbance after addition of sample or standard was monitored at 517 nm in a Systronics spectrophotometer (model – 2202). Ascorbic acid was used as positive control and comparing it with samples, results were expressed as ascorbic acid equivalents (µg ascorbic acid/gm oil sample).

2.6 Statistical Analyses

All the experiments were performed in quadruplicate and data are presented as mean ± standard deviation. Data were analyzed by one-way ANOVA followed by Tukey's *post-hoc* test for multiple comparisons of the means (software 'Prism 4.0', GraphPad Inc., USA).

3. RESULTS AND DISCUTION

Use of oil for frying foods is an old and popular method of food preparation (Pedrechi *et al.*, 2005). During such treatment, vegetable oils undergo changes in terms of chemical and physical properties when they interact with the food as well as atmosphere. Types of reaction that are known to lead to degradation of vegetable oils include oxidation, polymerization and hydrolysis (Henna Lu and Tan, 2009).

All these reactions have telling effects on the quality of the oil. The changes in the oil quality can be adjudicated by changes in the parameters like acid value (AV), saponification value (SV) and iodine value (IV).

The acid values of the four oil samples before and after thermal treatments were given in Table 1. From the table, it was observed that acid values of all the samples increased significantly ($p < 0.05$) after thermal treatments. The most prominent increments were observed in case of samples obtained from Kachi Ghani ($p < 0.01$).

Table 1: Acid values of different types of mustard oils, after processing with different thermal conditions. Results are expressed as mg KOH/gm sample.

Sample code	Processing methods			
	CT	HT	ON	ON-HT
PMO - 1	1.44±0.08	2.72±0.12**	3.17±0.13**	4.60±0.10**
PMO - 2	2.06±0.12	2.73±0.17*	3.30±0.12**	6.32±0.39**
KG - 1	2.02±0.07	3.20±0.04**	4.12±0.09**	10.50±0.57**
KG - 2	3.03±0.35	3.75±0.24*	5.05±0.13**	11.35±0.08**

Results are mean ± SD (n=4), CT: control, without thermal treatment, HT: heating for 30 minutes and cooled, ON: kept overnight after heating for 30 minutes, ON-HT: ON sample heated again for 30 minutes.
* $p<0.05$ and ** $p<0.01$ in comparison with CT

Table 2: Saponification values of different types of mustard oils, after processing with different thermal conditions. Results are expressed as mg KOH/gm sample

Sample code	Processing methods			
	CT	HT	ON	ON-HT
PMO - 1	177.6±1.75	172.36±2.04	165.00±2.16	155.81±1.78*
PMO - 2	174.89±2.18	169.99±2.10	163.21±1.79	150.68±2.68*
KG - 1	168.18±2.03	159.00±2.22	158.00±1.14	147.11±1.79*
KG - 2	166.82±2.15	150.83±2.26	137.54±1.73	110.48±2.46*

Results are mean ± SD (n=4), CT: control, without thermal treatment, HT: heating for 30 minutes and cooled, ON: kept overnight after heating for 30 minutes, ON-HT: ON sample heated again for 30 minutes.
* $p<0.05$ and ** $p<0.01$ in comparison with CT

The AV is a measure of the free fatty acids content of the oil (Nita *et al.*, 2010). Increase in AV means oxidation of the oil sample (Tawde *et al.*, 2013). Results from the present study revealed that heating increased AV of oils obtained from both commercial and native sources, indicating the fact that the oils are prone to oxidation. However, the increase in AV was maximum for KG samples (almost 4-times in KG - 2 sample) after rigorous thermal treatment, indicating that the commercial samples could resist such oxidation, probably due to presence of fortified antioxidants within them. The saponification values of the four oil samples before and after thermal treatments were given in Table 2. From the table, it was observed that saponification values of all the samples decreased significantly ($p < 0.05$) after re-heating of the samples kept for overnight after heating. There were no significant decreases after thermal treatment for shorter periods.

SV is a measure of presence of high or low molecular weight fatty acids as esters. The lower SV indicates that the oil is replete with large molecular weight fatty acids. It could efficiently be utilized to measure the oxidation of oil during storage, and higher value of it indicates deterioration of the oils (Nita *et al.*, 2010). Results from the present study indicated that at lesser thermal stress, the oils contained lower molecular weight fatty acids over higher molecular weight ones. However, during rigorous thermal stress, the low molecular weight fatty acids probably were oxidized more, which left the higher molecular weight acids. Since high molecular weight fatty acids are not good for health, the thermal treatment would deteriorate the quality of the mustard oils, which was rightfully reflected in their SV.

The Iodine values of the four oil samples before and after thermal treatments were given in Table 3. From the table, it was observed that Iodine values of all the samples decreased significantly ($p < 0.05$) after re-heating of the samples kept for overnight after heating. There were no significant decreases after thermal treatment for shorter periods.

Similarly, IV is an indicator of the degree of unsaturation; the greater the degree of unsaturation (or high IV), the more rapid the oil tends to be oxidized, particularly during deep-fat frying (Choudhary and Grover, 2013). The unsaturated character affects the stability of oils, and, as a result, leads to the appearance of degradation effects during storage (Nita *et al.*, 2010). The present study revealed that IV decreased significantly on rigorous heating, probably due to oxidation of the useful unsaturated fatty acids.

The DPPH radical scavenging activities of the four oil samples before and after thermal treatments were given in Table 4. From the table, it was observed that antioxidant activities of all the samples increased significantly only after heating conditions ($p < 0.01$ after 30 minutes heating). There were no significant changes upon cooling and keeping overnight.

Table 3: Iodine values of different types of mustard oils, after processing with different thermal conditions. Results are expressed as gm I_2 absorbed/100 gm sample

Sample	Processing methods			
code	CT	HT	ON	ON-HT
PMO - 1	102.48±2.42	99.10±2.05	92.32±1.27*	84.67±2.26**
PMO - 2	98.80±1.28	96.55±2.98	90.35±1.77	82.15±2.19*
KG - 1	91.19±1.98	89.84±2.21	76.80±1.28	73.31±1.76*
KG - 2	90.11±1.96	84.70±1.20	81.53±1.72	77.40±1.80*

Results are mean ± SD (n=4), CT: control, without thermal treatment, HT: heating for 30 minutes and cooled, ON: kept overnight after heating for 30 minutes, ON-HT: ON sample heated again for 30 minutes.
* $p<0.05$ and ** $p<0.01$ in comparison with CT

Table 4: DPPH radical scavenging activities of different types of mustard oils, after processing with different thermal conditions. Results are expressed as ascorbic acid equivalents (□g ascorbic acid/gm sample).

Sample	Processing methods			
description	CT	HT	ON	ON-HT
PMO - 1	61.00±2.58	99.55±2.53**	69.75±1.71	78.11±2.17*
PMO - 2	33.25±2.75	64.63±2.06**	34.00±2.58	50.00±3.74*
KG - 1	39.55±0.44	60.00±1.78**	44.00±2.58	57.00±6.63*
KG - 2	44.90±0.84	72.75±2.22**	47.48±1.08	64.86±4.18*

Results are mean ± SD (n=4), CT: control, without thermal treatment, HT: heating for 30 minutes and cooled, ON: kept overnight after heating for 30 minutes, ON-HT: ON sample heated again for 30 minutes.
* $p<0.05$ and ** $p<0.01$ in comparison with CT

Oil seeds contain free radical scavengers like polyphenols, flavonoids and phenolic compounds (Rasheed *et al.*, 2013). These phenolic compounds are the main factor rendering nutritional importance to cooking oils during extraction techniques like cold-pressing (Henna Lu and Tan, 2009), and also a determinant for the antioxidant capacity of the oils. DPPH radical scavenging assay was chosen for a specific purpose as the subject assay measures antioxidant potential for non-polar antioxidants efficiently as the assay was performed in alcoholic medium (Chakraborty and Bhattacharyya, 2014). It was observed that antioxidant activity increased after 30 minutes heating and returned nearly to the base value. It was increased again when the samples were heated again after keeping overnight. This might be due to dissociation of the antioxidants form otherwise conjugated antioxidant molecules during thermal treatments.

4. CONCLUSIONS

The major conclusion arising out of this research was that the nutritional quality of mustard oils could be deteriorated by thermal processing methods that resemble cooking. Decrement in the oil quality parameters like acid value, saponification value and iodine value were observed both in case of fresh cold-pressed oils (e.g. Kachi Ghani) as well as their packaged counterparts. However, there were indications that the packaged oils could withstand the rigorous thermal treatments as were reflected in their lesser deterioration of

acid values. Results of antioxidant potential assay of the oils indicated that radical scavenging abilities were improved upon thermal processing, which indicated that the oils could have withstand the oxidative onslaught during thermal treatment in their own ways, probably by dissociating the conjugated antioxidant biomolecules. It was deciphered that there were downfalls in the oil quality upon heat treatment, and the oils should not be used repeatedly for deep-fat frying.

5. ACKNOWLEDGEMENT

The authors are grateful to Sarada Ma Girls' College authority for providing financial and infrastructural assistance.

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