J. Mycopathol. Res. 46(1) : 89-92 (2008)

Microbial and biochemical compositions of traditional fermented vegetable food (*Gundruk* and *Sinki*) and their fermentation procedure

SUCHETA LAIRENLAKPAM AND G. K. N. CHHETRY
Department of Life Sciences, Manipur University, Canchipur 795 003, Manipur

Traditional and indigenous method of *Gundruk* and *Sinki* preparation is elaborated in this paper. Assessment of microbes on mustard leaves and radish and their respective fermented products called *Gundruk* and *Sinki* revealed the presence of 16 microbes including yeast and bacteria. Variations of microbes with respect to seasons, leaf maturity, storage periods, etc. of *Gundruk* and *Sinki* and their respective raw materials ; mustard leaves and radish were observed. Microbial population of fermented *Gundruk* and *Sinki* and unfermented vegetables i.e. mustard leaves and radish was significantly different. *Trichosporan asahii, Bacillus pantothenticus* and *Bacillus pumilus* were invariably found associated with fermented *Gundruk* and *Sinki* and also with non-fermented mustard leaves and radish. However, composition and number of microbes varied between the two products. Also, significant differences in biochemical compositions between fermented *Gundruk* and *Sinki* and non-fermented mustard leaves and radish were observed. Alterations in biochemical constituents of both *Gundruk* and *Sinki* when compared to its non-fermented raw metarials were attributed to the role of microbes.

Key words : Mustard leaves, radish, *Gundruk, Sinki,* yeast, bacteria

INTRODUCTION

Gundruk is traditionally fermented food product of vegetables, mostly mustard leaves (*Brassica juncea* var. *rugosa*), radish leaves (*Raphanus sativus*.) etc. whereas *Sinki* is a fermented product of radish (*Raphanus sativus*) and *shalgam* (*Brassica rapa*). To overcome the scarcity of green vegetables during off-season pariod of the year, mustard leaves and radish (roots and leaves) are fermented by the local people of Nepali community residing in the hills of Manipur and elsewhere. Availability of these vegetables in abundance only for a brief pefiod of the year particularly has encouraged local enterpreneurs to bring about the fermented products for future use.

MATERIALS AND METHODS

Traditional fermentation process of gundruk from mustard leaves (Brassica juncea var. rugosa)

Fresh mustard leaves were plucked during winter months and were thoroughly washed in clean water. It was then exposed to sunshine for one or two days so that the leaves were wilted to some extent. The wilted leaves were then smashed or shredded gently in traditional rice pillar called '*dhiki*'. The shredded leaves were exposed to sunshine for a while and placed in fermenters. Basically fermenters may be classified into two types. (i) : underground soil fermenter (pit fermenter) :- pit called *gundruk khadal* were prepared by digging into the soil and was later burnt inside to make it warm and dry and (ii) overground fermenters were *doko* (basket), *balti* (bucket), *handi* (basket), etc.

Walls of these traditional fermenters were lined with traditionally woven bamboo mat followed by paddy straw and banana, onion, *bhuletro, nevaro* leaves, etc. Then, mildly shredded and sun dried rayo

(*Brassica juncea*) were placed layer after layer into the fermenters by using wooden pestle called *musli* or stamping by feet. The shredded raw materials were then covered properly to make it air and water tight by using bamboo mat, paddy straw and leaves of banana, onion, *nevaro, bhuletro* etc.

Finally heavy objects were placed on the mouth of covered pit and other fermenters in order to provide constant pressure. This arrangement was left for fermentation under anaerobic conditions for about 15 to 21 days after which the fermented products were ready. Then the fermented products called *gundruk* were taken out of the fermenters layer after layer mannually with care. After removing the over fermented spoilt parts, the rest were cut and spread over bamboo mat called *mandro* for exposure. Now the *gundruk* were ready for consumption and were stored in different air tight container like jar, tin etc. for future use.

Traditional methods of sinki preparation from mula/radish (Raphanus sativus)

Raphanus sativus, locally called *mula* were uprooted and washed thoroughly. The leaves were then trimmed off and only the underground stem portions were taken. These were then sun dried for 2-3 days so as to make its wilted. The wilted radishes were crushed by traditional rice pillar called *dhiki* (wooden stick) or by a wooden hand bat called *mungro.* These were then exposed to sunshine by spreading over bamboo mats called *mandro* for 1-2 days. The fermenters used for *sinki* preparation were similar to those of *gundruk* and were categorised into (i) overground fermenters like *doko, balti* etc and (2) underground fermenters that is pits dug into ground.

The process of fermentation of *sinki* was also similar to the process of fermentation of *gundruk* already described above.

A comparative biochemical account of fresh gundruk and sinki with its stored counterparts for one and two year.

It should be noted that fresh *gundruk* and *sinki* implied traditional fermented vegetable food of Nepali community which had been fermented for about 15 days and had just been taken out from the fermenters and were ready to be consumed. On the other hand, stored *gundruk* and *sinki* were those which had been dried and stored in special containers for future use during off season.

Variations in terms of types and nature of microbes could be seen in fresh and stored *gundruk* and *sinki* also between different stored *gundruk* and *sinki* depending upon storage duration.

Taking into consideration, the biochemical compositions of non-fermented fresh mustard leaves and fresh radish underground stems and their fermented products, i.e. *gundruk* and *sinki* respectively, variations were found in terms of quantity and numbers of various biochemicals.

RESULTS AND DISCUSSION

Although *gundruk* is commonly prepared from mustard leaves, it can also be prepared from many other plants including some wild plants. Manandhar (1998) listed nine domestic plants and four wild plants used in the preparation of *gundruk* in Terai and hill regions of Nepal. Different names are assigned to *gundruk* prepared from leaves of different plants. For example *gundruk* made from *Brassica juncea* var. *rugosa* and *Brassica juncea* are commonly called *Rayo gundruk, Gundruk* made up of *Brassica campestries* i.e. *Sarson* is called *Sarson gundruk,* etc. Similarly *sinki* made from *Raphanus sativus* is called *'mula sinki'.* Other roots used in making *sinki* is *B. rapa* of the family Brassicaceae. *Gundruk* and *sinki* are prepared mostly by using traditional Nepali methods and it is expected that this traditional techniques will improve with time. The fermenter types used are very crude and there are no specially designed fermenters in contrast to fermenters of some other fermented products like *Sauerkraut, Kimchis, Tempeh, Oncom etc.* Improvement is needed in this front.

Gundruk and *Sinki* constitute a very important food item for the people of Nepali community living in various places for the following reasons.

* Firstly no chemicals (not even salt) are added during the course of their preparation.
* Secondly, the process is very quick and usually takes a minimum of 15 days to a maximum of 23 days.
* Thirdly the process of making them is very simple.
* Fourthly the preparation can be done in a very cost effective and easy way as the raw materials such as mustard leaves and radish are obtained in plenty in the market during season time. The equipments and fermenters used are also very cheap and also can be readily made at home or obtained from market.

* Lastly, the fermented products are very tasty and palatable due to its pleasant aroma, flavour and sour taste. The vegetables used are also made available through out the year even during off season.

Table 1 : Isolation of microbes from the field collected samples of fresh and stored gundruk for two year 2001-2002 and 2001-2003

Microorganisms	Sample taken in 2002		Sample taken, 2003
	15 days fermented fresh gundruk	2001-2002 1 year stored gundruk	2001-2003 2 years stored gundruk
Aspergillus glaucus	+	+	+
Aspergillus niger	+	+	-
Alternaria brassicae	+	+	+
Aureobasidium pullulans	-	+	-
Cerratosporella deviata	+	+	-
Cladosporium brassicae	+	+	+
Cladosporium oxysporium	+	+	-
Curvularia lunata	+	+	-
Isarea creatacea	+	-	-
Myrothecium roridium	+	+	+
Penicillium lanosum	+	+	+
Spiromyces minutus	+	+	+
Rhodotorula	+	+	+
Trichorsporan asahii (MTCC-6180)**	+	+	+
Bacillus pantothenticus (MTCC-6392)**	+	+	+
Bacillus pumilus (MTCC-6391)**	+	+	+

Table 2 : Isolation of microbes from the field collected samples of fresh and stored sinki for two year 2001-2002 and 2001-2003

Microorganisms	Sample taken in 2002		Sample taken, 2003
	15 days fermented fresh sinki	2001-2002 1 year stored sinki	2001-2003 2 years stored sinki
Aspergillus glaucus	+	+	+
Aspergillus niger	+	+	+
Alternaria brassicae	+	+	+
Aureobasidium pullulans	+		+
Cerratosporella deviata	+		+
Cladosporium brassicae	+	+	+
Cladosporium oxysporium	+	+	+
Curvularia lunata	+	+	+
Penicillium lanosum	+	+	+
Myrothecium roridium	+	+	+
Spiromyces minutus	+	+	+
Rhodotorula	+	+	+
Trichorsporan asahii (MTCC-6180)**	+	+	+
Bacillus pantothenticus (MTCC-6392)**	+	+	+

Variation in the quality of the fermented food products due to different processing technique used in various parts of the world was reported by Oliver and Nunez (1999).

Fermented *gundruk* and *sinki* yielded microbes numbering 12 fungi, 2 yeasts and 2 bacteria.(Table 1 and 2) Of these, dominant fungi were *Cladospo-*

Table 3 : Biochemical composition of field collected mustard leaves and radish and their fermented product (*Gundruk* and *sinki*) in mg/g*

Biochemical parameters	Non-fermented* (fresh mustard leaves)	Fermented* (fresh gundruk)	t-value**	Non-fermented*	Fermented* (fresh radish)	t-value** sinki
Total sugar	183±0.039	4±0.002	18.29**	61±0.125	56±0.014	6.56**
Reducing sugar	18±0.004	48±0.001	0.416	16±0.002	56±0.001	1.87
Starch	198±0.012	20±0.015	3.600	130±0.023	24±0.015	2.75
Protein	11.4±0.080	20.8±0.022	1.330	12.8±0.020	13.4±0.000	6.20**
Amino acid	64±0.006	37.2±0.018	24.26**	22±0.000	0.8±0.015	4.33
Fat	0.08±1.73	0.62±0.024	0.860	0.03±0.000	0.9±0.000	0.63
Phenol	22±0.010	136±0.00	28.580	62±0.145	130±0.006	1.97
Phytosterol	1±0.023	8.02±0.108	6.420	1.60±0.051	1.68±0.00	0.95
Nitrate reductase	6±0.000	10.8±0.018	5.500	1.3±0.006	19±0.001	0.4
Protease	19.6±0.020	193.2±0.018	13.400	143.2±0.006	183.2±0.038	7.9
Invertase	38.8±0.115	102±0.095	7.000	1.68±0.024	99.6±0.031	4.67
Amylase	104±0.0314	139±0.004	4.620	20±0.009	87.6±0.001	0.44
Ascorbic acid	0.042±0.035	0.27±0.100	0.480	0.010±0.05	0.181±0.075	0.56
Moisture content(%)	1.382±0.069	1.02±0.051	24.79	2.502±0.025	0.96±0.047	27.50
pH	5.80	4.08		5.58	3.96	

* Mean of three replications

** t-value-sample test of fresh and fermented gundruk and sinki at 1% significant lavel

rium brassicae, Spiromyces minutus, Aureobasidium pullulans, Myrothecium roridum, Cladosporium oxysporium and few others like *Penicllium lanosum.* Some of the dominant microbes were either natural microflora or introduced during fermentation process and handling or had entered from the wrapping materials. Introduction of microbes from the fig leaves during *hawaijar* fermentations, (a local soybean product) was also reported.

Sundried *gundruk* and *sinki* showed presence of more number of microbes than fresh fermented products. Variation in the number of microbes with respect to its fermentation duration was depicted in the fermentation of *gundruk* and *sinki.* Similar findings were reported by Dahal *et al.,* (2003) in *Masyaurya. Sufu* a local fermented soyabean product was reported to be mould fermented.

Comparative assessment of biochemical content showed that total sugar, starch, amino acid, moisture content and pH were found more in the non-fermented raw meterials whereas other biochemical contents such as reducing sugar, protein, phenol, fat, phytosterol and ascorbic acid were found more in the fermented counterparts (Table 3). Low biochemical content was observed in case of enzymes i.e. amylase, invertase, protease and nitrate-reductase in non-fermented mustard leaves as compared to fermented *gundruk.* Decreased pH in the fermented food might be due to the acid production that inhibited the growth of saprophytic fungi and favoured more acid tolerant homofermentative bacteria such as *Bacillus pantothenticus.* The pH value was also related to the production of acid during fermentation. High ascorbic acid content also contributed to the level of low pH values in fermented food products. *Gundruk* and *sinki* also showed decrease pH values with the increase in production of ascorbic acid. Similar observations were also reported by Devi and Singh (1986) in the fermented bamboo shoots. Oliver and Nunez (1999) reported that the acid produced by bacteria during the fermentation process helped to control the process by inhibiting the action of putrifying organisms and thus avoiding unfavourable changes in *sauerkraut.*

The loss of moisture content in the fermented *gundruk* and *sinki* could be due to the process of osmosis. Report of low moisture content was observed in sundried *masyaura* (Dahal *et al.,* 2003). Increased in protein, phenol, phytosterol. nitrate-reductase, protease, invertase and amylase in fermented *gundruk* and *sinki* might also have been due to the activities of various microorganisms. Increased in protein content and decreased in carbohydrate might also be attributed to the consistent relationship between the per cent loss of carbohydrate and increased in protein content. Similar observation was reported in solid substrate fermentation of potato waste by *Rhizopus oryzae.* The rise in protein content in *masyaura* due to the microbial activities especially by lactic acid bacteria during fermentation was reported by Dahal *et al.* (2003). Similar observation was also reported by Devi and Singh (1986) in fermented bamboo shoots.

The increase in fat in fermented *gundruk* and *sinki* might also be due to the various physiological changes by the fermenting bacteria. Increased in phytosterol content might also be due to the breakdown of complex organic compounds to secondary products by the activity of microorganisms. Similar observation of breaking down of organic raw materials by the activities of microorganisms was also observed by Singh (1988).

From the above results and discussion it is found that the fermented products, *gundruk* and *sinki* besides playing an important role as an off-season products are also quite nutritious for consumption.

ACKNOWLEDGEMENT

The authors thank the Head of Life Sciences Department for providing the necessary facilities for conducting the experiments.

REFERENCES

Dahal, N.; Rao, R. E. and Swamylingappa, B. 2003. Biochemical and nutritional evaluation of *Masyaura* : A legume based traditional savoury of Nepal. *J. Food. Sci. Tecnol.* **40**(1) : 17722.

Devi, S. P. and Singh, H. T. 1986. Studies on the chemical and nutritional changes of bamboo shoots during fermentations. *J.Food Science and Technol.* **23** : 338-339.

Manandhar, P. and Narayan 1998. The preparation of *gundruk* in Nepal : a sustainable rural industry. *IK Monitor article 6(3) IKPM Home page* : 1-3.

Oliver, Guillermo and Nunez M. 1999. Fermented vegetable products. *Academic Press Encyclopedia of Food Microbiology,* Argentina.

Singh, I. 1988. Adaptation and yield of potato under low light intensity *Ind. J. Plant Physiol.* **31** : 114-16.

(Accepted for publication November 08, 2007)