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EVALUATION OF NUTRITIVE VALUE OF LABORATORY SCALE FERMENTED PEANUT PRESS CAKE BY *N. INTERMEDIA* MTCC 1230 and *R. OLIGOSPORUS* MTCC 556.

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Abstract: The residue that remains after oil extraction by expeller from Peanut (Groundnut= *Arachis hypogea*) is known as Peanut press cake or Groundnut cake. Peanut press cake (PPC) is the common substrate (raw material) used for the preparation of a traditional food in Indonesia known as oncom. Oncom is a popular traditional Indonesian food prepared by the fermentation of Peanut press cake. It is semisolid cake, like tempeh, consumed as native dish and most commonly served deep fat fried as a meat analogue. Traditionally Oncom is prepared by fermenting overnight soaked peanut press cake with the mold cultures of *Neurospora intermedia, Neurospora sitophila* or *Rhizopus oligosporus* at room temperature for 48 hours. The inoculated molds modify physical, biochemical and nutritive characteristics of the peanut press cake substrate during fermentation. The molds produce amylase, protease and lipase enzymes which bring about physico-chemical changes in PPC constituents such as carbohydrates, proteins and lipids and enhance its digestibility, flavor and nutritive value. The present study was carried out to evaluate the nutritive value of laboratory scale fermented Peanut press cake under standardized conditions to determine total Carbohydrate, Protein and Lipid content, using the mold cultures *N. intermedia* MTCC 1230 and *R. oligosporus* MTCC 556 for fermentation. The nutritive value of fermented PPC by *N. intermedia* MTCC 1230 show total Carbohydrate (25.5 %); Protein (55.2 %) and Lipid (2.0 %) whereas that fermented by *R. oligosporus* MTCC 556 show total Carbohydrate (27 %), Protein (54.4 %) and Lipid (1.6 %).

Keywords: Peanut press cake, Neurospora intermedia, Rhizopus oligosporus, Nutritive value

Introduction:

Peanut Press Cake (PPC) is the common traditional substrate (raw material) used for oncom preparation. In most of the countries PPC is used as animal feed or as organic manure. Its higher fiber content and indigestible components make it undesirable as a human food. However, centuries ago the Indonesians demonstrated a way to convert this animal feed to human quality protein rich food through a traditional fungal fermentation process. Peanut press cake fermented by the molds *Neurospora sitophila / intermedia* or *Rhizopus oligosporus* for 48 hr. at room temperature makes a food known as **oncom**, which is used in the daily diet of about 30 million peoples in Indonesia. *N.sitophila / intermedia* produce orange-red oncom whereas; *R. oligosporus* produce ash-gray oncom (Beuchat, 1987; Bigelis, 1992, Kumbhare et al., 2003).

Oncom is a traditional fungal fermented Indonesian food very much popular in West Java. It is also known as Ontjom, Lontjom, or Onchom and closely resemble to tempeh (van Veen and Graham, 1968). Like tempeh, oncom is a thick homogenous semisolid cake commonly served deep fat fried or cooked with other native dishes. It may also be roasted and cut into pieces (chunks) and covered with ginger sauce (van Veen and Steinkraus, 1970). Oncom has pleasant fruity odor. When fried it takes on a minced-meat flavor. It is consumed as a low-cost protein-rich meat analog in Indonesia. Oncom apparently makes a significant quantitative as well as a qualitative contribution to enrich protein in the diet of peoples in area where it is produced and consumed (Winarno, 1979).

The inoculated molds bring about physico-chemical and nutritional quality changes in PPC substrate. The molds produce flavor metabolites, vitamins and extracellular amylase, protease, lipase enzymes which modify the PPC constituents such as carbohydrates, protein, lipids and enhances its flavor, digestibility and nutritive value (Quinn et al., 1975; Beuchat, 1995).

The present investigation was carried out to analyze total carbohydrate, protein and lipid content of laboratory scale fermented PPC (oncom) under standardized

conditions using the mold cultures *N.intermedia* MTCC 1230 and *R.oligosporus* MTCC 556.

Materials and Methods:

Substrate: The common traditional substrate (raw material) used for the preparation of oncom is Peanut Press Cake (PPC) (Beuchat, 1976). The residue that remains after oil extraction by Expeller from Peanut (Groundnut = *Arachis hypogea*) is known as Peanut press cake or Groundnut cake. It was procured from a local oil mill in Ballarpur.

Mold Cultures: The mold cultures, *N.intermedia* MTCC 1230 and *R.oligosporus* MTCC 556 were used for laboratory scale fermentation of PPC to determine its proximate composition. The mold cultures were obtained from Microbial Type Culture Collection (MTCC), Chandigarh (India) and maintained by growing on Potato Dextrose Agar (PDA, Himedia M096).

Mold culture inoculum: Mold culture inoculum for PPC fermentation was prepared from seven day old sporulated culture of molds grown on PDA slants at 28^{o} C. Ten ml of sterile distilled water was added to prepare spore suspension and 1 ml of this spore suspension was used as inoculum to inoculate 25 gm. of overnight soaked PPC substrate for fermentation.

Laboratory scale fermentation of PPC: The PPC was fermented on laboratory scale under optimized process conditions. A 25 gm. of overnight soaked and autoclaved PPC in a 250 ml Erlenmeyer flask was inoculated separately with 1 ml spore suspension of *N.intermedia* MTCC 1230 and *R.oligosporus* MTCC 556. One flask containing 25 gm. of overnight soaked and autoclaved PPC without inoculation (unfermented) was kept as a Control.

Extract for Analysis: The fermented PPC by the mold cultures and Control after 48 hr. of fermentation at 28^{o}C was dried in oven at 60^{o}C for 8 hr. and ground to smooth flour. The dried and ground flour in each case was analyzed for its total carbohydrate, protein and lipid content.

ANALYTICAL METHODS FOR EVALUATION OF NUTRITIVE VALUE:

(a) Determination of Carbohydrate Content: One gram of respective dry flour of fermented PPC was homogenized with 5ml of sterile distilled water and 5 ml of ethyl alcohol. Samples were extracted for 30 min with occasional stirring. Suspension was centrifuged at 10000 rpm for 10 min and filtered through Whatman No.1 filter paper. Filtrates were used as test sample to analyze total carbohydrate. Total carbohydrate was determined by Anthrone method (Plummer, 1993). Total carbohydrate in test samples was determined from a curve using glucose (100 µg/ml) as a standard and reported as carbohydrate percent (gm. /100 gm.).

(b) Determination of Protein Content: One gram of dry flour of fermented PPC was first defatted by treatment with the mixture of 10 ml of ethyl-ether and ethanol (3:1 v/v). The sediment obtained was washed with ether and air dried to remove the solvent. The dry solid was dissolved in 10 ml of ice-cold 10 % Tri-chloro acetic acid to precipitate out the protein. The precipitate was centrifuged at 10000 rpm for 10 min and filtered through Whatman No.1 filter paper. The filtrates were used as test samples to analyze total protein. Total protein content in the test sample was determined by a method of Lowry et al. (1951) using bovine serum albumin (100 µg/ml) as standard protein and Follin-Ciocalteau as reagent and reported as protein percent (gm. /100gm).

c) Determination of lipid Content: Total lipid content was determined by ethyl-ether and ethanol extraction method (Jayaraman, 1981). The lipid was extracted from a 10 gm. of dry flour (fermented PPC / Test samples) using ethyl- ether and ethanol mixture (3:1 v/v) in Sox-let lipid extractor (Asgi-2445) for 8 hr. Extracted lipid was kept in a vacuum drier at 60^{o}C to remove extraction solvent. The weight of the solvent free extracted lipid was determined in a dried and pre-weighed silica crucible (110^{o}C for 1hr, thrice). The difference in weight of the crucible with extracted lipid minus the weight of empty dried crucible was the amount of total lipid in the test sample and reported in terms of gm. % (gm. / 100gm).

Results and Discussion:

The data of evaluated nutritive values given in Table 1 for i) Control (Fig.1A) - Total carbohydrate (28.6%), protein (58.8%) and lipid (2.8%). ii) Laboratory scale fermented PPC by *N.intermedia* MTCC 1230 (Fig.1B) - total carbohydrate (25.2 %), protein (55.2 %), lipid (2.0 %) and iii) Laboratory scale fermented PPC by *R.oligosprus* MTCC 556 (Fig.1C) - total carbohydrate (27.0 %), protein (54.4 %), lipid (1.6 %). This indicates reduction in total carbohydrate, protein and lipid content during fermentation (Kumbhare et al., 2000, 2003).

These findings coincided with those by Beuchat et al., (1974) and Quinn et al., (1975), who reported that reduction in carbohydrate and protein content of PPC during fermentation was due to their utilization and enzymatic hydrolysis to simple sugar, amino acids and free fatty acids**.** The PPC fermented by *N.intermedia* MTCC 1230 showed comparatively more reduction in total carbohydrate (3.1%) than that fermented by *R.oligosporus* MTCC 556 (1.6%) whereas, PPC fermented by *R.oligosporus* MTCC 556 showed more reduction in total protein (4.4%) and lipid (1.2%) than that fermented by *N.intermedia* MTCC 1230 (protein 3.6%, lipid 0.8%). This indicated that *N.intermedia* MTCC 1230 utilizes more carbohydrate than *R.oligosporus* MTCC 556 whereas; *R.oligosporus* MTCC 556 utilizes more protein and lipid than *N.intermedia* MTCC 1230 during PPC fermentation.

van Veen and Steinkraus (1970) reported that *N. intermedia* produce cellulase which hydrolyze fiber content of PPC making it more digestible. Quinn et al., (1975) reported that, utilization of flatulence causing sugars like stachyose and raffinose in PPC by *N. intermedia* during fermentation causes increased digestibility in oncom. Fardiaz and Markakis (1981) reported that *N. intermedia* ATCC 14151 exhibited strong α-galactosidase activity which hydrolyze PPC carbohydrate and utilize more sucrose, stachyose and raffinose than *R. oligosporus* ATCC 22959. Beuchat et al. (1974) reported reduction in protein content by *R. oligosporus* during PPC fermentation due to the production of acid protease which hydrolyze the PPC protein (arachin and con-arachin) and liberate amino acids particularly glutamic acid and aspartic acid responsible for flavor enhance in oncom. They further noted that, about 2.7% of amino acids are liberated from peanut protein within 48 hr. of fermentation. Wang and Heseltine (1970) reported that, extracellular lipase produced by molds hydrolyze lipids, liberates free fatty acids especially oleic, linoleic and palmitic acids, which give pleasant odor to the fermented product. Winarno (1979) reported that, enzymes produced by *R. oligosporus* and *N. sitophila* deeply penetrates the PPC substrates making carbohydrate, protein and lipids more digestible and at the same time more flavorful.

The nutritive value of laboratory scale fermented PPC shown in Table-1 by *N. intermedia* MTCC 1230 show, total carbohydrate (25.5 %); protein (55.2 %) and lipid (2.0 %) whereas that fermented by *R. oligosporus* MTCC 556 show total carbohydrate (27 %), protein(54.4 %) and lipid (1.6 %). These results closely coincide with those reported by Quinn et al. (1975).

Fig. 1. Laboratory scale fermented peanut press cake.

Fig.1 A. Control (Unfermented PPC)

Fig.1 B. PPC fermented by *N.intermedia* MTCC 1230

Fig.1 C. PPC fermented by *R.oligosporus* MTCC 556

Table 1: Nutritive Value of laboratory scale fermented peanut press cake.

Mold cultures inoculated	Carbohydrate % (gm./100gm)	Protein % (gm./100gm)	Lipid % (gm./100gm)
Control (Unfermented PPC)	28.6	58.8	2.8
N.intermedia MTCC 1230	25.5	55.2	2.0
R.oligosporus MTCC 556	27.0	54.4	1.6

Conclusion:

These results conclude that the fermentation of PPC by mold cultures causes reduction in the initial carbohydrate, protein and lipid content due to their utilization. However, liberated simple sugars, amino acids and fatty acids by enzymatic hydrolysis enhance flavor, digestibility and nutritive value in fermented peanut press cake (oncom). Further studies on the analysis of liberated simple sugars, amino acids, fatty acids, vitamins flavor metabolites etc. is needed to fully characterize valuable nutritional properties in fermented peanut press cake by *N. intermedia* MTCC 1230 and *R. oligosporus* MTCC 556 .

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