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Nutritional changes in soybean and safflower oil due to storage fungi

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Abstract
Present research work embodies the deteriorative changes in soybean and safflower oil due to storage fungi. Ten test fungi which were previously isolated from abnormal oilseeds inoculated separately into the double refined soybean and safflower oil. Great variation in the colour and odour of the biodeteriorated oils of soybean and safflower oil were observed. Colour variation from yellow to bright yellow, while that of odour variation from normal to rancid. *Curvularia pellescens* increased the saponification number of safflower oil. *Macrophomina phaseolina* showed decrease in saponification number of soybean oil. *Alternaria dianthicola* hampered the free fatty acid content while, *Penicillium chrysogenum* and *Fusarium equiseti* showed increased in free fatty acid content of safflower oil. Free fatty acid content in soybean oil was drastically decreased due to *Alternaria dianthicola* and *Rhizopus stolonifer*. *Curvularia lunata* decreased the iodine value of safflower and soybean. Peroxide value of safflower and soybean was found to be increased due to *Fusarium oxysporum* and *Fusarium equiseti*. Based on this study, it is proposed that efforts should be made to minimize the hazard of deterioration due to storage fungi during storage of such oils.

Keywords: Biochemical changes, oil, seed-borne fungi

INTRODUCTION

Soybean oil is low in saturated fat and high in monounsaturated fat and polyunsaturated fat. It is widely used oil and is commonly called 'vegetable oil'. Soybean oil also contains the essential fatty acids linoleic and linolenic. Linoleic and linolenic acids are required for human health. Soybean oil is also rich in omega-3 fatty acids. Omega-3 fatty acids are believed to reduce the risk for heart diseases and may prevent osteoporosis. Soybean oil also contains phytosterols which could lower LDL cholesterol. Soybean oil does not contain cholesterol. Soybean oil contains natural antioxidants which remain in the oil even after extraction. These antioxidants help to prevent the oxidative rancidity.

Like all plant oils, safflower oil contains no cholesterol. In addition, safflower oil contains plant sterols, which make it harder for the small intestine to absorb cholesterol. The Clevel and Clinic states consuming plant sterols may help lower the bad, or low-density lipoprotein, cholesterol by 6 to 15 percent. It also contains Omega-6 and vitamin E as antioxidant which reduces respiratory problems, helps blood circulation, and strengthens the immune system. The high concentration of vitamin E also helps the body eliminate free radicals in the body, lowering the risk of heart disease and cancer. It is great for cooking as the nutritional value is not lost even when heated to a high temperature-such as deep frying.

Edible oils from plant provide characteristic flavors and textures to foods as integral diet components. Chemical compositions determine the keeping quality of oil, for instance, the percentages of the degree of unsaturation [1]. Improper storage condition renders the seed oils for deterioration with the principal decomposition reaction being oxidation. Oils in general are known to be susceptible to microbial attack. The oils composition determines the extent and type of organisms likely to thrive in them [1]. Chemical damage to vegetable oils caused due to microorganisms that lead to deterioration in quality of oils derived from the seeds or fruits pulps of plants. Acid values are used to measure the extent to which glyceride in the oil has been decomposed by lipase [2]. Liberation of free fatty acids (FFA) from triglycerides is due to deterioration of a fat. The amount of FFA in a fat or oil is indicative of its level of spoilage [3]. Iodine value is a measure of the degree of unsaturation in oil. This value could used to determine the amount of double bonds present in the oil, which revealed the susceptibility of oil to oxidation. Oxidation of seed oil occurs through a free radical mechanism, initially characterised by the emergence of a sweetish and unpleasant odour which becomes progressively worse until it attains a characteristic smell of rancid fat [4]. Lipid oxidation is considered a principal mean of deterioration in the quality of foodstuffs. It not only imparts rancid and undesirable flavors to fat products, but also it generates reactive oxygen species, which are linked to carcinogensis, inflammation, aging and cardiovascular disorders [5, 6 and 7). Lipid oxidation also influences the chemical, sensory, and nutritional properties of edible oils and fatty foods and thus plays an important role in determining their use and shelf-life (3 and 6). Such seed oil are not fit for human consumption and rejected for pharmaceutical at industrial level. As little information is available about the deteriorative changes in oil due to fungi, an attempt was made to study the physical and chemical changes in deteriorated oil due to storage fungi.

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MATERIALS AND METHODS

The double refined and deodorized soybean and safflower oil samples were collected from market of Aurangabad in presterilized bottles. These samples were brought in the laboratory. Spores of ten

fungi which were previously isolated from abnormal oilseeds, were inoculated in conical flask containing 100ml oil each of soybean and safflower in asceptic condition. After one month, oil was filtered and these oils were used for the estimation of physical and chemical parameters.

Biodeaterioration of oil
Physical parameters
Estimation of moisture from oil

Moisture content was estimated [8]. About 1 gm of oil was taken into a moisture dish made of aluminum sheet provided with tight filling slip-over cover. Dish was dried previously, cooled in the desiccator (containing an efficient desiccant such as phosphorous pentaoxide) and weighed. The dish was placed in the air oven for approximately two hours at 105^{0}C. The dish was removed from the oven, cooled in the desiccator at room temperature and weighed. This procedure was repeated but the dish kept in the oven only for half an hour each time until the difference between the two successive weighing does not exceed one milligram.
Moisture content was calculated by following formula

$$\text{Moisture \%} = \frac{100\ (M1-M2)}{M1-M}$$

Where, M_1= mass in gm of the dish with the material before drying
M_2= mass in gm of the dish with the material after drying
M= mass in gm of the empty dish.

Estimation of colour of oil

Colour of biodeteriorated oil was determined by observing the grade of the yellow colour as yellow, bright yellow, dark yellow, amber yellow and pale yellow.

Odor of biodeteriorated oil

Odor of biodeteriorated oil was determined by smelling the sample.

Absorbance of biodeteriorated oil

By taking the O.D. at 420nm, absorbance of biodeteriorated oil was recorded.

Estimation of specific gravity

Specific gravity was estimated [8]. The weight of dry specific gravity bottle was taken (B). The dry specific gravity bottle filled with the 5ml of sample. After fixing the stopper, weight was taken (A). Weight of the specific gravity bottle containing 5ml of distilled water was taken (C). Specific gravity was calculated by the formula:

$$\text{Specific gravity at } 30^oC = \frac{A-B}{C}$$

Where, A=Weight in gm of specific gravity bottle with oil at 30^{0}c.
B=Weight in gm of specific gravity bottle at 30^{0}c.
C=Weight in gm of specific gravity bottle with distilled water at 30^{0}C.

Chemical parameters
Estimation of peroxide value

Peroxide value of biodeteriorated oil was calculated [9]. 1 gm of sample of oil was taken in test tube. 20 ml acetic acid- chloroform solution (2:3 volume) and 1g powdered potassium iodide was added. Tube was placed in boiling water bath until liquid boil vigorously. Contents were quickly transferred to the flask containing 20ml of 5% KI solution. Tube was washed quickly with 25ml Distilled water each time and collected in conical flask. Yellow colour was appeared. This was then titrated with 0.1 N sodium thiosulphate solution with constant and vigorous shaking. The titration was continued till the yellow colour almost disappeared. 0.5 ml of starch solution was added and continued titration till the blue colour just disappeared. A blank determination of reagent was conducted. Peroxide value was calculated by formula

$$\text{Peroxide value} = \frac{(S-B) \times N \times 100}{\text{Wt. of the sample}}$$

Where,
B= Titration of blank test ml.
S= Titration of sample ml.
N= Normality of sodium thiosulphate solution.

Estimation of iodine value

Iodine value was determined according to the titrometric method [10]. 2g of oil sample was weighed into a dry glass stopper bottle of 250ml capacity and 10ml of carbon tetrachloride was added to the oil. About 20ml of Wij's solution (Mix 1.5 % of Iodine monochloride and 98% of Gacial acetic acid) was then added and allowed to stand in the dark for 30 min. 15ml of (10%) potassium iodide and 100ml of water was added and then titrated with 0.1M sodium thiosulphate solution using starch as indicator just before the end point. A blank was also prepared alongside the oil samples. Iodine value was calculated from the formula:

$$\text{Iodine value (Wij's)} = \frac{(V_2-V_1) \times 1.269}{\text{Weight of sample (g)}}$$

Where: V_2 = titer value for blank, V_1 = titer value for sample (s)

Estimation of free fatty acid content

Free fatty acid content was estimated by the method recommended [9]. 2ml of oil was dissolved in 50ml of neutral solvent in 250ml conical flask. Few drops of phenolphthelein indicator were added and titrated against 0.1N potassium hodroxide. Constant shaking was done until pink colour was persisted for fifteen seconds. Acid value was calculated by formula

$$\text{Acid value} = \frac{\text{Titre value X Normality of KOH X 56.1}}{\text{Weight of sample (g)}}$$

Saponification value

The Saponification value was determined according to the titre metric method [11]. 2g of oil sample was weighed into a conical flask and 25ml of alcoholic potassium hydroxide was added. Solution was heated in boiling water for 1h. 1ml of 1% phenolpthalein was added and titrated with 0.5N HCl. A blank was prepared alongside the oil samples. The value was calculated by the formula:

$$\text{Soponification no} = \frac{56.1 \times (B-S) \times N}{W}$$

Where,

B=Volume in ml of 0.5 N Hydrochloric acid required for blank test
S=Volume in ml of 0.5 Hydrochloric acid required for the sample
N=Normality of Hydrochloric Acid
W=Weight of oil in gm.

RESULTS

Absorbance (O.D. at 470nm) of deteriorated oil due to storage fungi

Absorbance is the parameter which determines the transparency of the oil. Absorbance of safflower oil was decreased due to *Alternaria dianthicola*, *Curvularia lunata* and *Penicillium chrysogenum.* In case of soybean oil absorbance was low due to *Penicillium chrysogenum* while it was maximum due to *Macrophomina phaseolina.* (Table 1 and 3).

Change in colour of deteriorated oil due to storage fungi

From table 1 and 3, *Alternaria dianthicola* was found to be responsible for the bright yellow colour of safflower oil. Yellow colour of safflower oil was turned to bright yellow colour due to *Curvularia lunata* and *Penicillium digitatum.* On the other hand *Macrophomina phaseolina* and *Penicillium digitatum* turned the normal pale yellow colour of soybean to bright yellow colour (Fig. 1).

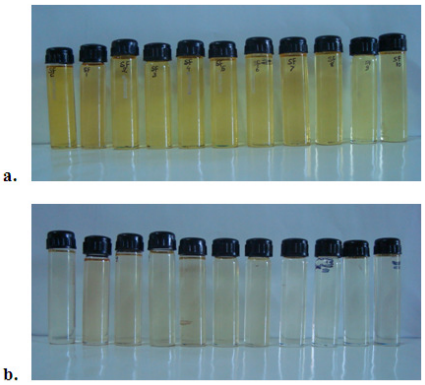

Fig 1. Biodeteriorated oil of a. safflower and b. soybean

Change in odour of deteriorated oil due to storage fungi

Change in odour of biodeteriorated was studied and results are given in table 1 and 3. *Alternaria dianthicola, Curvularia lunata, Rhizopus stolonifer* and *Penicillium chrysogenum* gave specific odour. *Alternaria dianthicola* caused rancidity to safflower oil. *Rhizopus stolonifer* and *Penicillium digitatum* gave specific odour to soybean oil while, *Fusarium oxysporum and Fusarium equiseti* gave specific odour to safflower. On the other hand, *Fusarium oxysporum, Macrophomina phaseolina* and *Trichoderma viride* did not give any specific odour to soybean oil.

Change in moisture content of deteriorated oil due to storage fungi

Moisture content of safflower and soybean was found to be drastically increased due to *Alternaria dianthicola* and *Fusarium oxysporum* respectively. All the fungi except *Trichoderma viride* and *Fusarium equiseti* increased the moisture content in safflower oil, while all the fungi other than *Curvularia lunata* and *Trichoderma viride* showed increase in the moisture content in soybean oil. (Table 1 and 3).

Change in specific gravity of deteriorated oil due to storage fungi

The specific gravity of safflower oil was increased due to *Penicillium digitatum* and *Penicillium chrysogenum* while *Fusarium equiseti* lowered the specific gravity of safflower oil. *Alternaria dianthicola* and *Fusarium equiseti* showed decrease in specific gravity while *Penicillium digitatum* and *Penicillium chrysogenum* increased the specific gravity of soybean oil. (Table 1 and 3).

Chemical parameters

Estimation of saponification number (mgKOH/g) of deteriorated oil due to storage fungi

In order to know the role of storage fungi to cause the saturation or unsaturation to oil, saponification number of fungal deteriorated soybean and safflower oils was calculated and results are summarized in table 2 and 4. *Penicillium chrysogenum* was found to be responsible for decrease in the saponification number while, *Curvularia pellescens* increased the saponification number of safflower oil. *Macrophomina phaseolina* showed decrease in saponification number while, *Rhizopus stolonifer* increased the saponification number of soybean oil.

Estimation of free fatty acid (%) of deteriorated oil due to storage fungi

Alternaria dianthicola hampered the free fatty acid content while, *Penicillium chrysogenum* and *Fusarium equiseti* showed increased in free fatty acid content of safflower oil. Free fatty acid content in soybean oil was drastically decreased due to *Alternaria dianthicola* and *Rhizopus stolonifer*. (Table 2 and 4).

Estimation of iodine number (wij's) of deteriorated oil due to storage fungi

Wij's method was adopted for the estimation of iodine number of all the five oil and results are given in table 2 and 4. Maximum increase in iodine value of safflower oil was observed due to *Penicillium digitatum, Macrophomina phaseolina* and *Rhizopus stolonifer.* On the other hand *Curvularia lunata, Curvularia pellescens* and *Fusarium oxysporum* showed decrease in iodine number of safflower oil. *Penicillium digitatum* in soybean showed maximum increase in the iodine value.

Estimation of peroxide value (mEq/kg) of deteriorated oil due to storage fungi

Change in peroxide value of five different types of oils due to storage fungi was studied and results are given in table 2 and 4. Peroxide value of safflower and soybean was found to be increased due to *Fusarium oxysporum* and *Fusarium equiseti*. *Alternaria dianthicola* was found to be responsible for the low peroxide of safflower and soybean. *Curvularia pellescens* hampered the peroxide value of saoybean oil while, *Macrophomina phaseolina*

hampered the peroxide value of safflower oil.

Table 1. Physical changes in biodeteriorated safflower oil

Fungi	Colour	Absorbance (470 nm)	Odour	Moisture content (%)	Specific Gravity
Alternaria dianthicola	Bright yellow	0.090	Rancid	6%	0.298
Curvularia lunata	Bright yellow	0.111	Normal	3%	0.298
Curvularia pellescens	Pale yellow	0.123	Normal	2%	0.323
Fusarium oxysporum	Pale yellow	0.121	Specific	4%	0.341
Fusarium equiseti	Pale yellow	0.130	Normal	1%	0.249
Macrophomina phaseolina	Yellow	0.119	Specific	4%	0.296
Rhizopus stolonifer	Yellow	0.124	Normal	5%	0.302
Penicillium digitatum	Bright yellow	0.135	Normal	3%	0.334
Penicillium chrysogenum	Pale yellow	0.115	Normal	2%	0.336
Trichoderma viride	Yellow	0.124	Normal	1%	0.289
Control	Yellow	0.153	Normal	1%	0.306

Table 2. Chemical changes in biodeteriorated safflower oil

Fungi	Saponification number	Free fatty acid (%)	Iodine no. (wij's)	Peroxide value (mEq/kg)
Alternaria dianthicola	265.07	1.122	126.90	10
Curvularia lunata	244.03	2.244	101.15	16
Curvularia pellescens	277.69	1.680	107.86	13
Fusarium oxysporum	230.01	2.805	107.86	65
Fusarium equiseti	222.99	3.366	114.21	62
Macrophomina phaseolina	266.47	1.680	152.28	10
Rhizopus stolonifer	239.82	2.805	145.90	13
Penicillium digitatum	221.59	3.260	158.62	54
Penicillium chrysogenum	193.51	3.927	139.59	58
Trichoderma viride	200.52	2.224	116.90	14
Control	200.53	1.680	114.21	17
S. E.	8.65	0.26	6.01	7.14
C.D. (p=0.05)	22.2	0.67	15.4	18.3

Table 3. Physical changes in biodeteriorated soybean oil

Fungi	Colour	Absorbance (470 nm)	Odour	Moisture content (%)	Specific Gravity
Alternaria dianthicola	Pale yellow	0.040	Normal	2%	0.268
Curvularia lunata	Pale yellow	0.037	Specific	1%	0.301
Curvularia pellescens	Pale yellow	0.038	Normal	5%	0.308
Fusarium oxysporum	Pale yellow	0.042	Normal	6%	0.298
Fusarium equiseti	Pale yellow	0.036	Normal	3%	0.246
Macrophomina phaseolina	Bright yellow	0.046	Normal	5%	0.298
Rhizopus stolonifer	Pale yellow	0.034	Specific	2%	0.301
Penicillium digitatum	Bright yellow	0.033	Specific	4%	0.335
Penicillium chrysogenum	Pale yellow	0.029	Normal	2%	0.336
Trichoderma viride	Pale yellow	0.036	Normal	1%	0.244
Control	Pale yellow	0.039	Normal	1%	0.296

Table 4. Chemical changes in biodeteriorated soybean oil

Fungi	Saponification number	Free fatty acid (%)	Iodine no. (wij's)	Peroxide value (mEq/kg)
Alternaria dianthicola	235.62	1.610	145.93	50
Curvularia lunata	238.42	2.810	143.21	68
Curvularia pellescens	245.55	2.820	144.55	21
Fusarium oxysporum	231.10	2.790	126.90	73
Fusarium equiseti	223.90	2.880	120.66	70
Macrophomina phaseolina	202.63	3.376	114.21	64
Rhizopus stolonifer	248.24	2.254	107.86	71
Penicillium digitatum	228.60	2.750	164.97	56
Penicillium chrysogenum	234.21	2.800	158.62	60
Trichoderma viride	221.59	2.810	145.90	65
Control	218.79	3.376	145.90	67
S. E.	3.91	0.15	5.48	4.45
C.D. (p=0.05)	10.0	0.38	14.0	11.4

DISCUSSION

The changes observed in the colour of the oil due to fungal invasion under present studies are in agreement with the findings of [12 and 13]. Similarly, [13] suggested that the changes in colour may be due to pigments synthesized by invading fungi. *Aspergillus flavus* and *Curvularia prasadii* were more effective in changing the oil colour, turning it green and orange [7].

Storage fungi like *Alternaria dianthicola* caused the rancidity to the safflower oil, this strongly suggests the production of certain volatile unpleasant compounds. *Rhizoctonia bataticola, Aspergillus flavus, Cladosporium herbarum* and *Botryodiplodia* sp. were responsible for producing unpleasant odour in groundnut oil [12]. Thus, it is evident that metabolites of invading fungi also causes odour in the oil. *Fusarium equiseti* caused decrease in specific gravity in soybean and safflower oil which indicates that texture of these biodeteriorated oil samples was hampered due to *Fusarium equiseti*.

Saponification number of edible oil was found to be deteriorated due to storage fungi. *Curvularia pellescens* in deteriorated safflower oil and *Rhizopus stolonifer* in deteriorated soybean oil showed increase in saponification number which indicate that presence of greater number of ester bonds of intact fat molecules which suggesting that oil may be use in production of liquid soap, shampoos, lather and shaving creams. Increase in saponification value due to fungal infestation has also been reported [12, 14 and 15]. On the other hand, there was decrease in the saponification number of biodeteriorated oil due to storage fungi which indicate that less number of ester bond.

Alternaria dianthicola was responsible to decrease the free fatty acid content of safflower oil. Similarly, soybean oil was decreased due to *Alternaria dianthicola*, *Rhizopus stolonifer* and *Fusarium oxysporum*. The low free fatty acid value shows that this oil is stable. Similar results were reported in groundnut [16]. He found that deterioration of oilseeds due to seed-borne fungi decreased the free fatty acid content of groundnut.

Iodine value is a measure of the degree of unsaturation in oil. This value could be used to determine the amount of double bonds present in the oil, which revealed the susceptibility of oil to oxidation. The iodine value obtained is high which indicate the presence of unsaturated fatty acid and this places the oil in the drying groups. This oil may find application as a raw material in industries for the manufacture of vegetable oil-based ice cream. Decrease in iodine value under infestation of fungi shows that change of unsaturated fatty acid into saturated fatty acid to facilitate the easy consumption of fatty acid by the storage fungi. Similar results were reported earlier by [15].

Increase in free fatty acids positively correlated with the infestation of seeds by *P. chrysogenum* at 10°C, and *A. flavus* and *A. niger* at 25°C [17]. On the contrary we found that, Biodeteriorated soybean and safflower oil showed low peroxide value. The peroxide value is lower than that expected of rancid oil, which shows that the oil is not rancid and considered stable. In addition to that, the low peroxide value indicates slow oxidation of these oils. These results are agreement with the results obtained by [18]. They reported that in case of sunflower oil, *Penicillium, Alternaria*, *Trichoderma, Stemphylium* and *Absidia* species significantly deteriorated the quality of oil with respect to acid value, peroxide value and fatty acid composition.

Lipid oxidation products make the oil unfit for human health, therefore in order to minimize the oxidation phenomenon, some antioxidants should be added to increase the storage and

shelf-life of oils and oil products. Better packing and storage conditions can also lead to an improvement in the oxidative stability of vegetable oils and other related products containing fats and oils. Only buy the quantity that will use within a couple of months as this will keep the oil the freshest.

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