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ORIGINAL ARTICLE

# Finger millet (*Eleusine coracana*) – an economically viable source for antihypercholesterolemic metabolites production by *Monascus purpureus*

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**Abstract** Rice, parboiled rice, finger millet, germinated finger millet, broken wheat, njavara (medicinal rice), sorghum and maize were used as substrates for solid state fermentation of *Monascus purpureus* at 28°C for 7 days using 2% seed medium as inoculum for the production of its metabolites. The fungus exhibited good growth in all the substrates. The fermented substrates were dried at 45°C and analysed for antihypercholesterolemic metabolite statins by standardized HPLC method and dietary sterol contents by spectrophotometric method using reference standards of statin (pravastatin and lovastatin) and cholesterol, respectively. Germinated finger millet yielded higher total statin production of 5.2 g/kg dry wt with pravastatin and lovastatin content of 4.9 and 0.37 g/kg dry wt respectively than other substrates which range from 1.04–4.41 g/kg. In addition to statin, monascus fermented germinated finger millet yielded dietary sterol of 0.053 g/kg dry wt which is 7.6 folds higher than the control. The value addition of finger millet by germination and fermentation with *Monascus purpureus* provides scope for development of functional food.

**Keywords** *Monascus purpureus* · Finger millet · *Eleusine coracana* · Antihypercholesterolemic · Statin · Dietary sterol

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## Introduction

Ascomycetes fungus, *Monascus purpureus*, traditionally known as red yeast rice is reported to produce metabolites like polyketides, antihypercholesterolemic agents, antihypertensive metabolite, lipid lowering fractions and other unsaturated fatty acids like oleic, linoleic and linolenic acids. The culture filtrate of *Monascus purpureus* is also contains antioxidant and antibacterial principles. *Monascus purpureus* is normally cultivated on cooked rice to produce a range of secondary metabolite, statin *viz*. lovastatin, monacolin J, pravastatin and mevastatin (Manzoni et al. 1999) known as Monacolins. These metabolites inhibit the enzymatic conversion of hydroxymethyl-glutarate to mevalonate by HMG- CoA reductase, which is the important step in the biosynthetic pathway of cholesterol (Heber et al. 1999, Manzoni and Rollini 2002). Statin inhibits the action of HMG-CoA reductase by its the structural similarity with the substrate, hydroxymethyl-glutarate. Statin is a common medicine in the therapy of hypercholesterolemia (Chen and Johns 1993). Fermented red rice of *Monascus purpureus* also contains lipid lowering constituents like beta-sitosterol and campesterol in addition to statins. Dietary sterol helps in decreasing low density lipids (LDL) serum level thereby reducing total cholesterol without affecting ligh density lipids (HDL) and triglycerides level (Law 2000, Ostlund 2002). Statin in combination with dietary sterols produced by the fungi is more effective in lowering cholesterol level than statin alone in the therapeutic use of anticholesterolemia (Plat and Mensink 2001).

Finger millet (*Eleusine coracana*), is cultivated in India and Africa for food and fodder. Millets can be cultivated in a wide range of soils and climates and because of their short growing season, they are of specific importance in semi-arid regions. Finger millet is consumed mainly as '*porridge*' and '*mudde'*. Abundant investigations have been reported in the past on antihypercholesterolemic metabolites production using rice as substrate. However, no information is

available on metabolite production by *Monascus purpureus* in relation to finger millet. Finger millet is cheaper than other cereals and millets (Anon 2009), rich in calcium, also called poor man's food, widely consumed in Asia and Africa and thus value addition to such a food will eliminate malnutrition. Hence in this study, the yield of total statin and dietary sterol produced by *Monascus purpureus* on alternate substrates were investigated. The objective of this study was to find out a cheaper substrate for antihypercholesterolemic metabolites production by *Monascus purpureus.*

## Materials and methods

Standards of lovastatin, pravastatin and cholesterol were obtained from Sigma Chemical Co. (St.Louis, MO., USA). Methanol and other solvents used for HPLC were of highest purity from Ranbaxy Fine Chemicals Ltd, Mumbai, India.

*Monascus purpureus* 'MTCC-410' isolate was obtained from Microbial Type Culture Collection (MTCC), Indian Institute of Microbial Technology, Chandigarh, India. *Monascus purpureus* 'MTCC-410' culture was maintained on PDA slopes and sub-cultured every 30 days and stored at 4°C.

*Oryza sativa* (raw rice, parboiled rice and njavara rice), *Eleusine coracana* (finger millet and germinated finger millet) *Triticum aestivum* (wheat), *Sorghum vulgare* (jowar), *Hordeum vulgare* (barley) and *Zea mays* (maize) were procured from the local market.

*Germination of finger millet:* Finger millet seeds were weighed and germinated in large Petri dishes. Wet country made filter papers were placed in the dish. The seeds were uniformly spread and sprinkled with water at a ratio of 2:1 w/v. The lid of the dish was closed and incubated at 28 ± 2°C for 72 h. Germinated seeds were dried in a dryer at 40°C for 24 h to a moisture content of ~12%. Germinated and raw finger millet were coarsely ground and used as substrate for metabolite production.

*Preparation of substrates for SSF:* For substrates other than finger millet, 20 g of dried substrate were taken in 500 ml Erlenmeyer flask and 40 ml of distilled water were added and in case of finger millet (germinated and ungerminated), 20 g of dried finger millet was taken in 500 ml Erlenmeyer flask and 35 ml of distilled water were added. Then the substrates were sterilized at 121°C for 20 min. Sterilized substrates were allowed to cool over night in room temperature (27 ± 2°C)

*Preparation of inoculum:* Seed culture was prepared by inoculating a loopful of spores from PDA slopes into 500 ml Erlenmeyer flask containing 100 ml of sterile basal medium (Glucose-10%, peptone 1%, $KNO_3$ 2%, $NH_4H_2PO_4$ 2%, $MgSO_4.7H_2O$ 0.5% and $CaCl_2$ 0.1% pH adjusted to 6.0). Culture was incubated at 28°C for 48 h at 110 rpm.

*Cultivation of Monascus purpureus by SSF:* Sterilized substrates were mixed well using a sterile glass rod under sterile condition to facilitate separation of cooked grains. Seed medium of *Monascus purpureus* at the rate of 2% was added as inoculum to the substrates and the flasks were shaken well to uniformly distribute the spores within the substrate. Then the flasks were kept in slanting position in an incubator maintained at 28°C for 7 days with intermittent mixing of substrates by hand shaking.

A digital pH meter (APX-175E, CD Instrumentation Pvt. Ltd., Bangalore, India) with a glass electrode was used for measuring the pH of substrates (before and after fermentation). Five g of substrate (both fermented and unfermented) and 25 ml of de-ionized water were homogenized individually using a mortar and pestle, and then filtered through a single layer cheesecloth to measure pH (No and Meyers 2004).

*Extraction and estimation of statin:* Fermented substrates were dried at 45°C for 24 h to a moisture content of ~12% and powdered using pestle and mortar. One g of the powdered material was extracted with 50 ml of methanol: water mixture (1:1, v/v) of pH 7 in 250 ml Erlenmeyer flask and incubated on a rotary shaker for 2 h at 150 rpm. The content was passed through Whatmann No.1 filter paper and the filtrate was flash evaporated using Roto evaporator (Buchi, Switzerland). The flash evaporated residue was washed with methanol and the washing was passed through 0.45 µ syringe filter and the resultant supernatant was collected. The statin content (pravastatin and lovastatin) was analysed by HPLC (isocratic) at 238 nm with C-18 Agilent column and acetonitrile : water 72:28 (v/v) as mobile phase. The flow rate was maintained at 1.0 ml/min (Valera et al. 2005). The peaks of standard pravastatin and lovastatin were obtained at a retention time of 2.063 min. and 7.097 min, respectively and the sample *Monascus* fermented germinated finger millet exhibited corresponding peaks of pravastatin and lovastatin at 1.850 min and 7.233 min (Fig. 1).

*Extraction and estimation of sterol:* One g of dried powdered fermented substrate was taken in 50ml Erlenmeyer's flask with 10 ml of 2.5 N alkali and autoclaved at 15 lb for 1 h (Kieber et al. 1955). After cooling, equal volume of diethyl ether was added to the contents and kept on incubator shaker for 1 h. The mixture was allowed to separate at 4°C. Ether layer containing sterol was separated using a separating funnel. Trace amount of sodium sulphate was added to the content and dried using nitrogen gas to remove any excess moisture. The dry residue was dissolved in 1 ml of chloroform and the content was passed through 0.45 µ membrane filter. The filtrate was collected in a tube and the solvent was evaporated by keeping the tube in an incubator maintained at 40°C. The cholesterol residue in the tube was dissolved in 1 ml of choloroform and the total sterol content was measured spectrophotometrically (Model: UV-1001A, Shimadzu, Japan) at 640 nm using cholesterol as standard (Sabir et al. 2003). The sterol concentration was derived from the standard calibration graph prepared using standard cholesterol with concentrations of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg.

## Results and discussion

The fungus exhibited good growth on all 9 substrates studied. After seven days of fermentation, statin and dietary sterols were extracted from the *Monascus* fermented substrates and quantified.

*Statin production by Monascus purpureus:* After seven days of solid state fermentation (SSF), the statin was extracted and quantified by HPLC. The germinated finger millet fermented with *Monascus purpureus* yielded higher total statin of 5.2 g/kg (pravastatin and lovastatin content of 4.9 and 0.37 g/kg, respectively) when compared to other substrates like broken wheat (4.4 g/kg) and parboiled rice (4.1 g/kg) (Table 1). *Monascus pupureus* 'MTCC 369' when grown on synthetic medium under shake flask culures yielded lovastatin of 351 g/l (Sayyad et al. 2007). Pyo (2007) reported that soybean fermented with *Monascus pilosus* 'KFRI 1140' yielded 2.9 mg mevinolins/g of dry wt. *Monascus pilosus* 'M12-69' when grown under SSF with rice as substrate, inoculum level of 5 ml spore suspension and incubation temperature of 30°C yielded 2.5 mg monocolin K/g of substrate (Chen and Hu 2005). *Monascus pupureus* 'NTU601' was grown on SSF with rice as substrates, the production of monocolin K at 30 °C was 530 mg/kg (Wang et al. 2003). Su et al. (2003) reported that *Monascus pupureus* 'CCRC 31615' exhibited monocolin K yield of 378 mg/kg under SSF with the long grain rice. *Monascus purpureus* 'NTU 301' with *Dioscorea* as the substrate has resulted in 2584 mg monocolin K which is 5.4 times more when rice is used as a substrate (Lee et al. 2006). It is inferred from the above reparts that the yield of statin was very high (5.2 g/kg) when *Monascus purpureus* was cultivated by SSF on germinated finger millet. This may be due to availability of essential nutrients and growth factors, promoted by germination of finger millet, for the growth of *Monascus purpureus*.

*Sterol production by Monascus purpureus:* The *Monascus* fermented barley, maize and raw rice yielded

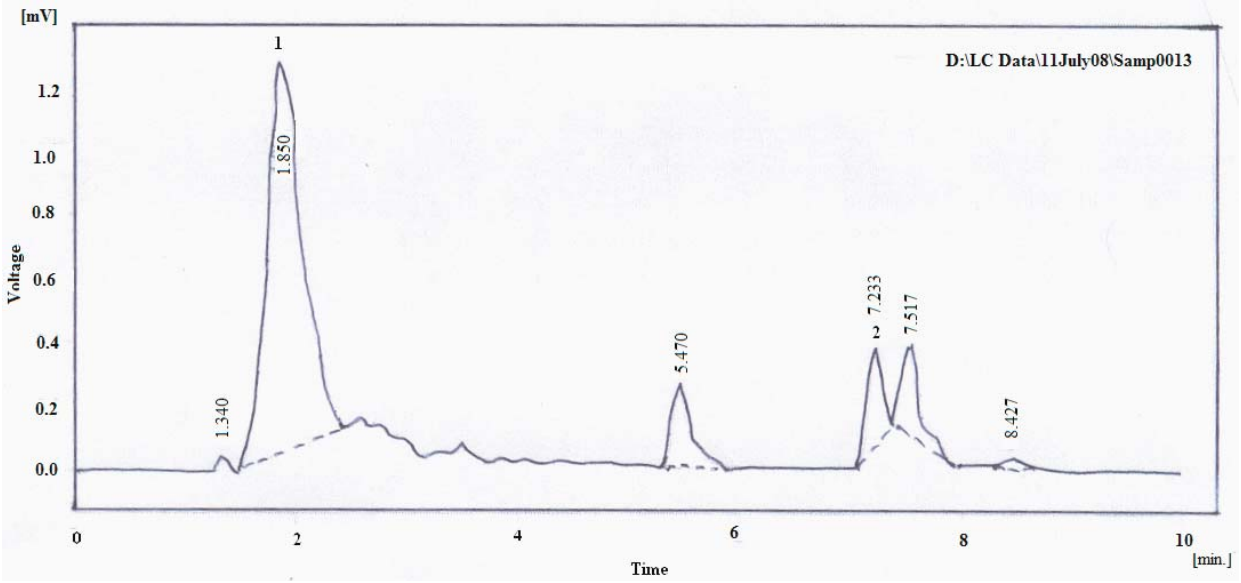


**Fig. 1** HPLC of pravastatin (1) and lovastatin (2) extracted from *Monascus purpureus* fermented germinated finger millet

**Table 1** Yield of statin by *Monascus purpureus* on different substrates and pH of substrates before after fermentation

| *Monascus* fermented substrate | Statin yield g/kg of dry wt. pH values | | | |
|---|---|---|---|---|
| | Lovastatin | Pravastatin | Before fermentation | After fermentation |
| Raw rice | 0.64 ± 0.01 | 2.6 ± 0.01 | 6.8 ± 0.09 | 5.01 ± 0.04 |
| Parboiled rice | 0.52 ± 0.02 | 3.6 ± 0.02 | 6.7 ± 0.11 | 4.8 ± 0.07 |
| Finger millet | 0.08 ± 0.00 | 0.96 ± 0.02 | 6.1 ± 0.07 | 5.1 ± 0.02 |
| Germinated finger millet | 0.37 ± 0.01 | 4.9 ± 0.03 | 5.8 ± 0.08 | 4.4 ± 0.06 |
| Barley | 0.22 ± 0.02 | 1.7 ± 0.02 | 6.0 ± 0.05 | 5.5 ± 0.08 |
| Wheat (broken) | 0.06 ± 0.01 | 4.3 ± 0.02 | 6.7 ± 0.02 | 4.7 ± 0.10 |
| Njavara (medicinal rice) | 0.16 ± 0.01 | 1.8 ± 0.01 | 6.6 ± 0.10 | 5.1 ± 0.03 |
| Sorghum | 0.17 ± 0.01 | 1.8 ± 0.01 | 6.5 ± 0.07 | 5.5 ± 0.06 |
| Maize | 0.47 ± 0.01 | 3.6 ± 0.02 | 6.8 ± 0.03 | 5.0 ± 0.01 |

(n=2)

total sterol of 0.14, 0.09 and 0.08 g/kg, respectively. Germinated finger millet al.so exhibited good yield of 0.053 g/kg sterol as against 0.02 g/kg sterol yield of finger millet (Fig. 2). The substrates germinated finger millet, barley and broken wheat when fermented with *Monascus purpureus* exhibited 7.6, 10.7 and 13.7 folds significantly higher sterol production than the control. The remarkable increase in sterol yield is due to the production of sterol by *Monascus purpureus* during SSF. The results obtained in this study were supported by the report of Heber et al. (1999), wherein it was stated that the red yeast rice produces sterols such as β- sitosterol and campasterol in addition to other bioactive molecules. The structural similarity of the plant sterols to cholesterol enables them to compete with cholesterol for incorporation into micelles and thereby lowering the dietary cholesterol and the cholesterol accumulated in the gastrointestinal tract (Clifton 2002, Lichtenstein 2002). Simons (2002) demonstrated that the effect of using a plant sterol ester in combination with statin is equivalent to doubling the dose of statins for the treatment of antihypercholesterolemia.

*Effect of pH on statin and sterol production:* pH of all the substrates before fermentation was 6.1 to 6.8 except germinated finger millet, which exhibited pH of 5.8. After 7 days of fermentation, all the substrates except germinated finger millet showed pH of 5.3–4.8. *Monascus* fermented germinated finger millet exhibited pH of 4.4, which was much lower than other fermented substrates (Table 1). The production of organic acids during fermentation could be the reason for steep drop in pH. The results are comparable to the observations of Sripriya et al. (1997) on pH during germination and fermentation of finger millet, where the initial pH of finger millet (5.8) was dropped to (5.7) due to germination and further maximum drop was observed from 6 to 12 h of fermentation (5.6–4.6), which was attributed to the production of organic acids like lactic acid, acetic acid and citric acid during fermentation.

In this study, after 7 days of fermentation, the fermented substrates viz. finger millet, barley, sorghum, medicinal rice exhibited pH ranging from 5.1–5.5 with statin production ranging from 1.04–1.95 g/kg dry wt. Fermented raw rice, maize, parboiled rice, broken wheat showed pH ranging from 4.8–5.0 with statin yield ranging from 3.3–4.4 g/kg dry wt. However, fermented germinated finger millet with pH 4.4 exhibited higher yield of statin (5.2 g/kg dry wt.) than other substrates used in SSF. This increase in statin yield could be attributed to the drop in pH. The results are comparable to the observations of Valera et al. (2005) on increased lovastatin production of 16.6 mg/g dry solid obtained in the 2-litre bioreactor with intermittent stirring and 2 vvm airflow rate at pH 5.0.

*Monascus* fermented barley with pH 5.5 exhibited 0.14 g/kg dry wt of total sterol yield i.e. 10.4 folds increase than control. Other substrates viz. rice, broken wheat, sorghum and maize with pH ranging from 4.8–5.4 exhibited sterol yield ranging from 0.02–0.09 g/kg. Germinated finger millet which showed initial pH of 5.8 reached 4.4 on 7$^{th}$ day of fermentation yielded an appreciable amount of sterol of 0.53 g /kg dry wt, 7.6 folds higher than control of 0.07 g /kg dry wt. It is inferred from the above results that low pH has no positive effect on sterol production. The results obtained in this experiment is compared to

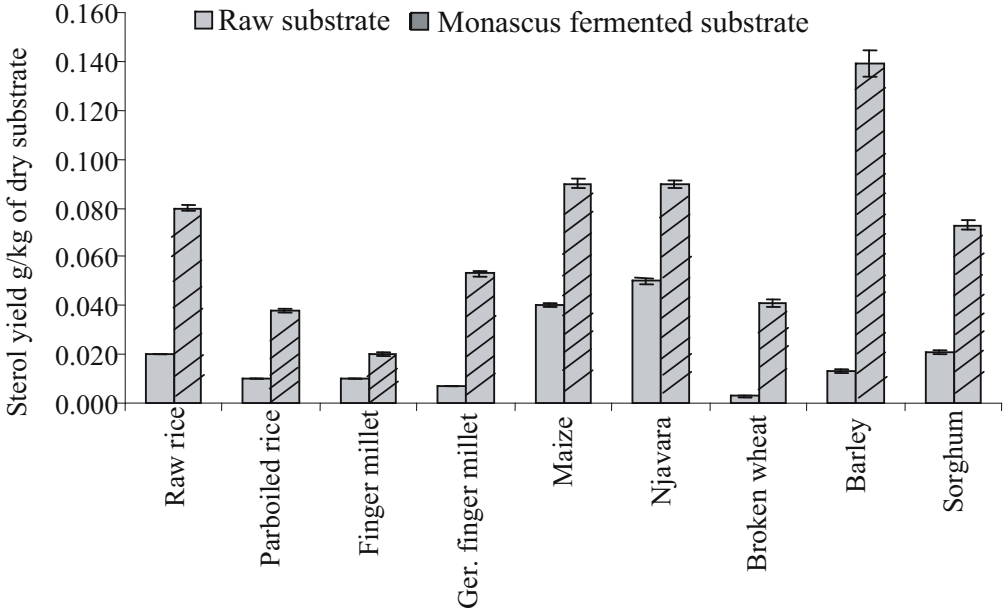


**Fig. 2** Sterol production by *Monascus purpureus* on different substrates (n=3)

report of Ghanem et al. (1990) wherein *Penicillium crustosum* utilized a cheap and economic fermentation medium (g/l) containing $H_2SO_4$-treated beet molasses, 90; corn steep solid, 30 with initial pH 7.0 and incubation period for 8 days that favoured maximum production of sterol (160 mg) 9.9% to dry wt. and ergosterol (98.5 mg) 6.1% to dry wt. Egorova et al. (2009) reported that the formation of testosterone from androstadiendione by sterol-transforming strains of *Mycobacterium* spp reached maximum level of 0.21 mM (6%) at neutral pH, and decreased at the acid or basic pH values, which clearly correlates with the results obtained in the present study.

*Statin production in SSF with germinated finger millet as substrate:* Germination helps in the degradation of antinutrient factors like phytic acid and tannins present in the finger millet grain, hydrolysis of complex carbohydrate to simple sugars and availability of other nutrients and minerals (Mbithi-Mwikya et al. 2000), which are needed by the fungus for its growth and metabolites production. In this study, the results showed that the yield of statin and sterol are considerably high when germinated finger millet was used as a substrate. Hence, germinated finger millet was used to study the production of statins at different intervals of fermentation.

The statin yield by *Monascus purpureus* fermented germinated finger millet on different days of fermentation was estimated (Fig. 3). From day 3 to day 5 the yield of statin showed increasing trend and reached maximum of 5.2 g/kg on the 7$^{th}$ day of fermentation which was significantly higher. However, from 9$^{th}$ day of fermentation, the statin yield showed decreasing trend. Manzoni et al. (1999) reported that *Monascus paxii* 'AM12M' spontaneous mutant yielded 127 mg lovastatin per litre and 53 mg pravastatin per litre at 21 days when whole soybean flour medium was used. The results obtained in the present study exhibited higher production of statin 5.2 g/kg dry wt. (lovastatin 0.37 g /kg dry wt and pravastatin 4.9 g/kg dry wt.) at lower fermentation period of 7 days, which was found to be better when compared to earlier reports.

The finger millet sprouted for 72 h and fermented with *Monascus purpureus* for 10 days exhibited major biochemical changes when compared to raw finger millet. The germination of finger millet was more effective in starch and protein hydrolysis resulting in the formation of free sugars and soluble proteins. Further, germination reduced pH, tannin and phytate content of the finger millet thereby increasing mineral bioavailability. The availability of free sugars, soluble protein and minerals for the growth of *Monascus purpureus* might have attributed to the increased production of antihypercholesterolemic metabolite under SSF.

## Conclusion

This study was aimed at evolving a cheaper substrate for maximum production of antihypercholesterolemic metabolites by *Monascus purpureus* in SSF. Germinated finger millet, besides supported higher yield of antihypercholesterolemic metabolites (statin 5.24 g/kg and sterol 0.053 g/kg) in a short period of 7 days of fermentation than other substrates used in the study. Germination of finger millet enhanced nutrient and mineral availability for *Monascus* and lowered the pH from 6.1 to 5.8 resulting in higher yield of statin and good yield of sterol production by *Monascus purpureus,* thus forming an alternate economically viable substrate for antihypercholesterolemic

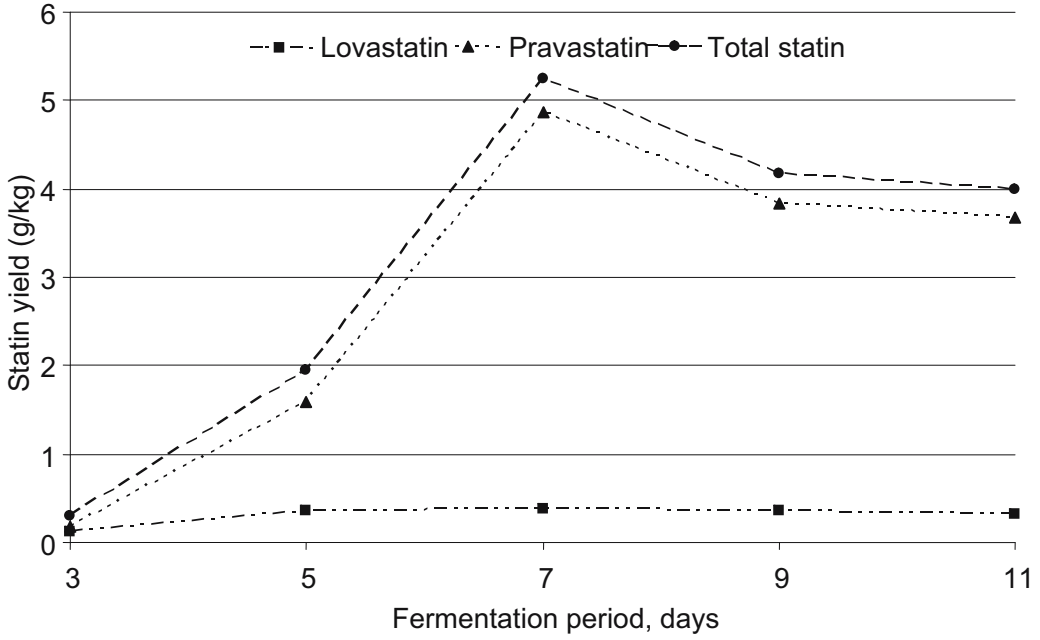


**Fig. 3** Statin production by *Monascus purpureus* grown on germinated finger millet (n=3)

metabolites production. In addition, sprouting helps in the degradation of antinutrient factors like phytic acid and tannin present in the finger millet grain. The sprouting of finger millet and fermenting with *Monascus purpureus* adds value for effective utilization of finger millet and provides scope for development of functional food.

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