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Total Lipids and Storage Quality of Oil Seeds and Nuts as Influenced by Yeast Fermentation

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Abstract

Oilseeds and the products made from them have a role in a healthy balanced diet, even though they are energy dense and contain a high proportion of fat. Most oilseeds are made up of protein and oil. In general, fat is 18–45% by weight of oilseeds and protein is about 16–40%. Fluctuations in oil yields can cause significant fluctuations in their rates. The oils contain fat-soluble vitamins and unsaturated fats such as omega-3 and omega-6, making them nutritionally useful. It is especially important in vitamin D and E supplementation and vitamin A utilization. Several yeast strains, including the genus Pichia have been used in fermentation process of many food stuffs. In the present study, the powdered samples of oilseed and nuts such as chia seeds, flax seeds, sesame, peanuts, and almonds were fermented using two yeast strains such as Pichia fermentans and Pichia kluyveri. After fermentation the culture broth were centrifuged and the pellets containing the seed samples were dried and subjected for extraction of oil. The results showed higher oil yield with fermented samples when compared to their respective raw samples. There was significant decrease in the peroxide value of 32.5%, 15.8%, and 16.7% in peanut, almond, and flax seeds fermented with P. kluyveri, respectively, while with P. fermentans, flax seeds, and peanut showed 16.7% reduction among all the tested oil samples. However, chia seed oil showed no change between the samples when compared to its raw. Hence, yeast fermentation plays an important role in reducing the oxidative rancidity of different cooking oils and thus improving their shelf life.

Keywords: Oil seeds and nuts, yeast fermentation, rancidity, *Pichia fermentans*, *Pichia kluyveri*

INTRODUCTION

Oilseeds are the major suppliers of vegetable oils even though oil-rich tree fruits such as olives, coconuts, and palm trees yield large amounts of oil rich stores its energy as starch, but seeds store their energy primarily as oil for germination [1].

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Seed oil can be used for different purposes: for human consumption as food, for cooking, as a source of bioactive compounds or nutraceutical (e.g., vitamins and polyphenols), and used in the pharmaceuticals and cosmetic industries for preparation of different products. The high-protein content also makes it useful as animal feed. The major oilseed crops grown worldwide are: soybean, rapeseed, cottonseed, peanut, sunflower seed, palm kernel, copra, etc. Once the oil is extracted from the seeds, a refining process is needed to improve the preservation conditions and nutritional properties. Scientific evidence have shown that there is a relation between excessive consumption of foods with partially or fully hydrogenated fats and increasing the levels of cholesterol and plasma triglycerides [2, 3].

Lipids are a group of heterogeneous compounds that include free fatty acids, mono-, di-, and triacylglycerols, phospholipids, sterol esters, glycolipids, and lipoproteins [4, 5].

In the present work peanut (*Arachis hypogaea*), almond (*Prunus dulcis*), sesame seeds (*Sesamum indicum* L.), chia seeds (*Salvia hispanica*), and flax seeds (*Linum usitatissimum*) powder were fermented using two yeast isolates such as *Pichia fermentans* and *Pichia kluyveri* followed by estimation of total lipid content and their storage quality assessment.

MATERIALS AND METHODS

Healthy oilseeds and nuts (Sesame, flax seeds, chia, almond, and peanut) were procured from a local market in Kushalnagar, Kodagu district, Karnataka washed, shade dried, and powdered. All the chemicals, reagents were of analytical grade procured from SRL, India, Hi-Media, India, and standards were from SIGMA, USA.

The cultures of *P. fermentans* (Pf) and *P. kluyveri* (Pk) were collected from Food Microbiology Laboratory, Mangalore University, Jnana Kaveri Campus, Chikka Aluvara, Kodagu, Karnataka, India. The cultures were sub-cultured in YEPD and maintained at 4ºC for further work.

Yeast Extract Peptone Dextrose (g/l)

Yeast Extract- 10, Peptone- 20, Dextrose- 20, Agar- 15, pH- 7.0

Yeast Fermentation of the Seeds

The Yeast Extract Peptone Dextrose (YEPD) broth was prepared, sterilized, and 100 ml medium was mixed with 10 g each of oilseed and nuts powder followed by inoculation with *P. fermentans* and *P. kluyveri* and incubated at room temperature for 3 days in a rotary shaker [6, 7]. The culture broth was centrifuged after incubation to remove the pellets from the metal surface. The fermented pellets were dried in a hot air oven at 60ºC to extract the lipid and supernatant was discarded [6, 8].

Extraction of Lipid

The dried pellets were taken in thimble and oil was extracted for 6 hours using petroleum ether in a Soxhlet apparatus. The total lipid was calculated by using the formula:

$$\%\text{ lipid yield} = \frac{weight\ of\ lipid}{weight\ of\ dry\ cell\ biomass} \times 100 \qquad [9]$$

Estimation of Peroxide Value (Rancidity)

0.3 g of the oil was mixed with 10 ml of chloroform:acetic acid ratio (2:3) to dissolve the fats and 1 ml of 10% KI was added and allowed to stand for 5 minutes in dark and then added 20 ml of distilled water. This solution was titrated against 0.0l N Sodium thiosulphate until it becomes light yellow and then added 1 ml of 1.5% starch as indicator that turns the mixture into blue color. The titration was continued until this blue color vanishes, which is the end-point. The same procedure is used for the blank titration, except that no fat is added [8]. Peroxide value (POV) was calculated using the formula:

$$\text{POV (meq/g)} = \frac{(Vs - Vb) \times N \times F \times 10}{W} \times 100,$$

where,

V_s= titration value of the sample (ml);
V_b= titration value of the blank (ml);
F-= factor of 0.01N $Na_2S_2O_3$ solution;
W = weight of fat in volume of extract used (g);
N = normality of $Na_2S_2O_3$ solution.

RESULT AND DISCUSSION

The oil seeds and nuts used in the present study are shown in Figure 1(a–e).

Figure 1. (a) Chia seeds (*S. hispanica*), (b) flax seeds (*L. usitatissimum*), (c) sesame seeds (*S. indicum* L.), (d) peanut (*A. hypogaea*), and (e) almond (*P. dulcis*).

The extracts obtained were incubated at 30°C after subculture on YEPD agar plates. After incubation the *Pichia* colonies were observed on petri plates that are shown in Figure 2(a) and (b).

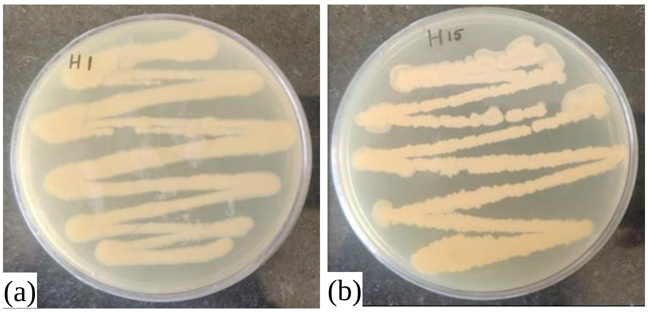

Figure 2. Pure cultures of (a) *P. fermentans* and (b) *P. kluyveri*.

FERMENTATION

The oil seeds and nuts powder were used as substrates for fermentation by *P. fermentans* and *P. kluyveri* (Figures 3 and 4).

The lipids were extracted from dried pellets of raw and fermented oil seeds and nuts used in this study (shown in Figures 5, 6, & 7) The obtained oils from all samples are shown in Figure 8(a)–(e).

In this study, (Figure 9) when compared to raw and fermented oilseeds and nuts samples, the fermented samples showed high percentage of lipid yield. Among the fermented samples, the peanut fermented with *P. kluyveri* shows the highest percentage of lipid yield (73.14%) and sesame fermented with *P. fermentans* shows the lowest percentage of lipid yield (22.84%). The work carried out by Ndomou et al. [10], proved that on heat treatment the lipid yield of peanut was higher (33.23%) than the sesame seeds (27%). The work carried out by Eddy et al. and Hwang et al. [11, 12] confirmed that the pumpkin seeds showed highest lipid content (58.41%) than the cashew nut (42.15%). All the tested seeds and nuts fermented with *P. kluyveri* showed higher yield of lipids when compared to *P. fermentans.*

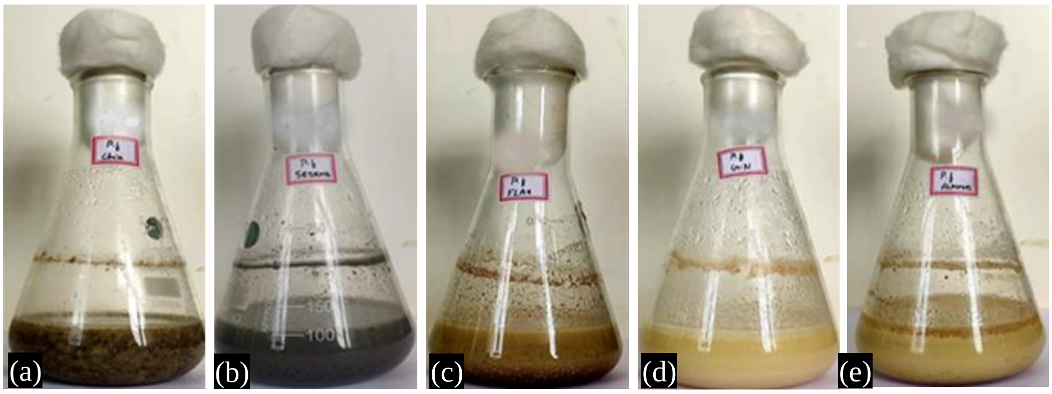

Figure 3. Fermentation of different oilseeds (a, b, c) and nuts (d, e) powder in YEPD broth with *P. fermentans.*

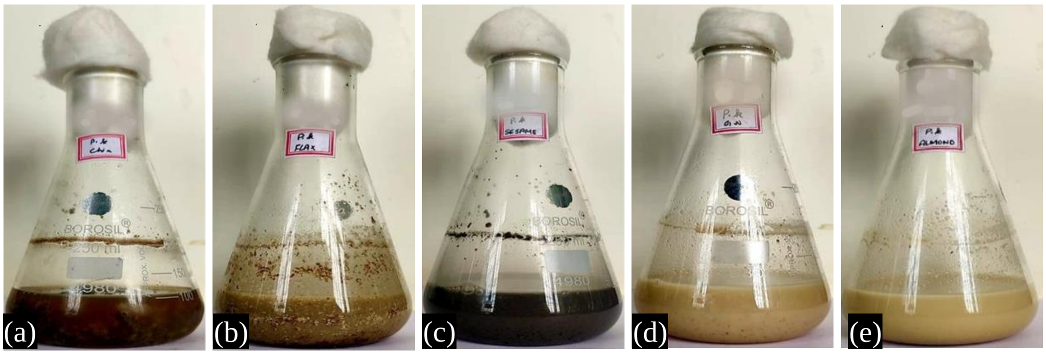

Figure 4. Fermentation of different oilseeds (a, b, c) and nuts (d, e) powder in YEPD broth with *P. kluyveri*.

Figure 5. Raw powder of different oilseeds and nuts.

Figure 6. Dried pellets of *P. fermentans* fermented oilseeds and nuts.

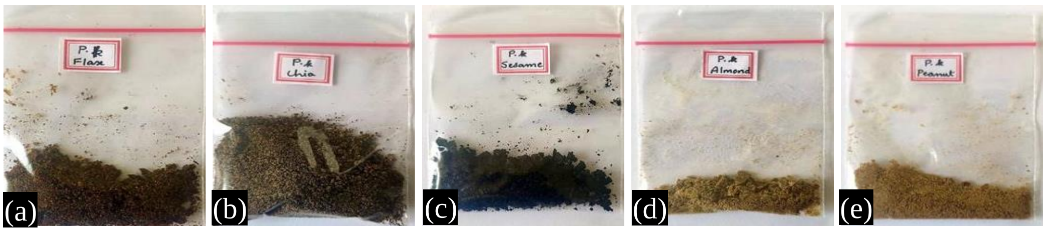

Figure 7. Dried pellets of *P. kluyveri* fermented oilseeds and nuts.

Figure 8. Lipid extraction from oilseeds of (a) raw and (b) fermented with *P. fermentans* and (c) *P. kluyveri*.

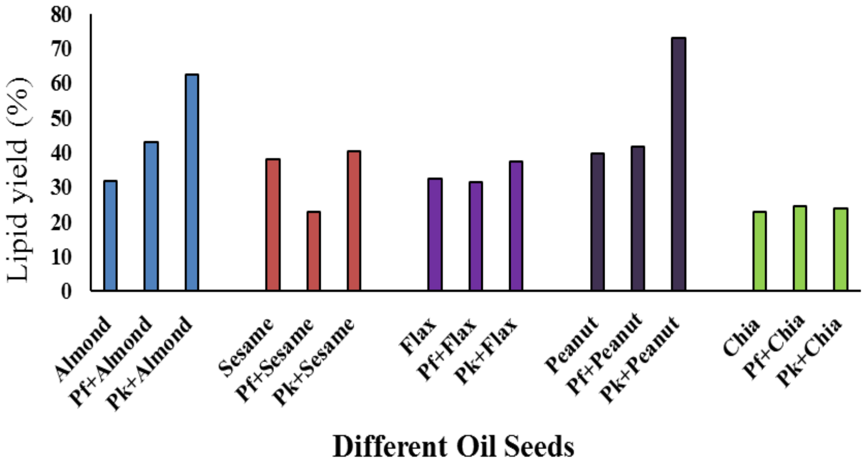

Figure 9. Lipid content from raw and yeast-fermented oilseeds and nuts.

Estimation of POV (Rancidity)

POV was estimated by titrimetric method for raw and *Pichia* sp. (H1 and H15) fermented oils and results are shown in Table 1.

Table 1. POV of both raw and fermented oils on day 1.

Oil Samples	**Raw**	***P. fermentans***	***P. kluyveri***
Sesame	22.86	32.66	49.00
Almond	22.86	35.93	45.73
Flax	52.26	35.93	29.40
Peanut	52.26	49.00	39.20
Chia	35.93	35.93	35.93

The POV of freshly extracted oil should be less than 10 units, however in the present study the POV was more than 10 units. This may be due to the stock of oil seeds and nuts that were procured. Raw sesame fermented with *P. fermentans* and *P. kluyveri* showed 42.86% and >2-fold increase in the POVs, respectively, while almond was 57.1% and 2-fold higher with *P. fermentans* and *P. kluyveri* [13, 14]. In case of peanut, there was a reduction of 6.3% and 25% with *P. fermentans* and *P. kluyveri* fermentation, whereas in flax seeds there was 31.3% and 43.8% reduction with *P. fermentans* and *P. kluyveri* fermentation, respectively.

POVs in chia seeds did not change.

The results showed that the POV of sesame oil increased to 5.8% when fermented with *P. fermentans* and there was 5.9% reduction with *P. kluyveri* after storing for 20 days. Almond seeds also showed similar trend of increased POV of 15.7% with *P. fermentans* and 15.8% reduction with *P. kluyveri*. Flax seeds and peanuts showed reduction in both yeast fermentations. There was 19.33% reduction in both *P. fermentans* and *P. kluyveri* fermented flax seed samples. While in peanut there was 19.33% and 32.5% reduction in *P. fermentans* and *P. kluyveri* fermented samples. All three samples of chia seeds showed similar POVs (Table 2).

Table 2. POV of raw and fermented oils stored for 20 days.

Oil Sample	**Raw**	***P. fermentans***	***P. kluyveri***
Sesame	55.53	58.8	52.26
Almond	62.06	71.86	52.26
Flax	78.4	65.33	65.33
Peanut	78.4	65.33	52.92
Chia	58.8	58.8	58.8

Eddy et al. [11] reported that the POVs of pumpkin seeds were lower (11.75) than the POV of cashew oil (15.23), Kola et al. [15] reported that the POV gives the evidence of rancidity in unsaturated oil, in their study the highest POV was observed in pistachio oil (10 meq of active oxygen kg^{-1}). POVs of walnut and acorn oils were 1.20 and 0.20 meq of active oxygen kg^{-1}, respectively.

CONCLUSION

Oils and fats derived from oilseeds have multiple uses in food, medicine, cosmetics, fertilizers, biodiesel, paints, and animal feeds. Hence, in the present study, we can conclude that when the oilseeds and nuts undergoes fermentation process we get high content of lipid yield and it can be stored for longer period as the degree of rancidity was lower which was evident from the reduced POV in fermented oil samples.

Acknowledgment

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Conflict of Interest

The authors have declared no conflicts of interest for this article.

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