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Fatty acid composition of cooked and fermented beans of the wild legumes (*Canavalia*) of coastal sand dunes

[1]Niveditha, V. R., [1*]Sridhar, K. R. and [2]Chatra, S. K. R.

[1]*Department of Biosciences, Mangalore University, Mangalagangotri, Mangalore 574 199, Karnataka, India*
[2]*Tribology Laboratory, Department of Mechanical Engineering, Indian Institute of Science, Bangalore 560 012, Karnataka, India*

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Abstract

Changes in total lipids and fatty acid methyl esters (FAMEs) of dry beans of two wild legumes of coastal sand dunes (CSD) (*Canavalia cathartica* and *C. maritima*) using different treatments (cooked and cooked + solid-state fermentation with *Rhizopus oligosporus*) and extraction methods (Soxhlet and cold extraction) were evaluated. Significant variations in total lipids as well as FAMEs were found between beans, treatments and extraction methods. Cold extraction (Bligh and Dyer method) resulted in significantly highest quantity of total lipids in both beans. The polyunsaturated/saturated ratios were ≥ 0.45 in cooked as well as fermented beans. Stearic acid was significantly elevated in fermented beans of both species in Soxhlet and cold extraction, while palmitic acid in both beans was significantly increased only in Soxhlet method. Oleic acid was significantly raised in *C. maritima* beans on Soxhlet extraction. There is scope for value-addition by following solid-state fermentation of protein-, carbohydrate-, energy-rich and low lipid *Canavalia* beans using *R. oligosporus*. Further studies required to evaluate the yield and acceptability of FAMEs in beans of CSD *Canavalia* spp. to human and or livestock using *R. oligosporus* at different temperature regimes, incubation periods and amendment of minerals.

Introduction

The goal of food security is perpetual accessibility of adequate, safe and nutritious food by the population to meet the diet and health requirements (FAO, 1996). The risk of food insufficiency and malnutrition continues especially in developing countries due to increased population, scarcity of animal-based foods and shrinking agricultural lands. The legumes (Fabaceae) constitute a major alternative and serve as an important source of proteins and offer economically viable traits (Vietmeyer, 1986; Lewis *et al*., 2005). Little known underexplored wild legumes (also known as tribal legumes/pulses) will be valuable in nutrition, health and soil fertility (Bressani *et al*., 1987; Seena *et al*., 2007). These legumes widen the food as well as environmental security due to their inbuilt traits to withstand the adverse conditions like elevated temperature, drought and soil erosion. A variety of habitats serve as natural repositories of wild legumes deserve serious attempts for germplasm collection, nutritional features and pharmaceutical values (Bhat and Karim, 2009).

In the recent past, one of the important habitats received a little attention for exploration of flora is the coastal sand dunes (CSD) (Rao and Sherieff, 2002; Bhat, 2003; Martinez and Psuty, 2004; Maun, 2009). The CSD are dwindling throughout the world due to human interference, pollution, raise in sea level and soil erosion. Among the coastal wild legumes of Southwest coast of India, the indigenous landraces of *Canavalia* exhibit fast growth, tolerance to coastal environment, disease resistance and gives high seed yield. Some studies on the seeds of coastal sand dune *Canavalia* revealed their adequacy in protein, fibre, amino acids and fatty acids (Seena and Sridhar, 2006). Although seeds of *Canavalia* of CSD are endowed with a few antinutritional components (e.g. concanavalin and canavanine), judicious processing methods help to decrease their concentration to serve as nutraceuticals. Leaves, roots and seeds of CSD *Canavalia* have traditional uses to cure skin diseases and to promote healing of burns (Chock, 1968; Bhagya and Sridhar, 2009). Roasted *C. maritima* seed powder substitutes coffee, leaves consists of L-betonicine and roots are useful to treat ciguatera poisoning (Rageau,

*Corresponding author.
Email: *sirikr@yahoo.com*

1973; Bourdy *et al*., 1992; Bhagya and Sridhar, 2009). Thermally treated seeds of *Canavalia* showed better fatty acid profile compared to fresh seeds (Seena and Sridhar, 2006). Solid-state fermentation (SSF) of food stuffs using many microorganisms has been followed to enhance the enzymes, antioxidants, biosurfactants, organic acids and polyunsaturated fatty acids (Silveira and Badiale-Furlong, 2007; Jang and Yang, 2008). Therefore, the present study aims at evaluating the changes in fatty acid profile of cooked beans and cooked + SSF beans (*Rhizopus oligosporus*) of *Canavalia* species growing on CSD.

Materials and methods

Seeds and processing

Canavalia plants of CSD were identified based on verification of fresh plant materials in using taxonomic descriptions, keys and herbarium (Bhat, 2003). The seed samples of *Canavalia cathartica* Thouars and *Canavalia maritima* Thouars were collected from three locations of the CSD of Someshwara, Southwest India (12º47′ N, 74º52′ E) during summer season (February-March, 2010) as the pods dehisce and dry seeds are available. Healthy seeds without damage were separated from dry pods and sun-dried for two days. Each seed was cut into two halves longitudinally using a nut-cracker to get four pieces of split beans and they were separated from seed coat. Split beans processed with two treatments such as pressure cooking and fungal fermentation. The split beans (25 g) in triplicate were transferred to conical flask (250 ml), soaked in distilled water (1:3 w/v) and pressure-cooked (6.5 L, Deluxe stainless steel; TTK Prestige™, Prestige Ltd., India). The cooked split beans were spread on aluminum foil, dried at 40ºC in an incubator, milled (Wiley Mill, 30 mesh) and stored in air-tight glass containers. To perform solid substrate fermentation, another set of cooked split beans in triplicates were allowed to attain laboratory temperature, inoculated with two 5 mm plugs of 3-day-old cultures of *Rhizopus microsporus* var. *oligosporus* (MTCC # 556; strain designation # 22959; Institute of Microbial Type Culture Collection, Chandigarh, India) and allowed to ferment for one week at 37ºC, later fermented split beans were dried, powdered and stored.

Total lipids

To follow Soxhlet method of lipid extraction (AOAC, 2003), dry split bean flours (1 g) were packed in thimbles covered with glass wool were extracted with petroleum ether (200 ml) (60-80ºC) in a soxhlet extractor. The rate of condensation was fixed to 150 drops min^{-1}, extracted up to 7 hr, cooled, transferred to a pre-weighed beakers and evaporated to dryness at room temperature (28±2ºC) for gravimetric estimation of total lipid content.

For cold extraction, Bligh and Dyer method was followed (Bligh and Dyer, 1959). Dry split bean flours (1 g) were homogenized in a chloroform-methanol mixture (2:1 v/v) and preserved overnight. The mixture were transferred to a separatory funnel, deionised water (10 ml) was added, mixed and allowed to separate. The lower chloroform layer containing lipids was drained into pre-weighed beaker, evaporated to dryness at room temperature and the quantity of extracted lipid was determined gravimetrically.

Methyl esters

Fatty acid methyl esters (FAMEs) were processed by the methods outlined by Padua-Resurreccion and Benzon (1979) and Nareshkumar (2007). The HCl reagent (5%) was prepared by addition of 8.3 ml acetyl chloride drop-wise to 100 ml absolute methanol in an ice jacket to avoid bumping. This reagent (2 ml) was added to total lipids (0.2 g) of *Canavalia* in a screw cap glass vial (15 ml), vortexed, incubated (70ºC) in a hot air oven (10 hr) and cooled to laboratory temperature. To this mixture, distilled water (5 ml) and hexane (1 ml) were added and vortexed. On separation of two layers, the top hexane layer was aspirated out into microtubes and stored for the gas chromatographic analysis.

Gas chromatography

The FAMEs were diluted (40 ml + 960 ml HPLC grade n-hexane) in the sample vial. One ml FAMEs was injected into the gas chromatograph (GC-2010, Shimadzu, Kyoto, Japan), by an auto injector (AOC-20i, Shimadzu), capillary column (BPX 70, SGE Analytical Science, Austin, TX) and the elutants were detected in flame ionization detector (FID, Shimadzu). The injection mode was in split (ratio 1:50); terminal temperature was 225ºC; carrier gasses were nitrogen and air; pressure, 114.9 kPa; total flow, 68.9 ml min^{-1}; column initial temperature, 100ºC with temperature elevation rate of 5ºC min^{-1}. The amplified signals were recorded in a computer with GC-Solutions software (Shimadzu). Quantitative estimation was followed using an external standard mixture of FAMEs (C6-C26; Sigma-Aldrich, Supelco, Bellefonte, PA). The concentrations and area of each peak was computed by a data analysis method developed using different concentrations of standard FAMEs. The data acquired were assessed using the GC Post-Run analysis software (Shimadzu, Japan).

Data analysis

The difference in the quantity of total lipids and FAMEs between seeds, treatments and methods were assessed by t-test (STATISTICA Version # 8) (StatSoft Inc., 2008).

Results

The color of the pressure-cooked *Canavalia* bean flours on fermentation changed from egg white to light brown based on visual observations. Fermented samples of both beans possess chocolate aroma, which persisted on drying (45-50°C), milling and storage. The taste of cooked and fermented bean flours was similar to milk powder, which needs further systematic sensory evaluations.

Total lipids

The total lipid extraction in split beans varied between plant species (*C. cathartica* and *C. maritima*), treatments (cooked and cooked + SSF) and extraction methods (Soxhlet and cold extraction) (Figure 1). Total lipid content between cooked and fermented split beans of *C. cathartica* did not differ significantly in both methods, while significant difference was seen in the beans of *C. maritima* ($p < 0.01$). In both beans, cold extraction of lipids was significantly higher in cooked as well as fermented split beans than in Soxhlet extraction ($p < 0.05$). The overall quantity of total lipids was highest in cooked and fermented beans of *C. maritima* extracted by Bligh and Dyer method.

Methyl esters

In *C. cathartica*, Soxhlet method yielded more unsaturated than saturated fatty acids in cooked split beans, while it was reverse in fermented beans (Table 1). Cooked as well as fermented split beans yielded more of unsaturated fatty acids in cold extraction with highest quantity in fermented beans. The polyunsaturated acid/saturated fatty acid (P/S) ratio was higher in cooked beans with significant decrease on fermentation in both methods ($p < 0.001$). Both methods of extraction yielded the highest quantity of palmitic acid in cooked and fermented beans with highest concentration in cold extraction. The second most common saturated fatty acid in Soxhlet method was stearic acid, while pentadecanoic (cooked) and stearic (fermented) acids in cold extraction. Among the unsaturated fatty acid, oleic acid was highest in cooked as well as fermented beans with high concentration in cold extraction. The second most common unsaturated fatty acid was palmitoleic acid in both extraction methods. Linoleic acid was confined to cooked beans in both methods.

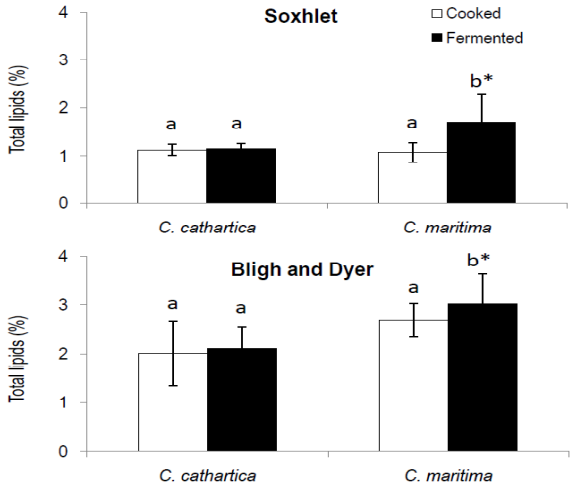

Figure 1. Total lipids extracted from split beans of *Canavalia* by Soxhlet and Bligh and Dyer methods (n=3, mean±SD). Different alphabets between cooked and fermented split beans significantly differed (*, $p < 0.01$)

In *C. maritima*, both methods yielded more of unsaturated than saturated fatty acids except for fermented beans in Soxhlet method (Table 2). Like *C. cathartica*, cold extraction yielded more of unsaturated fatty acids in fermented beans. As seen in *C. cathartica*, the P/S ratio was higher in cooked beans, which was significantly decreased on fermentation in both methods ($P < 0.001$). The beans of *C. maritima* showed the highest quantity of palmitic acid by Soxhlet method, which was followed by stearic acid (cooked) and lignoceric acid (fermented). In cold extraction, palmitic acid was highest in cooked as well as fermented beans ($p < 0.01$) followed by stearic acid. The quantity of linoleic acid was higher in cooked as well as fermented beans on cold extraction compared to Soxhlet method. Soxhlet method yielded enanthic acid, erucic acids (*C. cathartica*), eicosadienoic acid and erucic acid (*C. maritima*) only in fermented beans, while 11-octadecenoic acid (*C. maritima*) only in cooked beans. Among the beans, treatments and methods, fermented *C. maritima* beans on cold extraction exhibited the highest quantity of unsaturated fatty acids (Table 2).

Discussion

The quantity of total lipids and the P/S ratio of uncooked split beans of *C. cathartica* and *C. maritima* were elevated on thermal treatments (pressure-cooking and roasting) (Seena and Sridhar, 2006). Soxhlet extraction of lipids involves high temperature leading to changes in the fatty acid profile of beans compared to cold extraction and the latter method retains the

Table 1. Fatty acid methyl esters (mg 100 g^{-1} lipid) of cooked and fermented split beans of *Canavalia cathartica* (n=3, mean±SD)[a]

	Soxhlet method		Bligh and Dyer method	
Fatty acid	Cooked	Fermented	Cooked	Fermented
Saturated fatty acids				
Enanthic acid (C7:0)	–	0.05±0.003	–	–
Caprylic acid (C8:0)	0.68±0.06	0.82±0.05	0.52±0.17	0.42±0.09
Nonanoic acid (C9:0)	1.80±0.18	0.78±0.05**	1.81±0.58	0.63±0.13*
Capric acid (C10:0)	1.80±0.18	1.34±0.09*	1.20±0.39	0.84±0.18
Lauric acid (C12:0)	9.01±0.90	9.54±0.64	4.68±1.52	3.58±0.77
Tridecanoic acid (C13:0)	0.11±0.01	–	–	0.42±0.09
Myristic acid (C14:0)	17.58±1.77	48.13±3.27**	46.35±15.13	31.55±6.86
Pentadecanoic acid (C15:0)	15.89±1.60	42.61±2.90**	53.58±17.49	33.65±7.32
Palmitic acid (C16:0)	299.02±30.18	426.74±29.06*	622.47±203.23	485.45±105.62
Heptadecanoic acid (C17:0)	4.05±0.40	15.20±1.03**	17.46±5.69	11.36±2.47
Stearic acid (C18:0)	30.53±3.08	94.47±6.43**	47.76±15.59	124.52±27.09*
Nonadecanoic acid (C19:0)	–	0.75±0.05	–	0.83±0.18
Arachidic acid (C20:0)	6.76±0.68	35.16±2.39**	13.44±4.38	42.49±9.24*
Heneicosanoic acid (C21:0)	-	-	0.80±0.26	1.05±0.23
Behenic acid (C22:0)	3.27±0.32	31.29±2.13**	6.02±1.97	26.71±5.81**
Tricosanoic acid (C23:0)	1.80±0.18	5.96±0.40**	4.01±1.31	6.73±1.46*
Lignoceric acid (C24:0)	9.58±0.96	82.10±5.59**	18.46±6.02	76.35±16.61**
Pentacosanoic acid (C25:0)	3.83±0.38	31.14±2.12**	10.23±3.34	14.09±3.06
Cerotic acid (C26:0)	0.68±0.06	75.69±5.15***	7.63±2.48	61.63±13.40**
Unsaturated fatty acids				
Cis-7-hexadecenoic acid (C16:1)	2.93±0.29	–	0.20±0.06	–
Palmitoleic acid (C16:1)	31.43±3.17	81.06±5.52**	76.66±25.02	116.74±25.40*
Cis-10-heptadecanoic acid (C17:1)	–	16.84±1.14	6.82±2.22	6.52±1.41
Oleic acid (C18:1)	588.23±59.37	361.18±24.60*	912.83±298.03	898.96±195.59
Linoleic acid (C18:2)	19.83±2.00	–	15.45±5.04	–
Eicosenoic acid (C20:1)	11.49±1.15	36.65±2.49**	19.67±6.42	33.49±9.11*
Erucic acid (C22:1)	–	4.47±0.30	–	–
	406.39±40.94	901.77±61.35**	856.42±279.55	923.35±200.84
Total saturated fatty acids				
	653.91±65.98	500.2±34.05	1031.63±336.79	1055.71±231.51
Total unsaturated fatty acids				
P/S ratio	1.61	0.55***	1.20	1.14***

[a] Asterisk across the columns between cooked and fermented samples denotes significant difference (t-test: *, p < 0.05; **, p < 0.01; ***, p < 0.001)
-, Not detectable

Table 2. Fatty acid methyl esters (mg 100 g^{-1} lipid) of cooked and fermented split beans of *Canavalia maritima* (n=3, mean±SD)[a]

	Soxhlet method		Bligh and Dyer method	
Fatty acid	Cooked	Fermented	Cooked	Fermented
Saturated fatty acids				
Caprylic acid (C8:0)	0.38±0.07	1.87±0.62*	0.54±0.07	3.02±0.61**
Nonanoic acid (C9:0)	1.49±0.27	1.02±0.34	2.15±0.27	1.21±0.24**
Capric acid (C10:0)	0.85±0.15	1.70±0.56	1.35±0.16	4.53±0.91
Lauric acid (C12:0)	5.74±1.05	23.8±7.95*	5.65±0.70	57.38±11.52
Tridecanoic acid (C13:0)	0.22±0.03	–	0.27±0.03	0.60±0.12*
Myristic acid (C14:0)	19.99±3.66	49.3±16.47*	29.59±3.71	99.66±20.02**
Pentadecanoic acid (C15:0)	18.08±3.31	36.21±12.10	61.33±7.70	61.31±12.31
Palmitic acid (C16:0)	263.81±48.38	436.56±145.83*	703.70±88.36	307.93±63.74**
Heptadecanoic Acid (C17:0)	4.79±0.87	6.97±2.33	18.83±2.36	15.40±3.09*
Stearic acid (C18:0)	37.00±6.78	67.32±22.49*	94.69±11.89	246.43±49.50*
Nonadecanoic acid (C19:0)	0.43±0.07	19.89±6.64*	1.08±0.13	–
Arachidic acid (C20:0)	10.10±1.85	57.97±19.37*	32.28±4.05	100.87±20.26**
Heneicosanoic acid (C21:0)	0.64±0.11	–	2.15±0.27	–
Behenic acid (C22:0)	4.04±0.74	44.88±14.99*	14.26±1.79	85.47±17.16**
Tricosanoic acid (C23:0)	2.13±0.39	11.22±3.75*	7.26±0.91	22.65±4.55**
Lignoceric acid (C24:0)	11.06±2.02	97.07±32.43*	37.66±4.72	196.00±39.37**
Pentadecanoic acid (C25:0)	–	19.89±6.64	15.06±1.89	40.47±8.12*
Cerotic acid (C26:0)	3.30±0.60	68.17±22.77*	11.02±1.39	151.00±30.33**
Unsaturated fatty acids				
Palmitoleic acid (C16:1)	21.48±3.93	21.25±7.10	80.7±10.13	–
Cis-10-heptadecanoic acid (C17:1)	1.28±0.23	–	25.56±3.20	20.23±4.06*
Oleic acid (C18:1)	2.87±0.52	567.46±189.56*	1331.82±167.23	1368.36±274.90
11-octadecenoic acid (C18:1)	561.02±102.89	–	–	–
Linoleic acid (18:2)	20.52±3.76	–	26.36±3.31	23.56±4.73
Eicosenoic acid (C20:1)	9.25±1.69	59.33±19.82*	28.51±3.58	119.29±23.96**
Eicosadienoic acid (C20:2)	–	3.23±1.08	–	–
Erucic acid (C22:1)	–	4.93±1.65	–	–
Total saturated fatty acids	384.05±70.35	943.84±315.28	1038.87±130.40	1393.93±281.85*
Total unsaturated fatty acids	616.42±113.02	656.2±219.21	1492.95±187.45	1531.44±307.65
P/S ratio	1.61	0.70***	1.44	1.10***

[a] Asterisk across the columns between cooked and fermented samples denotes significant difference (t-test: *p, < 0.05; **, p < 0.01; ***, p < 0.001)
-, Not detectable

original fatty acid content (Oliveira *et al*., 2011). Our study supports this view as fatty acid composition and P/S ratio were consistent in beans on cold extraction than on Soxhlet extraction method. Moreover, there was no significant change in the total unsaturated fatty acids between cooked and cooked + fermented beans of both *Canavalia*. However, the difference in total lipids and fatty acid profile in beans based on cold extraction between *Canavalia* spp. depicts their differential inherent traits although exists in the same CSD habitat. In addition, this study confirms that the cold extraction (Bligh and Dyer method) serves better than hot extraction (Soxhlet method) in assessment of FAMEs of CSD *Canavalia*. This view has been supported by the earlier investigations on electron beam irradiated dry beans of *Canavalia* spp. of CSD (Supriya *et al*., 2012) as well as unirradiated ripened split beans of *Canavalia* spp. of CSD and mangroves of Southwest coast of India (Shreelalitha *et al*., 2011).

Increase in total lipids by fungal fermentation may be due to the dissociation of lipoprotein complexes and synthesis of their own lipids during growth on substrate (Wang *et al*., 1975; Oliveira *et al*., 2011). Increase in ether-extractable lipids in peanut flours fermented by *R. oligosporus* is predicted due to synthesis of lipids or utilization of non-lipid materials during fermentation (Beuchat and Worthington, 1974). In the present study, fermented beans of both *Canavalia* showed significant elevation in total lipids especially by cold extraction ($p < 0.05$). Although *Canavalia* beans possess low lipids, they consist of moderate to high quantity of fibre (1.7-10%) (Seena and Sridhar, 2006). As fibre is known to hold considerable quantity of lipids (Silva et al., 2006), fermented beans of *Canavalia* might have retained fatty acids. It is postulated that *R. oligosporus* utilize the available fatty acids in beans to build their cell wall phospholipids leading to changes in fatty acid profile of fermented beans (Oliveira *et al*., 2011). The lipid content of cell walls of fungi usually ranges from 1-10% of dry matter. Oleic, linoleic and palmitic acids are the major fatty acids in several fungal species. Palmitic and stearic acids dominate in cell wall, while myristic acid exists in minor quantities (Ruiz-Herrera, 1992; Oliveira *et al*., 2011). Palmitic, stearic, oleic, linoleic and linolenic acids were abundant in mycelia of *Rhizopus* spp. (Shaw, 1966). The SSF of rice bran using *R. oryzae* showed significant elevation in palmitic and linoleic acids, while significant reduction in stearic and linolenic acids compared to unfermented bran (Silveira *et al*., 2010). However, oleic, linoleic and palmitic acids

of rice bran were unaltered even after fermentation with *R. oryzae* as reported by Oliveira *et al*. (2011). In our study, stearic acid was significantly elevated in fermented beans of both species (7.1-17.7%) on Soxhlet and cold extraction methods ($p < 0.05$), while palmitic acid was significantly elevated in both beans (67.7-73.6%) only in Soxhlet extraction ($p < 0.05$). Oleic acid was significantly elevated in *C. maritima* beans in Soxhlet method ($p < 0.05$) (cooked, 0.46%; fermented, 86.5%).

Unprocessed seeds of *Canavalia* spp. showed highest quantity of oleic acid followed by stearic acid (Seena and Sridhar, 2006). But thermal treatments (cooking and roasting) and fermentation with *R. oligosporus* considerably changed the fatty acid profile. Lipases produced during fermentation of temph are known to breakdown glycerides into easily assimilable fatty acids (Mital and Garg, 1990; Hering *et al*., 1991). Increased activity of lipases in cooked beans might be one the reasons for liberation of fatty acids on fungal fermentation (Sarkar *et al*., 1996). Lipase activity in *R. oligosporus* was reported better than *R. oryzae* by Sudaryatiningsih and Supyani (2009). According to Teng *et al*. (2008), activity of lipases increases during fermentation by *Rhizopus* spp. resulting in formation of oleic acid. Oleic acid gets converted into linoleic and linolenic acids by the desaturase enzymes, which are dependent on temperature, moisture and oxygen regimes (Sudaryatiningsih and Supyani, 2009). The SSF of soybean flour by *Rhizopus* spp. at 25-26ºC resulted in production of ω-3 and ω-6 fatty acids, but increased temperature terminates production such essential fatty acids as it affects the activity of desaturases. As water content is known to govern the activity of lipase, slightly higher amount of water during SSF facilitates lipase to hydrolyze fats into glycerol and fatty acids. As SSF was carried out at 37ºC might have resulted in no synthesis or least synthesis of essential fatty acids in our study. However, a small quantity of linoleic (cold extraction) and eicosadienoic (Soxhlet extraction) acids were showed up in fermented beans of *C. maritima*. Linoleic acid present in cooked samples of both beans was eliminated by fermentation as shown in Soxhlet extraction. Interestingly, there was no significant change in the quantity of linoleic acid in cooked beans of *C. maritima* on fermentation as depicted by cold extraction ($p > 0.05$). Assessment of lipases especially in fungal fermented *Canavalia* beans is necessary to throw light on the changes in fatty acid profile more precisely.

On fermentation of low fatty acid (~2.2%) cowpea flour (*Vigna unguiculata*) with *R. oligosporus*, elevation of myristic, palmitic, stearic, and oleic acids was evident (Prinyawiwatkul *et al*., 1996). Our study also showed the elevation of only myristic acid in both methods of extraction in *C. maritima* ($p < 0.05$) and only in Soxhlet extraction in *C. cathartica* ($p < 0.01$). *Canavalia* beans with low quantity of fats (~2-3%) and high amount of carbohydrates (Seena and Sridhar, 2006) might have facilitated *R. oligosporus* to utilize carbohydrates as primary source of energy and thus overall fatty acid profile was not significantly altered in our study. This can be correlated to the decreased quantity of carbohydrates in *Canavalia* beans (Niveditha and Sridhar, unpub. obs.) as well as *Vigna unguiculata* (Prinyawiwatkul *et al*., 1996) on fermentation with *R. oligosporus*.

Dietary saturated fatty acids are known to prevent damage of liver by alcohol (Cha and Sachan, 1994). Among natural fats, palmitic and stearic acids are the best saturated fatty acids for mammalian nutrition (Hayes, 2002). Even though stearic acid of *Canavalia* beans was significantly elevated on SSF by *R. oligosporus* ($p < 0.05$), effect of stearic acid on blood cholesterol is neutral on consumption along with natural fats. Palmitic acid has an intermediate impact on lipoprotein profile and on its consumption along with MUFA or PUFA shows neutral effect. Food stuffs possessing P/S ratio below 0.45 is not advisable for human consumption as it leads to cardiac diseases (Department of Health, 1994). Before (1.2-1.61) and after (0.55-1.14) fermentation, beans of *Canavalia* spp. showed significantly higher P/S ratio (> 0.45) ($p < 0.001$) indicates their adequateness.

Fungi are known to respond differently to the changes in the edaphic factors (e.g. temperature, pH and salinity). For instance, membrane fluidity and desaturation of fatty acids increases by stress caused by temperature, pH, salt and hydrogen peroxide (Guerzoni *et al*., 1999, 2001). Besides edaphic factors, fatty acid composition seems to be dependent on the production of organic acids by *R. oryzae* during fermentation (Liou *et al*., 2001). Oxygen is essential for the desaturation of fatty acids by aerobic and facultative microbes. As increased oxygen during fermentation is directly proportional to the formation of linoleic acid (Sudaryatiningsih and Supyani, 2009), it is necessary to consider aeration/agitation during SSF of *Canavalia* by *R. oligosporus* in future studies. In addition, evaluation of changes in color, aroma and sensory features of fermented *Canavalia* beans by sophisticated techniques are warranted.

Conclusions

Among *Canavalia* of coastal sand dunes (CSD) of Southwest India, *C. maritima* is abundant, widely

distributed and grows along with other CSD flora, while *C. cathartica* is less abundant than *C. maritima* and usually grows in pure stand. Under optimum environmental conditions, the average seed yield of *C. maritima* was estimated to be about 720-1,500 kg ha^{-1} (Bresseni *et al*., 1987). For consumption, tribes of Southwest coast of India process dry and ripened beans of CSD *Canavalia* traditionally (e.g. soaking and extrusion cooking) to eliminate antinutritional components. Soaking also supports natural fermentation by microflora in beans. There is ample scope for value addition through fermentation of protein- and carbohydrate-rich *Canavalia* beans using *R. oligosporus* in favor of human and or livestock nutrition as evidenced in the present work. This study showed that cold extraction of lipids will be more useful in evaluation of total lipids and fatty acid methyl esters of beans of *Canavalia* of CSD. Further studies are necessary to evaluate the yield and acceptability of fatty acids in *R. oligosporus* fermented beans of *Canavalia* at different temperature regimes, incubation periods and amendment of minerals. Nevertheless, nutritional, antinutritional and functional properties of fermented beans of CSD *Canavalia* also assume prime importance.

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