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# ANNOUNCEMENT

## *JOURNAL OF FOOD SCIENCE AND TECHNOLOGY*

In view of the high increases in production costs, we have been forced to increase the subscription rates from Volume 19, 1982.

The new rates are given on inside front cover and are operative from January 1982. Membership rates are not increased. Subscribers are requested to renew their subscriptions for 1982 at the new rates and cooperate.

**Hony. Secretary**

# Improvement of the Traditional Process for Rice Flakes

T. K. Ananthachar, H. V. Narasimha, R. Shankara, M. S. Gopal and H. S. R. Desikachar
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**Traditional methods of making rice flakes (*Beaten rice, Poha, Avalakki*) gave 62-64 per cent yield in four varieties of paddy tested. Causes for the low yield have been traced to non-standardised process conditions and the high breakage occurring in the final stages of flaking. Soaking paddy (variety: 'China' 'CH45)' in water for 18 hr at 70°C, roasting in hot sand at 260°C for 30-35 sec, tempering for 4 min followed by flaking in edge-runner for 40-45 sec were found to be optimal for flaking and resulted in 2% increased yield as compared to traditional method. Making thick flakes in the edge-runner followed by thin flaking in a roller type flaker further increased the yield by 3 per cent.**

Rice flakes (*beaten* or flattened *rice*, *Poha*, *Avalakki*) is an important rice product prepared from paddy in India. It is traditionally prepared from soaked paddy after roasting with hot sand in a 'Bhatti' and immediate flattening either by hand pounding with pestle or in an edge-runner type machine[1-4]. It has been the general experience that the yield of rice flakes from paddy is low—much lower compared to the milling yield of rice from paddy. This is somewhat surprising as rice flakes are essentially a form of flattened parboiled rice and high yields upto 68 per cent as in parboiling could have been expected.

A study was therefore, undertaken to examine the traditional process to find out the causes of low yield and explore the possibilities of improving the process to enhance the yield and quality. The results of such a study are reported in the paper.

## Materials and Methods

Four different commercial varieties of paddy traditionally used for preparing rice flakes were collected from the local market and rice flakes were prepared in a local traditional mill by the conventional methods. The method consisted of soaking paddy in water at 40-45°C overnight (18 hr), draining and roasting in hot sand (220-240°C) over a conventional 'Bhatti' type roasting pan. The flaking was done in an edge-runner type flaking machine to get fine grade flakes. The roasting and flaking were carried out in 2 kg batches as is conventionally practised.

The effects of temperature of soak water and sand used for roasting and length of time needed for flaking with edge-runner were next investigated with 'CH-45' (locally called as China var.) variety paddy which is commonly used for flaking in Karnataka State. Soaked paddy was roasted with equal volumes of sand (1:2 of paddy and sand by weight) and roasting was terminated when a few grains started puffing. The material was sieved to separate the grains from the hot sand. This procedure was followed in the following studies.

*Soaking conditions:* Ten kg lots of paddy were soaked for 18 hr in water having initial temperatures ranging from 27°C (RT) to 80°C. The soaked paddy was roasted in sand, flaked in edge-runner and the per cent yield of head flakes, brokens and other byproducts (fractions) was determined.

*Roasting conditions:* Ten kg lots of paddy were soaked for 18 hr in water at 40°C and roasted with fine sand heated to 220, 240, 260, 280 and 300°C until a few grains started puffing, sieved to separate sand and the time needed for roasting was recorded. The tempering time between roasting and flaking varied between treatments due to moisture variations in the roasted paddy. The minimum tempering time needed for each treatment for easy flaking without undue lumping/breakage was determined. The yield data were also recorded for samples flaked after giving the optimal or minimal tempering time.

*Flaking time:* Ten kg lots of paddy processed by conventional method, were roasted at 220-240°C for 40-45 sec and flaked in an edge-runner for 15-60 sec to obtain different commercial grades of flakes. The yield of head flakes and brokens was determined in each case.

Moisture in soaked paddy and roasted paddy, ether extractive by Soxhlet method and thiamine by thichrome method were determined in rice flakes as per cereal laboratory methods[5]. Brokens were separated using a 8 mesh (BSS) sieve.

TABLE 1. YIELD OF DIFFERENT FRACTIONS DURING FLAKING OF PADDY IN THE TRADITIONAL METHOD

| Variety | Flaked rice (%) | Broken flakes (-8 BSS) (%) | Awns and powdered flakes (%) | Rice powder, husk and bran (%) |
|---|---|---|---|---|
| IR-8 (new) | 62.6 | 5.6 | 1.70 | 29.1 |
| IR-8 (old) | 63.8 | 4.8 | 1.50 | 28.8 |
| T-65 | 63.6 | 4.9 | 1.80 | 29.0 |
| China paddy (CH-45) | 64.0 | 5.0 | 1.92 | 29.4 |
| Long paddy (SR-26-B) | 63.8 | 4.3 | 1.65 | 30.4 |

## Results and Discussion

*Yield in traditional method:* The data presented in Table 1 show that the yield of flakes from the four varieties of paddy varied from 62.6 to 64 per cent, with the brokens amounting to about 5 per cent. It was found that a small percentage (1.5-2 per cent) of powder and very small brokens got mixed up with awns of the paddy and could not be separated from it. Fine husk (husk powder) and coarse husk fraction comprised 27-29 per cent. This also contained rice powder. These results indicate that the low yield of rice flakes in these studies is due to heavy loss, as powder which gets mixed up with husk fractions; this phenomenon is common to all the varieties studied.

*Variation of temperature of soak water/moisture content of soaked paddy:* Higher the initial temperature of soak water, greater was the moisture imbibed by the paddy during 18 hr soaking which varied from 33 to 58 per cent (Table 2). When soaked paddy was processed by the conventional method, it was found that low temperature of soaking resulting in low moisture content of soaked paddy caused higher breakage and lower yield of flakes. In contrast, high temperature soaking (as at 80°C and boiling water) reduced the breakage and gave higher yield of flakes, but the product became excessively lumpy and disfigured during flaking causing operational difficulties. Optimal condition for easy operation and to get minimum brokens was soaking paddy at an initial temperature of 70° C which gave 64.6 per cent yield and 3 per cent brokens.

*Variation of roasting temperatures:* Roasting of paddy at different temperatures showed that the roasting time (as judged by the initiation of puffing of a few grains) decreased with increase in temperature of roasting, as a result of which the moisture content in the paddy soon after roasting increased and it was in the range of 18.5 to 23.5 per cent depending upon the roasting temperature. High moisture in roasted paddy caused problems of lumping during flaking in the edge-runner and needed longer tempering time between roasting and flaking. Data presented in Table 3 show that although higher roasting temperature (280-300°C) reduced the brokens and increased the yield of whole flakes, lumping

TABLE 2. RELATIONSHIP BETWEEN SOAKING CONDITIONS, YIELD AND BREAKAGE OF RICE FLAKES

| Soaking temp. (°C) | Moisture content* | | Yield of flakes (%) | Brokens (-8 BSS) (%) | Observations on flakes |
|---|---|---|---|---|---|
| | Soaked paddy (%) | Roasted paddy (%) | | | |
| 27 | 33.0 | 15.8 | 56.2 | 10.5 | Opaque/bright flakes may be due to white belly kernels |
| 40 | 36.5 | 16.6 | 60.0 | 8.5 | Fissured edges, more breakage |
| 50 | 40.0 | 19.0 | 61.2 | 6.3 | - do - |
| 60 | 41.8 | 20.8 | 63.5 | 4.2 | Some grains had fissured edges |
| 70 | 42.5 | 21.5 | 64.6 | 3.0 | Normal flakes |
| 80 | 55.0 | 24.2 | 65.0 | 2.8 | Lumps, some kernels not fully flaked and had embedded husk |
| Boiling | 58.5 | 26.6 | 65.3 | 2.6 | Highly lumpy, some grains not fully flaked. Admixed with lot of husk in the lumps. |

All samples were soaked overnight (about 18 hrs) at the initial temperature of water indicated in the column without maintaining the soaking temperature as in the traditional procedure.

Roasting conditions: 260°C for 35-40 sec.

*Per cent dry basis.

TABLE 3. ROASTING TEMPERATURE AND YIELD OF RICE FLAKES

| Roasting temp. (°C) | Roasting time (sec) | Tempering* time (min) | Moisture in roasted paddy (% d.b.) | Yield of head flakes (%) | Brokens (%) | Characteristics of flakes |
|---|---|---|---|---|---|---|
| 200 | 45 | 3 | 18.5 | 60.0 | 5.8 | Highly fi sured |
| 240 | 40 | 3 | 19.4 | 61.2 | 3.9 | - do - |
| 260 | 35 | 4 | 20.8 | 63.9 | 3.2 | Slightly fissured edges |
| 280 | 25 | 5 | 22.5 | 64.5 | 2.5 | Lumpy |
| 300 | 20 | 10 | 23.5 | 65.1 | 2.1 | Highly lumpy |

*Minimum time needed between roasting and flaking for minimal breakage/lumping.

during flaking even after 10 min tempering precluded the use of high temperatures. Maximal yield (63.9 per cent) of flakes without lumping was obtained at a roasting temperature of 260°C with about 3 per cent brokens.

*Thickness of flakes in relation to yield:* It is interesting to note from Table 4 that the flake thickness has profound influence on the yield of flakes. With increased time of flaking, the flakes became thinner and thinner and had a whitish opaque appearance. The yield of flakes suddenly decreased after 30 sec of flaking. It fell from 70 to 65 per cent between 30 and 40 sec of flaking and finally declined to 60 per cent at 60 sec of flaking. This demonstrated that low yield obtained in flaking is caused mainly at the final stages of flaking during conversion from medium to fine and superfine grades. Increasing flaking time and producing improved commercial grades of flakes reduced the content of thiamine and ether extractives in the flakes which was mainly due to the loss of bran/germ during the flaking process. Inspite of that the thinner flakes had $19\mu/g$ of thiamine which is comparable to milled parboiled rice.

It is also interesting to note that when coarse flakes obtained after flaking for 25-30 sec in the edge-runner were subjected to further flaking in a roller type flaking machine, which is commonly used for making corn flakes, the yield remained constant at 70 per cent, thereby confirming that the heavy breakage and powdering is taking place in the final stages of flaking in the edge-runner and this is responsible for the reduction in yield. When brown rice obtained from roasted paddy was flaked in the edge-runner, yield of flakes was not improved because of high breakage. The probable cause for high breakage with prolonged flaking is due to the drying of the material and also lowered plasticity caused by cooling. This is overcome in a roller flaker where the flaking is done instantaneously.

The present results show that if flaking is stopped at medium grade stage, high yields of 70 per cent flakes could be obtained even in the edge-runner. Further flaking to very fine or superfine grade could be done in a roller flaker without reduction in commercial yield.

TABLE 4. YIELD OF RICE FLAKES IN RELATION TO FLAKING TIME AND FLAKE GRADE

| Flaking time (sec) | Flake thickness (cm) | Commercial grade | Yield of flakes (%) | Total brokens (-8 BSS) (%) | Husk and bran (%) | Ether extractives (%) | Thiamine (mg %) |
|---|---|---|---|---|---|---|---|
| 15 | 0.18 | Coarse | 72 | 1.0 | 26.0 | 1.86 | 0.23 |
| 25 | 0.12 | Coarse | 70 | 2.6 | 27.5 | 1.45 | 0.21 |
| 30 | 0·10 | Medium | 70 | 3.5 | 28.0 | N D | N D |
| 40 | 0.08 | Fine | 65 | 4.5 | 30.0 | 1.22 | 0.21 |
| 60 | 0.06 | Superfine | 60 | 6.0 | 34.1 | 1.05 | 0.19 |
| 25-30* | 0.055 | Superfine | 70 | 3.1 | 27.0 | 1.20 | 0.20 |

N D - Not determined
*Flaking done in edge-runner followed by roller flaking

TABLE 5. YIELD OF RICE FLAKES UNDER OPTIMAL SOAKING AND ROASTING CONDITIONS

| Method | Whole flakes (%) | Broken flakes (%) | Awns and small brokens (%) | Rice powder, husk and bran (%) |
|---|---|---|---|---|
| Commercial (Long paddy) (SR-26B) | 63.3 | 4.5 | 1.92 | 29.70 |
| Standardised (commercial) | 65.6 | 3.9 | 1.70 | 28.00 |
| Commercial (China Var.) (CH 45) | 64.0 | 5.0 | 1.90 | 28.10 |
| Standardised (commercial) | 66.0 | 4.0 | 1.50 | 27.80 |

All samples were flaked for 40-45 sec in the edge-runner.

*Yields under optimised process conditions:* Studies reported above indicate that soaking of paddy for 18 hr at an initial temperature of 70°C, roasting in sand at 260°C, tempering for 4-5 min and flaking for 40-45 sec are the optimum operating conditions with the edge-runner type flaking machines. When the seoptimal conditions were employed for 2 varieties of paddy, yield of flakes was about 2 per cent higher than that obtained with the traditional (commercial) process (Table 5). Still the yields are much lower than the theoretical yields. The losses were caused by heavy breakage and powdering while making fine and superfine grades. This heavy loss, however, could be completely eliminated if roller type of flaking machine is used for final thinning of the medium grade flakes. Yields upto 70 per cent could be realised when the optimal conditions for processing are combined with instantaneous flaking machine like the roller flaker.

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# Extractives of Medicinal Plants as Pulse Protectants Against *Callosobruchus chinensis* L. Infestation


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**Petroleum ether extractives (PEE) of nine plant species—*Carum roxburghianum* Benth. & Hook, *Saussurea lappa* Clarke, *Embelia ribes* Burm., *Withania somnifera* Dunal, *Psoralea corylifolia* Linn., *Clerodendron inerme* (Linn.) Gaertn., *Cestrum nocturnum* Linn., *Caesalpinia bonduc* (Linn.) Roxb. and rice bran oil have been tested against *Callosobruchus chinensis* Linn. infesting green gram, *Phaseolus mungo* Linn. PEE of *C. roxburghianum* at 0.1 per cent level, rice bran oil at 0.2 and 0.5 per cent levels and *P. corylifolia* at 0.5 per cent level, gave almost complete protection of the pulse up to 135 days, while *S. lappa* and *W. somnifera* at 0.1 per cent and *E. ribes* at 0.5 per cent protected up to 90 days. However, *C. bonduc*, *C. nocturnum* and *C. inerme* comparatively were less effective against the bruchid attack. The germination test showed that the treatments did not have any adverse effect on seed viability.**


Mixing of synthetic organic insecticides like DDT and malathion to check grain damage in stored seed was practised in the past decades, but due to persistent residues of such insecticides, the emphasis was shifted to mixing non-toxic grain protectants like inert dusts, talc and lime[1]. As early as 1920, Fletcher and Ghosh[2] have reported the use of aromatic plant powders as antifeedants and repellents against pests of stored grains. Treatment of grains with vegetable oils had also been practiced as effective protectants against pulse beetles[3–5]. Similarly, plant materials either as powders or extracts have also been reported to possess repellent properties[6,7]. In the present studies, the extractives of nine indigenous medicinal plants[8] were screened for their effectiveness

as protectants against the pulse beetle, *Callosobruchus chinensis* Linn. infesting green gram, *Phaseolus mungo* Linn.

## Materials and Methods

Petroleum ether extractives of nine plant materials, seeds of *Carum roxburghianum* Benth. & Hook, *Embelia ribes* Burm. and Babchi (*Psoralea corylifolia* Linn.); leaves of garden quinine (*Clerodendron inerme* Linn. Gaertn) and night jessmine (*Cestrum nocturnum* Linn. root and rhizome of kut root (*Saussurea lappa* Clarke) and winter cherry (*Withania somnifera* Dunal); fruits of bonduc nut (*Caesalpinia bonduc* Linn. Roxb.) and rice bran oil were tested.

The plant materials were procured locally, dried in a hot air drier and powdered to 60 mesh in Raymond's Hammer mill. The powders were extracted thoroughly in petroleum ether (b.p. 60-80°C) by cold extraction and excess of solvent was evaporated in a vacuum flash evaporator at 40°C. Uninfested green gram samples were treated with 0.1, 0.2 and 0.5 parts of extract per 100 parts of green gram (v/w). For homogenous coating of grains, the required quantity of extract was dissolved in petroleum ether and solvent was evaporated by constant rotating of the jars (120 × 50 mm). The treated samples were kept in glass jars covered with muslin cloth at 27 ± 2°C and 70-75 per cent RH. Three replicates with 50 g. of treated green gram were maintained for each treatment along with an equal number of untreated controls.

Initially, ten pairs of 1-day old adult insects were released in all the treatments including controls. After 45 and 90 days, fresh batches of ten pairs of insects were introduced at all concentration levels of *C. roxburghianum*, rice bran oil, *S. lappa*, *W. somnifera* and 0.5 per cent level of *E. ribes* and *P. corylifolia*. However, after 135 days a third batch of ten pairs of insects were released in all the concentrations of *C. roxburghianum* and 0.5 and 0.2 per cent concentrations of rice bran oil. Observations on the number of damaged grains were recorded at intervals of 45, 90, 135 and 180 days. At the end of the experiment, germination test was carried out according to International testing methods.[9]

The efficacy of the extractives as protectants against the bruchid attack was judged by calculating the per cent damage. The per cent damage in each treatment was recorded by counting the number of visibly damaged grains in 50 g sample of each concentration. The data on the final observation were statistically analysed by analysis of variance. As there was wide variation in damage at different levels of treatment, the original values were subjected to angular transformations before carrying out the statistical analysis.

## Results and Discussion

The data presented in Table 1 indicate that *C. roxburghianum* at 0.1, 0.2 and 0.5 per cent; rice bran oil at

TABLE 1. COMPARATIVE EFFICACY OF VARIOUS PLANT EXTRACTIVES AGAINST *C. CHINENSIS* L. ATTACKING *PHASEOLUS MUNGO*

| Protectant | Concn. % (v/w) | *Average % damage after indicated days | | | |
|---|---|---|---|---|---|
| | | 45 | 90 | 135 | 180 |
| *Carum roxburghianum* | 0.0 | 17.00 | 70.33 | 87.67 | 100.00 |
| | 0.1 | 0.00 | 0.00 | 0.00 | 7.00 |
| | 0.2 | 0.00 | 0.00 | 0.00 | 4.67 |
| | 0.5 | 0.00 | 0.00 | 0.00 | 2.83 |
| Rice bran Oil | 0.0 | 17.00 | 70.33 | 87.67 | 100.00 |
| | 0.1 | 0.00 | 5.67 | 59.00 | 87.33 |
| | 0.2 | 0.00 | 0.33 | 1.67 | 35.67 |
| | 0.5 | 0.00 | 0.00 | 0.00 | 4.17 |
| *Psoralea corylifolia* | 0.0 | 22.33 | 50.67 | 100.00 | — |
| | 0.1 | 0.73 | 38.67 | 79.00 | |
| | 0.2 | 0.00 | 17.67 | 65.66 | |
| | 0.5 | 0.00 | 0.00 | 1.16 | |
| *Saussurea lappa* | 0.0 | 25.00 | 71.67 | 82.33 | 99.00 |
| | 0.1 | 0.00 | 0.00 | 16.83 | 74.00 |
| | 0.2 | 0.00 | 0.00 | 12.83 | 64.00 |
| | 0.5 | 0.00 | 0.00 | 8.50 | 49.67 |
| *Embelia ribes* | 0.0 | 17.00 | 70.33 | 87.67 | 100.00 |
| | 0.1 | 0.00 | 11.67 | 71.67 | 100.00 |
| | 0.2 | 0.00 | 6.67 | 58.33 | 93.33 |
| | 0.5 | 0.00 | 1.33 | 10.00 | 51.33 |
| *Withania somnifera* | 0.0 | 24.33 | 62.33 | 85.67 | 100 00 |
| | 0.1 | 0.50 | 0.90 | 31.00 | 80.33 |
| | 0.2 | 0.50 | 0.50 | 27.67 | 68.33 |
| | 0.5 | 0.10 | 0.10 | 21.00 | 49.00 |
| *Cestrum nocturnum* | 0.0 | 26.35 | 80.67 | 100.00 | — |
| | 0.1 | 20.67 | 68.67 | 83.00 | |
| | 0.2 | 11.00 | 31.67 | 61.67 | |
| | 0.5 | 2.33 | 10.50 | 43.00 | |
| *Caesalpinia bonduc* | 0.0 | 24.33 | 63.00 | 100.00 | — |
| | 0.1 | 8.67 | 56.33 | 81.66 | |
| | 0.2 | 1.50 | 42.50 | 70.00 | |
| | 0.5 | 0.00 | 36.67 | 61.00 | |
| *Clerodendron inerme* | 0.0 | 27.00 | 76.35 | 99.00 | — |
| | 0.1 | 0.50 | 69.00 | 96.00 | |
| | 0.2 | 0.40 | 57.33 | 88.33 | |
| | 0.5 | 0.40 | 48.67 | 73.33 | |

*The averages of three replicates.

0.2 and 0.5 per cent and *P. corylifolia* at 0.5 per cent gave complete protection from *C. chinensis* upto 135 days. Similarly, *E. ribes* at 0.2 and 0.5 per cent, *S. lappa* and *W. somnifera* at 0.1, 0.2 and 0.5 per cent levels protected the grain up to 90 days. However, *C. inerme* and *C. nocturnum* at 0.5 per cent and *C. bonduc* at 0.2 and 0.5 per cent levels gave good protection upto 45 days only and the damage increased progressively beyond this period. The increase in damage with progress of time indicates the decrease in potency of such extractives with time. The difference in per cent damage among various control treatments could be due to slight difference in egg laying capacity of females. F-test showed high significant differences between control and treatments (Table 2). It is observed that per cent damage of the grains was inversely proportional to concentration of the protectant mixed with grains.

It is confirmed from the germination test that treatment of grains did not affect the viability of grains to any significant extent. The average per cent germination recorded for green gram treated with various extractives was *C. roxburghianum* 99; rice bran oil, 94; *P. corylifolia*, 91; *S. lappa*, 93; *C. bonduc*, 93: *E. ribes*, 92; *W. somnifera*, 94; *C. nocturnum*, 99; *C. inerme*, 95 and control, 100.

Thus, from the results indicated in Table 1, it may be concluded that *C. roxburghianum* even at the lowest concentration level and rice bran oil or *P. corylifolia* at higher concentrations proved effective in controlling the bruchid attack up to 4-5 months. However, slight damage due to insects occurred in all the concentrations of *C. roxburghianum* and higher concentrations of rice bran oil after 5 months due to loss in potency of the extractives. Extractives of *E. ribes* at higher and *S. lappa* or *W. somnifera* at lower concentrations provided protection upto 3 months. It was found that in all the extractives, egg laying was either nil or insignificant for a specified period which subsequently reduced the reproductive capacity of the females thereby reducing the grain damage. This phenomenon may be attributed to the oviposition inhibitory action or ovicidal nature of such extractives. Saramma and Varma[10] have reported that the powdered roots of *S. lappa* at 2 per cent level checked the damage of wheat from *Trogoderma granarium* effectively for 2-3 months. Mammigatti and Raghunathan[3] have observed that coating of green gram with castor, gingely and mustard oils at 0.3 per cent level and coconut or groundnut oils at 0.5 per cent level inhibited the multiplication of *C. chinensis* for 45 days. Similarly use of some vegetable oils at 5 ml/kg of pulse against *Zabrotes subfasciatus*[4] and groundnut oil against *C. maculatus*[5] provided high level of control. The extractives and oily fractions tested can safely be used in storing pulses for prolonged period without affecting the seed quality and viability.

TABLE 2. EFFECT OF PLANT EXTRACTIVES AS PROTECTANTS OF *PHASEOLUS MUNGO* AGAINST *C. CHINENSIS* L. AT THE END OF THE EXPERIMENT

| Protectant | *Average (%) damage at indicated concn. | | | | | |
|---|---|---|---|---|---|---|
| | 0.0 | 0.1 | 0.2 | 0.5 | S.Em ± | CD at 5% |
| *C. roxburghianum* | 90.00 | 15.83 | 12.4 | 9.69 | 0.37 | 1.20 |
| Rice bran oil | 90.00 | 69.21 | 36.67 | 1[illegible]5 | 0.78 | 2.54 |
| *P. corylifolia* | 90.00 | 62.78 | 54.15 | 5.06 | 1.63 | 5.31 |
| *S. lappa* | 85.38 | 59.39 | 53.13 | 44.80 | 1.73 | 5.64 |
| *E. ribes* | 69.50 | 57.84 | 49.81 | 17.90 | 1.81 | 5.90 |
| *C. nocturnum* | 90.00 | 65.71 | 5[illegible].75 | 40.94 | 0.97 | 3.16 |
| *W. somnifera* | 90.00 | 63.69 | 55.76 | 44.73 | 0.70 | 2.28 |
| *C. bonduc* | 90.00 | 64.66 | 56.80 | 51.36 | 0.99 | 3.23 |
| *C. inerme* | 85.38 | 78.68 | 68.09 | 59.01 | 3.09 | 10.08 |

*Values obtained by angular transformation.

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# Staphylococcal Incidence in Market *Khoa* and Their Enterotoxins Production

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**The average staphylococcal counts of 100 market *Khoa* (a heat concentrated Indian milk product) samples collected from cities of Bangalore and Mysore were $400\times10^6$ and $360\times10^6$ colony forming units (CFU)/g respectively. Preformed, thermostable deoxyribonuclease (TDNase) was detected in 24 of these samples. The isolates were characterised for pigmentation, mannitol fermentation and production of acetoin, ammonia, phosphatase, coagulase and TDNase and nitrate reduction. Among the 300 isolates, 125 were identified as *Staphylococcus aureus*. Only 64 isolates produced staphylococcal enterotoxins (SE)—SEA, SEB, SEC, SED and SEE, either singly or in combinations. All the enterotoxigenic isolates were strains of *S. aureus*.**

Among the several indigenous milk products of India, *Khoa* is most popular especially in Northern and Western parts. This forms the base of several sweetmeats such as, *Gulab jamoon*, *Pedha*, *Burfi*, etc. *Khoa* is mainly produced in the rural areas, often under unhygienic conditions. During handling, storage and transportation of *Khoa* to consuming centres, there is ample scope for microbial contamination and their growth. One of the common contaminants is *Staphylococcus* spp., of which *Staphylococcus aureus* is important. Several strains of *S. aureus* are known to elaborate thermostable enterotoxins during their growth. Five antigenically different staphylococcal enterotoxins viz., A, B, C, D and E (SEA, SEB, SEC, SED and SEE) have been identified, with the sixth type 'F' being not fully established[1].

Information on the incidence of *Staphylococci* and their enterotoxins in milk, cheese, icecream and dried milk, have been reported[2–5]. Information on the incidence of *Staphylococci* in market *Khoa* samples collected in North India are available[6–8], but no information is available on the enterotoxins produced by them.

The present study was initiated to determine the extent of incidence of *Staphylococci* in *Khoa* marketed in the urban areas of Bangalore and Mysore. Further, the Staphylococcal isolates were screened for enterotoxins production and also to find out the relationship with some important characteristics such as, production of thermostable deoxyribonuclease (TDNase) and coagulase.

## Materials and Methods

*Khoa samples:* One hundred samples of market *Khoa* collected from in and around cities of Bangalore (60 samples) and Mysore (40 samples) were enumerated for Staphylococcal and total bacterial counts as per the procedure given for hard and processed cheese by Indian Standard Institution[9]. The samples were collected in sterilised bottles and brought to the Laboratory in a small ice-box within 30 min in Bangalore city and within 3 to 4 hr in Mysore city and subjected to analysis.

*Staphylococci and total bacterial counts:* Staphylococcal counts were carried out by surface plating of the dilutions in 2 per cent citrate buffer on egg yolk tellurite glycine pyruvate agar (ETGPA) of Baird-Parker[10]. Triplicate plates were used for each dilution and incubated for 24 to 48 hr at 37°C and all the colonies were counted as Staphylococcal and the average of agreeing plates were expressed as colony forming units per gram (CFU/g).

Total bacterial counts were done by pour plate method using tryptone yeast extract dextrose (TYD) agar. Colony counts were expressed as total bacterial CFU/g.

*Presence of TDNase in market samples of Khoa:* A $10^{-1}$ dilution of the samples in 2 per cent citrate buffer were centrifuged at 10,000 rpm for 10 min and the supernates were steamed for 10 min, cooled and screened for TDNase by toluidene blue deoxyribonucleic acid agar (TB-DNA) plate method of Lachica *et al.*[11].

*Isolation of Staphylococci:* Colonies appearing on ETGPA plates were isolated at random with a preference to those colonies that had cleared the surrounding egg yolk.

*Characteristics of Staphylococci:* Isolates obtained from *Khoa* samples were tested for production of catalase, acetoin, ammonia and phosphatase and nitrate reduction as described by Baird-Parker[12], while aerobic and anaero-

bic fermentation of sugars was tested by a slight modification of the method of Ghosh[4]. The sugars tested included glucose, lactose, maltose and mannitol.

*Coagulase production:* This was performed on the lines of Sperber and Tatini[13] using 24 hr old Difco brain heart infusion (BHI) broth cultures and Difco bacto-coagulase plasma-ethylene diamine tetra acetic acid.

*TDNase production:* Steamed BHI supernates of 24 hr old cultures were screened for TDNase as per the method of Lachica *et al.*[11].

*Staphylococci cultures:* The test cultures included were (*i*) wild Staphylococcal isolates obtained from market *Khoa* samples, and (*ii*) standard strains of *S. aureus* viz., $A_{100}$, $B_{5-6}$, $C_{137}$, $D_{472}$ and $E_{326}$ producing their respective enterotoxins—SEA, SEB, SEC, SED and SEE, obtained with courtesy from Dr. M. S. Bergdoll Food Research Institute, Wisconsin.

*Reference enterotoxins and their corresponding antisera:* Preliminary titration of the 5 enterotoxins and their respective antisera were carried out by the single gel-diffusion tube (SGDT) technique of Oudin as described by Ghosh[4] and optimal sensitivity plate (OSP) method of Robbins *et al.*[14] to find out the appropriate dilutions of sera necessary to detect minimum concentration of respective toxins.

*Screening of cultures for enterotoxin(s):* Enterotoxins were extracted from Staphylococcal cultures by growing them in BHI broth and agar using modified cellophane-over-method of Jarvis and Lawrence[15]. The supernates obtained by centrifuging the harvested cells were screened for the detectable level of the 5 enterotoxins individually by the above two serological methods.

**Results and Discussion**

Staphylococcal incidence: *Staphylococci* and total bacterial counts in market *Khoa* samples are shown in Table 1. It may be seen that there was considerable variation in the Staphylococcal counts of individual *Khoa* samples collected from Bangalore and Mysore cities, which ranged from $6\times10^6$ to $9000\times10^6$ CFU/g. The average counts for Bangalore and Mysore were $400\times10^6$ ($\pm$78.0) CFU/g and $360\times10^6$ ($\pm$60.9) CFU/g respectively.

These counts were higher than those reported by Ghodekar *et al.*[6] and Sharma *et al.*[8] Most of the samples would be considered 'poor,' as per the grading system of Ghodekar *et al.*[6].

It is also evident that the total bacterial counts in *Khoa* samples ranged between $2\times10^6$ and $310\times10^6$ CFU/g and averaged $95\times10^6$ ($\pm$5.2) CFU/g and $90\times10^6$ ($\pm$4.1) CFU/g for the Bangalore and Mysore city samples respectively. These counts are higher than that reported earlier for merket *Khoa* samples[6,16]. Atmaram Naidu and Ranganathan[17] obtained a range value of $1.3\times10^3$ to $1.5\times10^6$/g for fresh market *Khoa*.

Ghodekar *et al.*[6] have enunciated the reasons for the wide variations in the counts. The high Staphylococcal and total bacterial counts in the present investigation could be attributed to the post production contamination and growth before marketing. Most of the lots were produced in rural areas and transported without refrigeration. Considerable time elapsed between the preparation of *Khoa* and its disposal to consumers which resulted in increase in the microbial growth.

It may also be noted that Staphylococcal counts of *Khoa* were higher than the total bacterial counts. This difference can be attributed to the differences in the isolation medium used. Since TYD agar is an all purpose medium, while ETGPA used for Staphylococcal enumeration is a selective medium for *Staphylococci* and contained additional nutrients. Further, the competition among the different microorganisms in *Khoa* might have retarded the growth of *Staphylococci* on TYD agar. The data in Table 1 also reveal the presence of TDNase in 24 out of 100 *Khoa* samples collected from the two cities. The presence of TDNase in *Khoa* samples is indicative of the Staphylococcal growth. According to Tatini *et al.*[18] a minimum cell population of $5\times10^5$ to $1\times10^6$ per g/ml is necessary to detect TDNase. The zone diameter of 10-12 mm observed in this investigation is a clear indication for the growth of *Staphylococci*.

*Characteristics:* From 100 samples of *Khoa*, 300 real isolates were identified and their important characteristics are given in Table 2. All the 300 isolates produced catalase and acid from glucose anaerobically,

TABLE 1. INCIDENCE OF *STAPHYLOCOCCI* AND PREFORMED TDNASE IN MARKET SAMPLES OF KHOA

| Collection place (city) | No. of samples analysed | *Staphylococci* (× 10⁶CFU/g) | | | Total bacterial count (× 10⁶CFU/g) | | | TDNase positive samples | |
|---|---|---|---|---|---|---|---|---|---|
| | | Min | Max | Average | Min | Max | Average | (No.) | zone diameter* (mm) |
| Bangalore | 60 | 6 | 9000 | 400(±78.0) | 3 | 310 | 95(±5.2) | 14 | 10-12 |
| Mysore | 40 | 9 | 8500 | 360(±60.9) | 2 | 220 | 90(±4.1) | 10 | 10-12 |

*Initial diameter of well on TB-DNA agar plates = 5 mm.

CFU = Colony forming units.

TABLE 2. CHARACTERISTICS OF STAPHYLOCOCCAL ISOLATES

| Characters | | Positive Isolates (No.) | (%) |
|---|---|---|---|
| Pigmentation (golden yellow) | | 160 | 53.3 |
| (white) | | 140 | 46.7 |
| Mannitol fermentation | + | 241 | 80.3 |
| Aerobic and anaerobic | 0 | 19 | 6.3 |
| aerobic | + | 28 | 9.4 |
| Anaerobic | + | 12 | 4.0 |
| Acetoin production | + | 220 | 73.3 |
| | 0 | 80 | 26.7 |
| Ammonia production | + | 240 | 80.0 |
| | 0 | 60 | 20.0 |
| Phosphatase production | + | 262 | 87.3 |
| | 0 | 38 | 12.7 |
| Nitrate reduction | + | 280 | 93.3 |
| | 0 | 20 | 6.7 |
| Coagulase production | $+^4$ | 82 | 27.3 |
| | $+^3$ | 43 | 14.3 |
| | $+^2$ | 08 | 2.7 |
| | 0 | 167 | 55.7 |
| TDNase production | + | 193 | 64.3 |
| | 0 | 107 | 35.7 |

+ = positive; 0 = negative $+^4$, $+^3$ and $+^2$ – degrees of coagulation.

TABLE 3. DISTRIBUTION OF ENTEROTOXIN(S) AMONG STAPHYLOCOCCAL ISOLATES

| Enterotoxin(s) | Positive Isolates (No.) | (%) |
|---|---|---|
| A | 8 | 12.5 |
| B | 27 | 42.1 |
| C | 9 | 14.0 |
| D | 2 | 3.1 |
| AB | 5 | 7.8 |
| AC | 2 | 3.1 |
| AE | 2 | 3.1 |
| ABD | 1 | 1.5 |
| ABDE | 2 | 3.1 |
| ABCD | 1 | 1.5 |
| ABCDE | 1 | 1.5 |
| BC | 2 | 3.1 |
| BE | 1 | 1.5 |
| CE | 1 | 1.5 |
| Total | 64 | 100.0 |

Standard strains produced their respective enterotoxins.

Enterotoxin(s) detected by both the serological techniques were same.

confirming them as *Staphylococci*. The golden yellow (53.3 per cent) and white (46.7 per cent) pigmented colonies were observed almost in equal numbers. Mannitol was fermented both aerobically and anaerobically by 80.3 per cent of the isolates. Acetoin, ammonia, phosphatase, coagulase and TDNase were produced by 73.3 per cent, 80.0 per cent, 87.3 per cent, 44.3 per cent and 64.3 per cent of the isolates respectively, while nitrate was reduced by 93.3 per cent of the isolates. Among the coagulase positive *Staphylococci*, 27.3 per cent, 14.3 per cent and 2.7 per cent of these produced $+^4$, $+^3$ and $+^2$ degrees of coagulation respectively. The present finding was similar to that reported by Ghosh[4] in regard to pigmentation. Batish *et al.*[19] found 29 (12.3 per cent) out of 236 Staphylococcal isolates obtained from various samples of milk and milk products were positive for TDNase.

The incidence of coagulase positive and TDNase positive *Staphylococci* in *Khoa* were 24.0 per cent and 37.0 per cent respectively. Samples possessing coagulase positive *Staphylococci*, also included TDNase positive cultures. Considerable variations in the incidences of coagulase and TDNase positive *Staphylococci* have been recorded among the various milk and milk products[20,21].

On the basis of their ability to produce TDNase, coagulase and phosphatase and ferment mannitol, 125 out of 300 Staphylococcal isolates were classified as *S. aureus* and considered to be potential producers of enterotoxins.

The different types of enterotoxins produced by Staphylococcal isolates are shown in Table 3. Enterotoxins produced by the isolates were same when detected by SGDT technique and OSP method. While, all the standard strains of *S. aureus* produced their respective enterotoxins 64 out of the 300 wild isolates were enterotoxigenic producing enterotoxins either singly or in combinations. Among the 46 isolates producing single enterotoxins, SEB was produced by 27, SEC by 9, SEA by 8 and SEE by 2 but SED was not produced singly by any of the isolates. Almost all the combinations contained either SEA or SEB or both.

Most of the earlier studies[19,22–25] have shown the predominance of SEA producing strains among *Staphy-*

*lococci* isolated from milk and milk products. Donnelly *et al.*[26] did not find SEB producing strains among coagulase positive *Staphylococci* isolated from cheese which contained only SEA producing strains. Variations among the type of enterotoxins produced by *S. aureus* strains depend mostly upon the nature of strains and the substrate/medium. Since *Khoa* is a heat concentrated milk product, the presence of enterotoxigenic *Staphylocicci* cultures may be due to contamination during handling, transportation and marketing of *Khoa*.

Although Payne and Wood[27] reported high incidence of SEA producing strains, many of them produced multiple enterotoxins containing SEA or SEB or both, as in the present investigation. In the present investigation, 22 strains out of 64 produced SEA either singly or in combination with other toxins SEB, SEC, SED and SEE.

Earlier studies[22,23] have shown that, SEA is the most potent in causing food poisoning followed by SED, SEC and SEB.

Considering that the Staphylococcal isolates were obtained from 100 samples, the incidence of enterotoxigenic *Staphylococci* in market *Khoa* works out to 10.0 per cent. The incidence in other dairy products reported by earlier workers[4,19-28] varied from 7.6 to 30.0 per cent.

All the enterotoxigenic Staphylococcal cultures were found to be strains of *S. aureus*, they fermented mannitol and produced acetoin, phosphatase, coagulase and TD-Nase. Out of 64 enterotoxigenic cultures, 33 were golden yellow pigmented and the rest produced white colonies.

The different degrees of coagulation of plasma ($+^4$, $+^3$, $+^2$) produced by the enterotoxigenic strains of *S. aureus* are shown in Table 4. No correlation was observed between the degree of coagulase reaction and the type of toxins produced. Five out of 64 strains produced weak reaction ($+^2$), but still they elaborated the toxins.

A definite relationship could not be drawn between the type of enterotoxins produced by *S. aureus* strains and the type of pigmentation (golden yellow/white) formed. However, 3 out of 9 strains producing SEC singly and also few out of 7 strains producing SEC in combination with other toxins were white pigmented.

It is evident from this study, that the strains of *S. aureus* present in market *Khoa* can produce all the 5 types of enterotoxins either singly or in combinations and thus be a source of food poisoning.

TABLE 4. DEGREES OF COAGULATION OF PLASMA BY ENTEROTOXIGENIC STRAINS OF *S. AUREUS*

| Enterotoxin(s) | No. of strains positive for degrees of coagulation | | | Total |
|---|---|---|---|---|
| | $+^4$ | $+^3$ | $+^2$ | |
| A | 4 | 4 | Nil | 8 |
| B | 16 | 10 | 1 | 27 |
| C | 2 | 6 | 1 | 9 |
| E | 1 | 1 | Nil | 2 |
| AB | 4 | Nil | 1 | 5 |
| AC | 2 | ,, | Nil | 2 |
| AE | 1 | 1 | ,, | 2 |
| ABD | Nil | 1 | ,, | 1 |
| ABDE | ,, | Nil | 2 | 2 |
| ABCD | 1 | ,, | Nil | 1 |
| ABCDE | 1 | ,, | ,, | 1 |
| BC | 2 | ,, | ,, | 2 |
| BE | 1 | ,, | ,, | 1 |
| CE | Nil | 1 | ,, | 1 |

Standard strains (5) were positive for $+^4$ coagulase reaction.

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# Influence of Some Growth Factors on the Production of Mushroom Mycelium in Submerged Culture

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**The effect of vitamins, hormones and fatty acids on the submerged mycelial yields of some mushrooms grown in synthetic media were investigated. None of the water soluble and fat soluble vitamins showed any significant growth-promoting activities for these mushrooms. Among the plant-growth hormones tested, kinetin is growth stimulatory for *T. clypeatus*, and *C. lagopus*, while indole-3-acetic acid is for *V. volvacea*, but gibberellic acid has no such activity. Caprylic, capric, palmitic and stearic acids present in the medium affect growth. Caprylic and capric acids acted as growth inhibitors at high concentration, but stimulated growth of *T. clypeatus*, *C. lagopus* and *V. volvacea* at low concentrations.**

Growth substances like vitamins, fatty acids and plant-growth hormones have been reported to influence the yields of mushroom mycelia. Jennison *et al.* made an extensive study of the nutritional requirements of more than 40 species of wood rotting Basidiomycetes[1]. According to these investigations, thiamine was required by almost all strains tested, while *Poria vaillantii* required biotin and riboflavin in addition to thiamine. Stimulation of growth of mushrooms either on compost[2] or in submerged culture[3] has been observed when the medium was

supplemented with various lipids and vegetable oils. Dijkstra *et al.*[4] reported both stimulation and inhibition of growth in the presence of different saturated and unsaturated fatty acids. The unsaturated free fatty acids were found to be inhibitory while oleic acid esters were stimulatory. Guha and Banerjee[5] studied the effect of indole-3-acetic acid and kinetin on the mycelial yield of *Agaricus campestris* S12 and the former only was found to be stimulatory for growth.

This paper reports the effect of various growth factors, including vitamins, plant growth hormones and fatty acids, on seven strains of mushrooms.

## Materials and Methods

*Chemicals:* Kinetin, gibberellic acid and all the members of the vitamin B group were purchased from Sigma Chemical Co. USA. Indole-3-acetic acid was the product of C. H. Boehringer Sohn, Germany. Vitamin E and C were purchased from E. Merck, Germany. All the fatty acids were obtained from Centron Research Laboratory, Bombay, (India). Vitamin A (acetate)-4, 95,000 IU/g, vitamin D (calciferol)-6, 90,000 IU/g and vitamin K (menadion, sodium bisulfite) were obtained from Central Drug Laboratory, Calcutta, Other chemicals used were either of AR or GR quality.

*Mushrooms and their propagation:* The mushroom strains used were: *Termitomyces clypeatus* (Heim), *Panafolus papillionaceus* (Bull. ex. Fr. Qu'el), *Gymnopilus chrysimyces* (Berk), *Coprinus lagopus* (Fr.) Fr, *Lentinus squarrosulus* (Mont.), *Volvariella volvacea* and *Agaricus bisporus*. Methods for the development of mycelial growth from their fruit bodies and their propagation have been described elsewhere[6-8].

*Growth substances:* Vitamin A, D and K were emulsified with a drop of refined groundnut oil and diluted to the required volume with ethanol. Concentration of these vitamins and vitamin C and E were formulated as needed by different[10] animals Since requirement of these vitamins by mushrooms were not available, B-vitamins, fatty acids and plant growth hormones were dissolved in absolute alcohol. Concentration of these growth promoting substances were derived from the previous reports on mushroom growth[1-5]. Composition of B vitamin mixture added in the medium and their concentrations are as follows (in mg/ml of medium): Thiamine-HCl ($5\times10^{-4}$), riboflavin ($5\times10^{-4}$), nicotinamide ($1\times10^{-6}$), calcium pantothenate ($5\times10^{-4}$), pyridoxal ($1\times10^{-4}$), pyridoxine-HCl ($2\times10^{-4}$), pyridoxamine ($2\times10^{-4}$), cyanocobalamine ($1\times10^{-6}$), folic acid ($3\times10^{-6}$), *p*-aminobenzoic acid ($2\times10^{-4}$), biotin ($5\times10^{-6}$) nicotinic acid ($5\times10^{-4}$), choline chloride ($1\times10^{-2}$) and *m*-inositol ($1\times10^{-2}$).

*Medium:* Media for the submerged propagation of different mushrooms are described Table 1[6-8].

TABLE 1 GROWTH MEDIA

| Organisms | Initial pH of the medium | N-source | | C-source | |
|---|---|---|---|---|---|
| | | Compounds | Amount (%, w/v) | Compounds | Amount (%, w/v) |
| *T. clypeatus* | 3.0 | $NH_4H_2PO_4$ | 2.463 | Dextrin | 10 |
| *P. papillionaceus* | 3.0 | $NH_4H_2PO_4$ | 2.463 | Soluble Starch | 9 |
| *G. chrysimyces* | 5.0 | Urea | 0.214 | ,, | 5 |
| *C. lagopus* | 5.0 | ,, | 0.032 | ,, | 6 |
| *L. squarrosulus* | 5.0 | $NH_4Cl$ | 0.057 | Dextrin | 8 |
| *V. volvacea* | 4.0 | $KNO_3$ | 0.108 | Soluble starch | 5 |
| *A. bisporus* | 8.0 | $NH_4Cl$ | 0.382 | Glucose | 6 |

Micronutrients present in g/100 ml: $KH_2PO_4$, 0.087; $MgSO_4$, $7H_2O$, 0.05; $FeSO_4$, $7H_20$, 0.025; $MnCl_2$, $4H_20$, 0.0036; $NaMoO_4$, $4H_2O$, 0.0032; $ZnSO_4$, $7H_2O$, 0.03; $CaCl_2$, $2H_2O$, 0.037; boric acid, 0.057; $CuSO_4$, $5H_2O$, 0.0039; with the omission of $Cu^{2+}$ for *T. clypeatus*, *C. lagopus* and *V. volvacea*; $NaMoO_4$ for *G. chrysimyces* and *L. squarrosulus*; $Zn^{2+}$ for *A. bisporus*. Omission of all except $KH_2PO_4$ and $MgSO_4$ were made for *P. papillionaceus.* Incubation temperature for all the mushrooms was maintained at 30±1°C except for *A. bisporus* which was 24±1°C.

*Growth measurement:* The mycelial growths 15 days after inoculation were recovered by filtration, washed thoroughly, dried (60-70°C) and weighed.

## Results and Discussion

The results in Table 2 show the effects of vitamins on the mycelial yields of mushrooms grown under submerged condition. It was observed that the vitamin B mixture did not cause any appreciable growth stimulation for any of the strains. Although it was reported by Eddy[9] that *Coprinus comatus* required thiamine for growth,

TABLE 2. INFLUENCE OF VITAMINS ON YIELD[a]

| Organism | Mycelial yield in Control (g/100 ml) | Mycelial yield (g/100 ml) with indicated vitamins* added | | | | | |
|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | K |
| *P. papillionaceus* | 2.086 | 2.130 | 2.086 | 1.136 | 2.318 | 2.016 | 2.126 |
| *C. lagopus* | 1.982 | 2.090 | 2.160 | 2.012 | 1.980 | 1.448 | 2.048 |
| *L. squarrosulus* | 0.470 | 0.522 | 0.554 | 0.512 | 0.464 | 0.446 | 0.478 |
| *V. volvacea* | 0.668 | 0.620 | 0.438 | 0.234 | 0.614 | 0.134 | 0.580 |
| *A. bisporus* | 1.220 | 1.174 | 1.182 | 1.216 | 1.100 | 1.394 | 1.000 |

*a*. Yield on dry wt. basis 15 days after inoculation. Results are average of triplicates.
*Vitamins added: Vitamin A: 0.001 mg/100 ml; vitamin B: See under text; vitamin C: 5.0 mg/100 ml; vitamin D, E and K: each 0.5 mg/100ml. 1 ml of ethanolic solutions were added.

TABLE 3. INFLUENCE OF PLANT GROWTH HORMONES ON YIELD[a]

| Organism | Mycelial yield in control‡ (g/100 ml) | Mycelial yield (g/100 ml) with indicated hormones* | | |
|---|---|---|---|---|
| | | Gibberellic acid | Indole-3-acetic acid | Kinetin |
| *T. clypeatus* | 1.320 | 1.340 | 1.564 | 2.140 |
| *C. lagopus* | 1.568 | 1.400 | 1.364 | 2.146 |
| *V. volvacea* | 0.212 | 0.180 | 0.404 | 0.150 |

*a*. Yield on dry wt. basis measured in triplicate after 15 days of inoculation.
‡. Basal medium (100 ml) as stated in text contained 1% (v/v) ethanol.
* 1 ml of ethanolic solutions were added.

the present strains did not respond to B-vitamins. Moreover, addition of B-vitamins inhibited growth of *V. volvacea*. Vitamins A, D and K did not affect growth, but vitamin C and vitamin E inhibited the growth of *V. volvacea*, *P. papillionaceus* and of *C. lagopus*, *V. volvacea* respectively. Growth of *T. clypeatus* and *G. chrysimyces* was not affected by any of the vitamins tested.

Although it seems rational to study the effect of plant growth hormones on mushrooms, limited information is already available[3]. Table 3 gives the effect of some plant growth hormones on the growth of selected mushrooms. It is evident from the Table that kinetin effected significant growth stimulation with *T. clypeatus* and *C. lagopus* and growth inhibition with *V. volvacea*. Indole-3 acetic acid was beneficial for the growth of *V. volvacea*, only. Addition of gibberellic acid was not effective.

The possible effect of fatty acids on mushroom growth was initially suggested by the observation that the yield of *Agaricus bisporus* fruit body is stimulated by the addition of vegetable oils to the compost. Wardle and Schisler[2] reported the same during submerged cultivation of mushroom mycelium. In the present study, free fatty acids were tested for their effects on the growth of different strains of mushrooms (Table 4). Capric acid is inhibitory for almost all the strains, except *P. papil-*

TABLE 4. INFLUENCE OF FATTY ACIDS ON GROWTH[a] IN SYNTHETIC MEDIA

| Organisms | Mycelial yield in control[b] (g/100 ml) | Mycelial yield (g/100 ml) with fatty acids | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Capric acid | Caprylic acid | Lauric acid | Myristic acid | Oleic acid | Palmitic acid | Stearic acid |
| *T. clypeatus* | 2.976 | 0.018 | 0.062 | 3.004 | 2.700 | 3.072 | 3.178 | 2.470 |
| *P. papillionaceus* | 2.224 | 2.636 | 2.604 | 2.640 | 2.674 | 2.552 | 2.802 | 2.376 |
| *C. lagopus* | 1.241 | 1.270 | 1.597 | 1.416 | 1.383 | 1.279 | 1.267 | 1.258 |
| *L. squarrosulus* | 0.464 | 0.355 | 0.341 | 0.379 | 0.410 | 0.534 | 0.448 | 0.534 |
| *V. volvacea* | 1.200 | 0.330 | 1.860 | 0.600 | 1.350 | 0.750 | 1.350 | 1.800 |
| *A. bisporus* | 1.209 | 0.983 | 0.875 | 0.979 | 1.173 | 1.159 | 1.098 | 1.133 |

*a* Growth, always in triplicate, was measured as dry wt. in g/100 ml after 15 days of inoculation.
*b* as stated in growth medium table, contained 1% (v/v) ethanol.

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*lionaceus* and *C. lagopus*. On the other hand, caprylic acid was stimulatory for *V. volvacea* and *C. lagopus*, but both the acids were extremely inhibitory for *T. clypeatus*. Lauric acid was inhibitory for *V. volvacea*, but myristic acid did not affect the growth of any of the mushrooms significantly. Oleic acid was slightly stimulatory for *L. squarrosulus*, but for *V. volvacea* it was inhibitory. Palmitic acid was stimulatory for *P. papillionaceus*. Though stearic acid showed a little inhibition with *T. clypeatus*, it was stimulatory for *V. volvacea*. Growth of *G. chrysimyces* was unaffected by any of the fatty acids. Growth inhibition of *A. bisporus* by oleic acid, stearic acid and palmitic acid was reported by Dijkstra *et al.*[4] We observed that oleic acid was also inhibitory for *V. volvacea* while palmitic and stearic acids were stimulatory for the growth of *V. volvacea*, *L. squarrosulus* and *P. papillionaceus*.

The growth inhibition by capric and caprylic acids is interesting and hence the mycelial yields with different levels were studied (Fig. 1). It was found that both the fatty acids were stimulatory for the growth of *T. clypeatus* at low concentrations of 1 mg/100 ml, but at higher concentrations they were strongly inhibitory. Growth stimulation of *C. lagopus* and *V. volvacea* by caprylic acid was also studied (Fig. 2). Similar growth inhibition was achieved with a larger amount (250 mg/100 ml) of fatty acid. In addition, growth inhibition of *V. volvacea* was noted at the substimulatory doses. In general, these fatty acids have stimulatory effect at a narrow range of concentrations which varies from strain to strain. It may be concluded that among all the growth substances tested, the $C_8$ and $C_{10}$ saturated fatty acids significantly alter the metabolism of mushrooms tested and affect their growth.

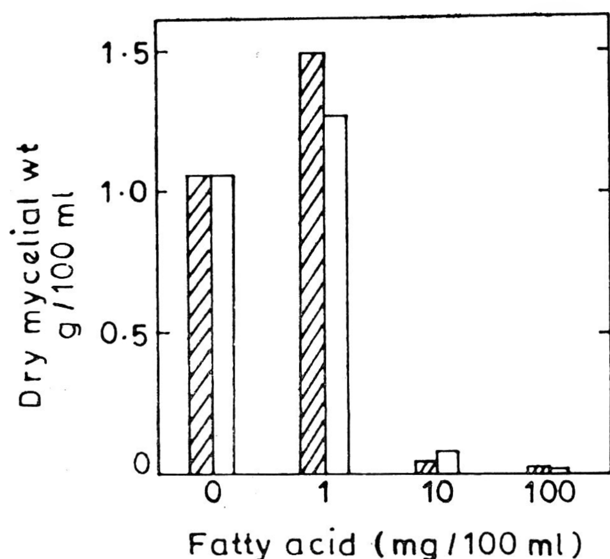


Fig. 1. Influence of caprylic acid (□) and capric acid (▨) on the growth of *T. clypeatus*. Growth was measured, after 15 days of inoculation.

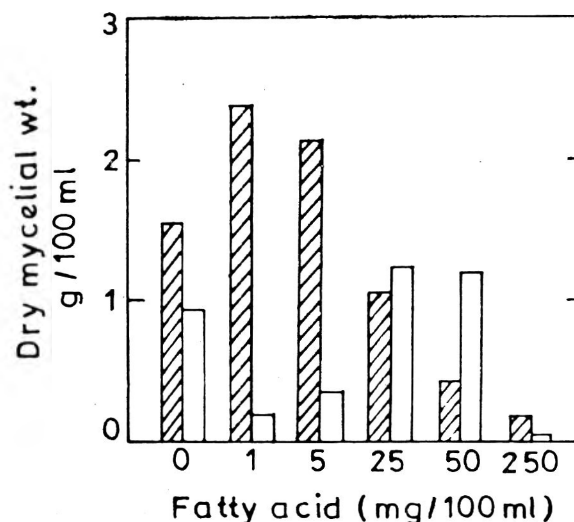


Fig. 2. Influence of caprylic acid on the growth of *C. lagopus* (▨) and *V. volvacea* (□). Growth was measured, after 15 days of inoculation.

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# Studies on the Nutritional Requirements of *P. citrinum* for the Synthesis of Citrinin Toxin

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**Studies on the nutritional requirements of *Penicillium citrinum* for the elaboration of toxin, citrinin in a synthetic medium show that dextrin and cornsteep liquor are the most suitable carbon and nitrogen sources respectively for the production of the toxin. There was no citrinin production in the medium containing ammonium salt due to high acidity of the broth, but addition of 0.1 per cent $CaCO_3$ to neutralise the acidity enhanced the citrinin production. Among the different raw materials tried, rice, soyabean and groundnut cakes are the most suitable for the production of citrinin.**

Citrinin, a toxic secondary metabolite of fungi was first isolated in 1931 from *P. citrinum* Thom[1]. Due to the nephrotoxicity[2] and teratogenic effect this compound has attracted special attention. It was isolated from yellow coloured rice imported from South east Asia. It has been found as a natural contaminant of rice, wheat, corn, rye, barley and oats[3-6]. Scott *et al.*[6] have obtained as much as 0.08 g of citrinin per kg. of wheat which was naturally contaminated.

Citrinin has been isolated from culture filtrates of fungi belonging to the genera *Aspergillus* and *Pencillium*[7] and from the plant *Crotalaria crispata*[8]. Thus, citrinin is a potentially important mycotoxin that may contaminate food and agricultural products and thus cause chronic disease in man and animals. Extensive research was carried out by several workers on the toxic effect of moldy cereals, infected with citrinin producing molds in animals specially on the kidney[9,10].

The present paper describes the study done on the nutritional requirements of *Penicillium citrinum* for citrinin production, using a simple and convenient cup-plate method of assay of the toxin.

## Materials and Methods

The toxin producing strain of *P. citrinum* used was isolated from oil cakes stored for one year[11]. The culture was maintained on potato-dextrose agar slants. For studying nutritional requirements, the organism was grown in 50 ml of a synthetic medium taken in a 250 ml Erlenmeyer flask. The medium was inoculated with 0.2 ml of spore suspension (containing $37.5 \times 10^8$ spores per ml), spores being collected from 7 days old potato-dextrose agar cultures. Triplicate flasks were used for each set. The flasks were kept on a rotary shaker (120 r.p.m.) for 10 days at $28° \pm 1°C$. The basal medium contained $KH_2PO_4$, 1.0 g; $MgSO_4 . 7H_2O$, 0.5 g; KCl, 0.5 g; $FeSO_4 . 7H_2O$, 0.001 g; $NaNO_3$, 2.00 g; distilled water, 1000 ml. The pH was adjusted to $5.8 \pm 0.1$. The carbon sources were sterilised separately and added just prior to inoculation at 5 per cent concentration. Citrinin concentration in the broth was determined by modified cup plate method with *Bacillus subtilis* var. *mycoides* as the test organisms. Citrinin (sample from USDA, Northern Regional Research Laboratory, Peoria, Illinois, U.S.A.) was used as the standard. The results are expressed in terms of micro grams of citrinin per ml broth as calculated from a standard curve. A standard preparation of citrinin containing 0.05 mg per ml of 50 per cent aqueous alcohol produces 20 mm zone of inhibition. Growth was determined as dry weight of cells, separated from the culture broth by centrifugation in the cold, washed 3 times with water and dried for 24 hr at $60° \pm 5°C$.

*Carbon sources:* A number of sugars including glucose, galactose, maltose, mannose, lactose, sucrose, arabinose, xylose, starch and dextrin were used at 5 per cent level as carbon sources and the citrinin elaborated after the 7th and 10th day along with the cell growth and pH of the broth were determined.

*Nitrogen sources:* The different nitrogen sources tested for the elaboration of citrinin included sodium nitrate, ammonium nitrate, ammonium chloride, ammonium hydrogen phosphate, urea, tryptone, peptone, corn steep liquor and yeast extract. The medium contained 4 per cent dextrin. The nitrogen sources were added at a concentration equivalent to 0.033 g nitrogen per 100 ml.

As the ammonium salts used reduced the pH of the broth, $CaCO_3$ at 0.1 per cent level was added to ammonium hydrogen phosphate, ammonium sulphate and ammonium chloride and the citrinin produced was determined.

The citrinin elaborated cell growth and pH of the broth were determined on the 7th and 10th day.

*Citrinin production on solid substrate:* The growth of *P. citrinum* and citrinin production were studied on different solid substrates. Several agricultural commodities were used as substrates for the mold growth. Humidity and temperature are the main controlling factors for the growth of *P. citrinum* and citrinin production. In this experiment, 10 g of solid samples with 50 per cent moisture taken in 100 ml flasks, were inoculated with 0.5 ml of spore suspension and incubated at 28°C in stationary state. Usually, triplicate flasks were used for each set three times. Citrinin was extracted from different food samples, with measured volume of 50 per cent alcohol. The combined extract was assayed by the usual method.

## Results and Discussion

*Effect of carbon sources:* Among the different carbon sources, dextrin is excellent for growth and citrinin production (Table 1). Maximum production of citrinin was obtained on the 10th day. There is, however, no direct relation between cell growth and citrinin production. Lactose does not favour synthesis of citrinin.

TABLE 1. EFFECT OF DIFFERENT CARBON SOURCES ON CITRININ PRODUCTION BY *P. CITRINUM* IN A SYNTHETIC MEDIUM

| Carbon sources | 7th day | | | 10th day | | |
|---|---|---|---|---|---|---|
| | Citrinin ($\mu$g/ml) | Cell growth (g/l) | pH of broth | Citrinin ($\mu$g/ml) | Cell growth (g/l) | pH of broth |
| Glucose | 98 | 7.90 | 5.0 | 165 | 6.04 | 5.2 |
| Galactose | 110 | 7.70 | 4.8 | 170 | 5.64 | 5.0 |
| Maltose | 110 | 8.54 | 4.7 | 216 | 9.28 | 4.9 |
| Mannose | 96 | 6.66 | 4.9 | 155 | 6.98 | 5.0 |
| Lactose | — | 3.16 | 4.1 | — | 2.76 | 4.2 |
| Sucrose | 120 | 9.60 | 4.3 | 180 | 10.78 | 5.0 |
| Arabinose | 88 | 4.34 | 4.8 | 98 | 4.76 | 5.1 |
| Xylose | 75 | 8.10 | 4.9 | 156 | 6.30 | 4.8 |
| Starch (soluble) | 200 | 9.70 | 5.2 | 275 | 8.54 | 4.6 |
| Dextrin | 245 | 10.30 | 5.1 | 320 | 10.76 | 5.5 |

TABLE 2. EFFECT OF DIFFERENT NITROGEN SOURCES ON CITRININ PRODUCTION BY *P. CITRINUM* IN A SYNTHETIC MEDIUM

| Nitrogen sources | 7th day | | | 10th day | | |
|---|---|---|---|---|---|---|
| | Citrinin ($\mu$g/ml) | Cell growth (g/l) | pH of broth | Citrinin ($\mu$ g/ml) | Cell growth (g/l) | pH of broth |
| Sodium nitrate | 265 | 9.10 | 4.5 | 325 | 8.70 | 4.8 |
| Ammonium sulphate | — | 4.74 | 2.0 | — | 4.78 | 2.0 |
| Ammonium chloride | — | 4.50 | 2.1 | - | 4.16 | 2.1 |
| Ammonium hydrogen phosphate | — | 4.98 | 2.0 | — | 5.70 | 2.0 |
| Urea | 230 | 8.22 | 4.9 | 265 | 8.90 | 5.2 |
| Tryptone | 315 | 9.94 | 5.5 | 355 | 9.18 | 5.9 |
| Peptone | 212 | 10.16 | 5.1 | 250 | 9.54 | 5.0 |
| Corn steep liquor | 530 | 13.76 | 6.1 | 616 | 11.62 | 6.4 |
| Yeast extract | 215 | 8.66 | 4.8 | 235 | 8.02 | 5.4 |

*Effect of nitrogen sources:* It is evident from Table 2 that there was no citrinin production in synthetic medium containing ammonium salt, whereas organic nitrogen compounds as well as nitrate are suitable. Corn steep liquor allows maximum production of toxin. There is sharp fall in pH of the broth containing ammonium salt.

*Effect of calcium carbonate:* Table 3 shows that addition of ($CaCo_3$) increases the synthesis of citrinin in ammonium salt medium. It is, therefore, evident that high acidity of the medium inhibits citrinin synthesis.

TABLE 3. EFFECT OF $CACO_3$ ON CITRININ PRODUCTION BY *P. CITRINUM* IN A SYNTHETIC MEDIUM CONTAINING AMMONIUM SALT

| Nitrogen sources | 7th day | | | 10th day | | |
|---|---|---|---|---|---|---|
| | Citrinin ($\mu$g/ml) | Cell growth | pH | Citrinin ($\mu$g/ml) | Cell growth | pH |
| Ammonium hydrogen phosphate | 235 | 11.82 | 4.8 | 350 | 11.62 | 4.9 |
| Ammonium sulphate | 96 | 10.10 | 4.4 | 130 | 9.62 | 4.6 |
| Ammonium chloride | 88 | 6.64 | 4.1 | 98 | 8.02 | 4.5 |

$CaCo_3$ added at 0.1%

TABLE 4. PRODUCTION OF CITRININ ON DIFFERENT GRAINS AND OIL-CAKES BY *P. CITRINUM*

| Solid substrate | Citrinin (μ g/g) | | |
|---|---|---|---|
| | 5th day | 7th day | 9th day |
| Rice | 602.5 | 592.5 | 643.0 |
| Wheat | 418.0 | 539.0 | 435.5 |
| Corn | 532.0 | 473.0 | 470.0 |
| Soyabean | 336.5 | 668.0 | 615.0 |
| Oat | 195.0 | 205.0 | 233.5 |
| Barley | 315.0 | 403.0 | 445.0 |
| Groundnut | 200.0 | 286.0 | 325.0 |
| Mustard Oil cake | 206.0 | 235.0 | 210.0 |
| Groundnut Oil cake | 495.0 | 639.0 | 615.6 |

*Citrinin production on solid substrate:* It is evident from Table 4 that although all grains, oilseeds and their meals supported growth of the mould and citrinin production in solid state fermentation, rice, soyabean and groundnut cakes are more suitable substrates for citrinin production. The study demonstrates the possibility of contamination of grains, legumes and oilseeds by the citrinin producing fungi and the production of the mycotoxin under favourable conditions.

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# Retention of Aflatoxin in *Ugali* and Bread Made from Contaminated Maize Flour

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**Maize flour was contaminated with aflatoxins by growing *Aspergillus parasiticus* (NRRL 3145). *Ugali*, a traditional staple dish in Africa, was cooked from the contaminated whole maize flour. Bread was baked from a composite flour that contained 10 parts of the aflatoxin contaminated maize flour and 90 parts of uncontaminated wheat flour. The ready-to-eat *ugali* still contained 199.1 mg/kg aflatoxin B ($B_1+B_2$) and 206 mg/kg of aflatoxin G ($G_1+G_2$), amounting to retention percentages of 88.5 and 82.4 respectively. In bread, retention was 83.8% for aflatoxin B ($B_1+B_2$) and 67.8% for aflatoxin G ($G_1+G_2$). In both *ugali* and bread, more of aflatoxin B was retained than G.**

Aflatoxins are potent carcinogens produced as secondary metabolites mostly by fungi belonging to the *Aspergillus flavus* group. Aflatoxin $B_1$ is one of the most potent carcinogens known[1]. As little as 0.3 $\mu$g/g of aflatoxin $B_1$ in the diet has been shown to induce tumor formation in the livers of experimental animals[2]. Epidemiological evidence from different countries[3-5] has shown the relation between the consumption of aflatoxin contaminated foods and the death from hepatomas in children and adults. Although aflatoxins were initially discovered in moldy peanut meal[6], foods stored under hot and humid conditions are also prone to aflatoxin contamination. The isolated aflatoxins are stable upto their melting points of around 250°C[7]. Consuming aflatoxin-contaminated grains could be hazardous. Liver cancer has been recognized as a serious disease in Africa for a long time[8] and in several cases correlated to the incidence of foodstuff heavily contaminated with aflatoxin[9]. The present paper describes the relation between cooking of *ugali*, baking of bread and retention of aflatoxin in these products.

### Materials and Methods

One kilogram of polished maize flour was locally purchased. The flour in 250 g portions was dispensed into Fernbach flasks of 2 l capacity, plugged with cotton and autoclaved for 20 min at 121°C. It was transferred to a chamber maintained at 90 per cent RH as described earlier[10], which contained an incubator set at 30°C. Following equilibration for two days, the flour in all flasks, except the control, was sprayed with 10 ml (per flask) spore suspension of *Aspergillus parasiticus* (NRRL 3145) in sterile distilled water, grown on potato-dextrose agar slants. The flasks were incubated for 10 days at 30°C maintaining a RH of 90±2 per cent.

*Ugali* was cooked in the traditional way in an aluminium vessel by adding the pooled, and uniformly mixed contaminated maize flour to boiling water. The changes in temperature during cooking were recorded till the product was fully formed. Bread was made by mixing 10 parts of the contaminated maize flour and 90 parts of aflatoxin free wheat flour, and baked at 240°C for 35 min. The bread temperature was noted after the bread was completely baked.

Aflatoxins were extracted from 50 g of maize flour that had been pooled from all flasks which showed the growth of *A. parasiticus*. Contents from the control flask were similarly extracted. The exraction procedure followed was that of Shannon *et al.*[11] using acetone: water (85:15 v/v) as the primary extractant and ammonium sulphate for cleanup. Aflatoxins were extracted in a similar way from portions representing 50g of maize flour from *ugali* and bread. Aflatoxin detection and separation were done for all extracts by spotting 20 $\mu$l on thin-layer chromatography plates coated with kieselgel 60 HR (article 7744 Merck). A solvent system of chloroform: acetone (90:10 v/v) in an unlined, unequilibrated tank was used for developing. After observing under a long wave UV light, different spots containing aflatoxins, recognized using co-developed aflatoxin standards (Supelco Inc., Bellefonte, PA16823, USA) were scraped ($B_1+B_2$ together, and $G_1+G_2$ together) into 5 ml of acetonitrile contained in test tubes. The test tubes were gently shaken and stored in dark for 30 min closing with

stoppers. Aliquots of aflatoxins in acetonitrile were quantified in a SV 30 UV spectrophotometer, reading absorbance at 335 nm for aflatoxin B and 358 nm for aflatoxin G. Aflatoxin concentrations were calculated after making standard graphs using known concentrations of aflatoxin standards.

## Results and Discussion

Levels of aflatoxin B and aflatoxin G in maize flour inoculated with *A. parasiticws* were 225 mg/kg and 250 mg/kg respectively. Aflatoxin was absent in the control, implying that the maize flour was initially free from aflatoxin. Quantities of aflatoxin retained in *ugali* and bread made from the contaminated flour are presented in Table 1. Elaboration by *A. parasiticus* of more aflatoxin G than aflatoxin B was prominent. A similar trend is also noted for this strain cultured on soybeans[12]. However, the total amount of aflatoxin formed in maize flour is far greater than the concentrations reported in few surveys of natural contamination of maize with aflatoxin in some parts of Africa[8], and North America[13]. The increased production of aflatoxin in the laboratory is perhaps due to optimal RH and temperature provided during incubation.

Although aflatoxins are known to be resistant to heat, the intensity and the duration of heat during cooking and baking appear to have some influence. During the cooking of *ugali* the temperature ranged from 80 to 85°C, and lasted for 15 min. As can be seen from Table 1, only 11.5 per cent of aflatoxin B is destroyed after cooking *ugali*, while 16.2 per cent of aflatoxin B is destroyed in the baking of bread. The final bread temperature was 95°C. This difference in part is due to the different temperatures employed in cooking and baking. Destruction of more aflatoxin G than aflatoxin B in both the cases is in agreement with the findings in feed by the U.S. Food and Drug Administration[14]. While it is known that higher moisture content would promote heat destruction of aflatoxin $B_1$[15] in cooking *ugali* where the contaminated flour was in contact with water, the destruction was only 11.5 per cent. Evidently, neither the conventional cooking nor the baking practices promise the total destruction of aflatoxins. A 10 to 15 per cent destruction of aflatoxin B and 15 to 30 per cent destruction of aflatoxin G was apparent from this study.

TABLE 1. AFLATOXINS IN *UGALI* AND BREAD BEFORE AND AFTER COOKING AND BAKING

| Food | Aflatoxin concentration (mg/kg) Before | | After | | Aflatoxin retention (%) | |
|---|---|---|---|---|---|---|
| | $B_1+B_2$ | $G_1+G_2$ | $B_1+B_2$ | $G_1+G_2$ | B | G |
| *Ugali* | 225.0 | 250.0 | 199.1 | 206.0 | 88.5 | 82.4 |
| Bread* | 225.0 | 250.0 | 187.6 | 169.5 | 83.8 | 67.8 |

*Made from 10 parts of aflatoxin contaminated maize flour and 90 parts of uncontaminated wheat flour.

In order to study the pattern of aflatoxin destruction in *ugali* by cooking and in bread by baking, it would have been necessary to prolong the time; but this would have only rendered the final products unacceptable.

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# Studies on Production and Quality of Queso Blanco Cheese

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**Preparation of Queso Blanco cheese from buffalo milk of 5 per cent fat and cow milk of 3.5 per cent fat using citric acid as a coagulant and addition of 2 per cent salt and, 10 and 20 per cent cane sugar was described and the shelf life was assessed by storing at 6-8°C for 6 days. Addition of 2 per cent salt resulted in higher yield when milk solids are more in milk and moisture retention was more in the case of Queso Blanco cheese made from cow milk. Further, addition of 2 per cent salt resulted in higher fat percentage in cheese from buffalo milk and low yeast and mould count. Addition of cane sugar at 10 or 20 per cent levels resulted in more losses of fat in the whey, and higher yeast and mould count, but the cheese was more acceptable when made from buffalo milk with addition of 10 per cent cane sugar.**

Queso Blanco cheese or Latin American white cheese made by direct acidification of cow milk with organic acids, is an unripened white cheese with bland acid flavour. Non-usage of rennet in the production of cheese may overcome the prevalent barriers of vegetarian population of India for cheese consumption and it may become good substitute for cheese of different types, since it is nutritious with bland acid flavour and good slicing properties. This cheese is commonly made by acidification of cow milk with glacial acetic acid at 82-83° C in Latin American countries. Many workers studied the different aspects of production including the development of factory method[1], quality of Queso Blanco cheese[2–4] and influence of various coagulating acids on yield and quality[5]. Since in these investigations only cow milk and addition of salt on quality were studied, an attempt has been made in the present study to compare the quality of Queso Blanco cheese made with buffalo milk by addition of salt as well as cane sugar at 10 and 20 per cent level with that of cheese made from cow milk. Of all the organic acid coagulants, pure citric acid is considered as the best, as it does not impart any flavour to the product[6] and hence, it is used in the present study.

## Materials and Methods

The milk obtained from healthy cows and buffaloes was standardised to 3.5 and 5 per cent fat, respectively, and were divided into three portions of 3 kg each and labelled as $C_1$, $C_2$, $C_3$ and $B_1$, $B_2$, $B_3$, respectively. All the six samples were heated separately to 82-83°C and coagulated with 5 per cent citric acid solution. The curd obtained after draining from $C_1$ and $B_1$ was cut into small cubes, and salt was sprinkled at the rate of 2 per cent of the curd and pressed overnight. Similarly, the curd obtained from $C_2$ and $B_2$ was cut and 10 per cent powdered cane sugar was added before pressing overnight. The curd obtained from $C_3$ and $B_3$ was also treated as above but with addition of 20 per cent powdered cane sugar. The curd obtained after overnight pressing was sliced and packed in chlorinated vegetable parchment paper and stored at refrigeration temperature of 6-8°C for 6 days to find out the chemical composition as well as microbial quality. During the manufacture of Queso Blanco cheese (in all 10 replications), the following tests were conducted as per the standard procedures.

*Chemical:* Fat in milk, skimmed milk and whey[7] and in cream and cheese[8] were determined as per the ISI procedure. Estimation of solids-not-fat (SNF) in cow and buffalo milks was done as per the procedure given in ISI[9]. The SNF was found to range from 8.89 to 9.54 for cow and from 9.42 to 10.05 for buffalo milk. Moisture in cheese was estimated as per the procedure given by Kosikowski[10].

*Microbial:* Coliform counts were determined on the first, third and sixth day of preparation according to the procedure of B.B.L. manual[11]. For dilution, 1:9 ml dilution blanks containing 2 per cent warm sodium citrate solution were used. Yeast and mould counts were determined as per the ISI procedure[12].

*Organoleptic evaluation:* This was carried out as per the score card recommended by Angivine *et al.*[13].

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## Results and Discussion

*Yield:* The data given in Table 1 indicate the mean per cent yield of Queso Blanco cheese. It is seen that buffalo milk gave higher yield of cheese irrespective of the additions. Tnis might be due to higher total solids in buffalo milk. It is also clear that addition of salt at 2 per cent level resulted in higher yield of cheese irrespective of type of milk which might be due to enhanced draining of whey by addition of cane sugar.

*Fat losses in whey:* The results in Table 2 indicate the fat losses in whey. The fat loss is more in whey from cheese made with buffalo milk indicating that the fat losses are directly proportional to the fat level. Addition of sugar resulted in more loss of fat than addition of salt and also the level of sugar added was found to be directly proportional to the fat losses in whey. The individual treatment values did not differ significantly at 5 per cent level of probability.

*Fat in cheese (on dry matter basis):* The values given in Table 3 indicate that salt-added cheese contained more fat than sugar added cheese. This might be the result of more of fat loss in whey when sugar was added to the cheese. Further, it was observed that at 20 per cent level of sugar addition, the fat level variations in the raw milk did not influence fat percentage (on dry matter basis) in the finished cheese.

*Moisture:* Data given in Table 4 indicate the

TABLE 1. MEAN VALUES OF PER CENT YIELDS OF QUESO BLANCO CHEESE

| Source of milk | 2% salt | 10% sugar | 20% sugar | Mean for milk type |
|---|---|---|---|---|
| Cow | $14.163^{c}$ | $11.576^{b}$ | $10.759^{a}$ | $12.166^{p}$ |
| Buffalo | $17.413^{e}$ | $15.269^{d}$ | $14.46^{c}$ | $15.714^{q}$ |
| Mean for additions | $15.788^{x}$ | $13.423^{y}$ | $12.61^{z}$ | |

Mean values of similar categories bearing the same superscript do not differ significantly ($P<0.05$)

TABLE 2. MEAN VALUES OF PER CENT FAT LOSSES IN WHEY OF QUESO BLANCO CHEESE

| Source of milk | 2% salt | 10% sugar | 20% sugar | Mean for milk type |
|---|---|---|---|---|
| Cow | $0.260^{a}$ | $0.434^{b}$ | $0.443^{b}$ | $0.379^{p}$ |
| Buffalo | $0.453^{b}$ | $0.588^{c}$ | $0.595^{c}$ | $0.545^{q}$ |
| Mean for additions | $0.357^{x}$ | $0.511^{y}$ | $0.519^{z}$ | |

Mean values superscripted by same letter do not differ significantly at $P<0.05$.

TABLE 3. MEAN VALUES OF PER CENT FAT (ON DRY MATTER BASIS) OF QUESO BLANCO CHEESE

| Source of milk | 2% salt | 10% sugar | 20% sugar | Mean for milk type |
|---|---|---|---|---|
| Cow | $46.613^{c}$ | $44.347^{a}$ | $44.831^{ab}$ | $45.264^{p}$ |
| Buffalo | $48.982^{d}$ | $45.367^{b}$ | $44.613^{a}$ | $46.321^{q}$ |
| Mean for additions | $47.798^{x}$ | $44.857^{y}$ | $44.722^{y}$ | |

Mean values superscribed by the same letter do not differ significantly at $P<0.05$.

TABLE 4. COMPARISON OF MEAN VALUES OF PER CENT MOISTURE OF QUESO BLANCO CHEESE ON STORAGE AT REFRIGERATION TEMPERATURE

| Storage period (days) | Cow milk | | | Buffalo milk | | | Mean for storage days |
|---|---|---|---|---|---|---|---|
| | 2% salt | 10% sugar | 20% sugar | 2% salt | 10% sugar | 20% sugar | |
| 1 | $51.300^{l}$ | $43.863^{i}$ | $42.213^{h}$ | $49.574^{k}$ | $40.721^{f}$ | $38.718^{c}$ | $44.398^{x}$ |
| 3 | $50.748^{l}$ | $43.461^{i}$ | $41.430^{g}$ | $48.885^{j}$ | $39.349^{d}$ | $37.152^{b}$ | $43.504^{y}$ |
| 6 | $50.037^{k}$ | $42.732^{h}$ | $40.089^{e}$ | $48.340^{j}$ | $38.458^{c}$ | $36.164^{a}$ | $42.600^{z}$ |
| Mean for additions within the milk | $50.695^{r}$ | $43.352^{p}$ | $41.244^{o}$ | $48.933^{q}$ | $39.509^{n}$ | $37.344^{m}$ | |
| Mean for milk type | | $45.097^{t}$ | | | $41.929^{u}$ | | |
| Mean for additions | $49.814^{A}$ (2% salt) | | $41.431^{B}$ (10% sugar) | | $39.294^{C}$ (20% sugar) | | |

Mean values of similar categories bearing the same superscript do not differ significantly at $P<0.05$.

TABLE 5. COMPARISON OF MEAN VALUES OF YEAST AND MOULD COUNT OF QUESO BLANCO CHEESE ON STORAGE AT REFRIGERATION TEMPERATURE

| Storage period (days) | Cow milk | | | Buffalo milk | | | Mean for storage day |
|---|---|---|---|---|---|---|---|
| | 2% salt | 10% sugar | 20% sugar | 2% salt | 10% sugar | 20% sugar | |
| 1 | $72^{a}$ | $112^{d}$ | $184^{h}$ | $74^{b}$ | $143^{e}$ | $227^{i}$ | $135^{x}$ |
| 3 | $101^{c}$ | $354^{i}$ | $432^{m}$ | $177^{g}$ | $271^{j}$ | $449^{o}$ | $297^{y}$ |
| 6 | $161^{f}$ | $443^{n}$ | $607^{q}$ | $334^{k}$ | $500^{p}$ | $697^{r}$ | $457^{z}$ |
| Mean for additions within milk | $111^{A}$ | $303^{C}$ | $408^{E}$ | $195^{B}$ | $305^{D}$ | $458^{F}$ | |
| Mean for milk type | | $274^{M}$ | | | $319^{N}$ | | |
| Mean for additions | $153^{u}$ | | $304^{v}$ | | $433^{w}$ | | |

Mean values of similar categories bearing same superscripts do not differ significantly at $P<0.05$.

TABLE 6. MEAN SCORES OF ORGANOLEPTIC QUALITY OF QUESO BLANCO CHEESE STORED AT REFRIGERATION TEMPERATURE

| Source of milk | Storage period (days) | 2% salt | 10% sugar | 20% sugar |
|---|---|---|---|---|
| Cow | 1 | 96 | 95 | 93 |
| | 3 | 92 | 95 | 92 |
| | 6 | 90 | 93 | 90 |
| Buffalo | 1 | 96 | 96 | 92 |
| | 3 | 95 | 96 | 92 |
| | 6 | 92 | 92 | 90 |

moisture content in cheese. The moisture retention was found to be more in salt added cheese than the sugar added cheese. Also the cow milk cheese retained more moisture than buffalo milk cheese during entire storage period with all the 3 types of additions.

*Coliform count:* Coliforms were not detected in Queso Blanco cheese made with different treatments, either on the first day of preparation or on the third and sixth day of storage at refrigeration temperature.

*Yeast and Mould Count:* The results presented in Table 5 indicate the yeast and mould count of cheese stored at 6-8°C for a period of 5 days. The results show a direct relationship between sugar levels, yeast and mould counts which indicate the role of cane sugar in contributing to the yeast and mould count. Salt added samples showed low initial count of yeast and mould.

*Organoleptic quality:* The mean scores for organoleptic quality of cheese are presented in Table 6, which indicate that addition of salt was preferred in cheese of cow milk on first day of storage, but as the storage period increased 10 per cent cane sugar added cheese was preferred. In the case of buffalo milk cheese, on first day, 2 per cent salt and 10 per cent cane sugar added samples were preferred over 20 per cent sugar added samples but in storage, preference was for cow milk. This indicates that as the storage period increased 10 per cent cane sugar added samples proved more acceptable over others.

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# Studies on Production and Quality of *Chhana* from Goat Milk

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**_Chhana_ was prepared from goat milk of 3, 4 and 5 per cent fat using lactic acid, sour whey and citric acid as coagulants and compared it with that of _Chhana_ made from cow milk of 4 per cent fat. The _Chhana_ was tested for moisture content, fat losses in whey and organoleptic quality apart from the yield. _Rasagolla_ made from _Chhana_ was also studied for the quality. The production cost was calculated. The results indicate that goat milk with 5 per cent fat and with citric acid as coagulant resulted in higher yields, but milk with 3 per cent fat gave _Chhana_ with higher moisture content. Fat losses in whey are minimum when lactic acid is used as coagulant and when milk fat was 3 per cent. Better quality _Rasagolla_ was obtained from _Chhana_ made from 4 per cent fat using lactic acid as coagulant. The cost of production of _Chhana_ suitable for _Rasagolla_ making is minimum when goat milk of 4 per cent fat was used.**

In India 8-10 million milch type goats are maintained which produce 7 lakh metric tonnes of milk annually[1]. Due recognition is not given for the utilization of goat milk in India. While methods are available for the preparation of *Chhana* from cow and buffalo milks, only recently Jailkhani and De[2] reported utilization of goat milk for some indigenous milk products including *Chhana*. Therefore, the present study was aimed to find out suitable fat levels in goat milk and the type of coagulant useful for the production of good quality *Chhana* suitable for the preparation of *Rasagolla* (a sweet meat prepared from *Chhana*).

## Materials and Methods

Standardized cow milk of 4 per cent fat and goat milk of 3, 4 and 5 per cent fat were prepared by using the required amounts of cream/skim milk from cow and goat milks.

*Chhana* was made from cow milk of 4 per cent fat with 0.25 per cent (by wt.) of citric acid as coagulant ($T_1$, control) as per the method of Rao[3]. *Chhana* from goat milk was made as per the method of Jailkhani and De[2] from the following combinations. Goat milk of 3 per cent fat was coagulated with 5 per cent lactic acid solution ($T_2$) or using sour whey (0.71 to 0.84 per cent lactic acid) ($T_3$) or 5 per cent citric acid solution ($T_4$). Similarly, goat milk with 4 per cent ($T_5$, $T_6$, $T_7$) and 5 per cent ($T_8$, $T_9$, $T_{10}$) fat were also coagulated with the above mentioned coagulants. respectively.

Fat in milk, whey and skimmed milk[4] and in cream[5] was analysed as per the ISI procedure. Solids-not-fat (SNF) in standardized cow and goat milk was determined as per the ISI procedure[6] and found to be 8.6-8.8 per cent in cow milk and 9 -9.3 per cent in goat milk used for the experiment. Moisture per cent in *Chhana* was estimated as that adopted for cheese by Kosikowski[7].

The ten types of *Chhana* made in each trial were judged for organoleptic quality by a panel of 6 judges using a 10-point scale by giving reverse values. *Rasagolla* was made from the *Chhana* as per the procedure recommended by Srinivasan and Anantakrishnan[8] and evaluated for its organoleptic quality by a panel of 6 judges by the 10-point scale. Cost of production was also calculated for *Chhana* prepared from cow and goat milks.

## Results and Discussion

*Yield:* The results presented in Table 1 indicate the mean per cent yield of *Chhana* under different treatments. It can be seen that the fat level in goat milk was directly related to the yield of *Chhana* irrespective of the coagulants used. Citric acid gave always higher yield of *Chhana* at all the fat levels of goat milk. From this, it can be concluded that goat milk with 5 per cent fat gives maximum yield of *Chhana* (17.35 per cent) when citric acid is used as coagulant.

*Moisture:* The mean values of moisture content in *Chhana* are presented in Table 2 which indicates that citric acid as coagulant retains more moisture in *Chhana* followed by sour whey and lactic acid. The fat contents in milk were found to be inversely related to the moisture

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content of *Chhana* irrespective of the coagulant used. For higher moisture retention in *Chhana* (58.21 per cent), goat milk with 3 per cent fat coagulated with citric acid solution was found to be more suitable.

*Fat losses in whey:* The mean values of per cent fat losses in *Chhana* whey obtained from different treatments are presented in Table 3 which indicate that fat losses are directly related to the fat levels of goat milk. Further, the fat losses are more when sour whey was used as a coagulant, but were less when lactic acid was used. For minimum fat losses in *Chhana* whey during manufacture of *Chhana*, 3 per cent fat goat milk coagulated with lactic acid solution would be preferred.

*Organoleptic quality of Chhana and Rasagolla:* The data presented in Table 4 indicate the average rank value of *chhana* given by panel of judges. The quality of *Chhana* from cow milk ($T_1$) was the best having the highest rank value followed by *Chhana* obtained from 3 per cent goat milk ($T_4$). The average rank value of *Chhana* made from the goat milk of 4 per cent fat ($T_6$) was the lowest.

The data presented in Table 4 also indicate the rank value for *Rasagolla* prepared from *Chhana*. The *Rasagolla* prepared from *Chhana* of 4 per cent fat goat milk with lactic acid as coagulant ($T_5$) scored highest and is preferred by judges over others. The *Rasagolla* prepared from control *Chhana* was adjudged as the next best.

*Cost of production:* The cost of raw materials for the production of *Chhana* per kg was Rs. 16.47 for the control and Rs. 11.48, Rs. 14.20 and Rs. 16.86 for the goat milk of 3, 4 and 5 per cent fat, respectively. In other words, the cost of production of *Chhana* suitable for *Rasagolla* making is less when goat milk of 4 per cent fat is used than cow milk of 4 per cent fat.

TABLE 1. MEANS OF PER CENT YIELD OF *CHHANA* FROM COW AND GOAT MILKS AT DIFFERENT COMBINATIONS

| Source of milk | Fat level (%) | % yield of *chhana* with: Lactic acid | Sour whey | Citric acid |
|---|---|---|---|---|
| Cow | 4 | — | — | $16.65^a$ |
| Goat | 3 | $14.06^p$ | $15.10^b$ | $15.25^b$ |
| Goat | 4 | $15.25^b$ | $16.15^c$ | $16.50^a$ |
| Goat | 5 | $16.14^c$ | $16.92^a$ | $17.35^q$ |
| Means for coagulants | | $15.15^x$ | $16.05^y$ | $16.36^z$ |

Mean values superscribed by common letters do not differ significantly at 5% level.

TABLE 2. MEAN VALUES OF MOISTURE CONTENT IN *CHHANA* FROM COW AND GOAT MILK AT DIFFERENT COMBINATIONS

| Source of milk | Fat content (%) | % moisture is *chhana* with: Lactic acid | Sour whey | Citric acid |
|---|---|---|---|---|
| Cow | 4 | — | — | $57.54^a$ |
| Goat | 3 | $55.25^b$ | $57.44^a$ | $58.21^q$ |
| Goat | 4 | $54.80^c$ | $55.86^b$ | $56.35^r$ |
| Goat | 5 | $54.13^p$ | $54.95^{bc}$ | $55.58^{bd}$ |
| Means for coagulants | | $54.773^x$ | $56.084^y$ | $56.715^z$ |

Mean values superscribed by common letters do not differ significantly at 5% level.

TABLE 3. MEAN VALUES OF PER CENT FAT LOSSES IN *CHHANA* WHEY AT DIFFERENT COMBINATIONS

| Source of milk | Fat content (%) | % fat loss in whey with: Lactic acid | Sour whey | Citric acid |
|---|---|---|---|---|
| Cow | 4 | — | — | $0.45^r$ |
| Goat | 3 | $0.29^p$ | $0.36^b$ | $0.35^b$ |
| Goat | 4 | $0.39^q$ | $0.46^{ac}$ | $0.44^a$ |
| Goat | 5 | $0.48^c$ | $0.45^d$ | $0.52^d$ |
| Means for coagulants | | $0.38^x$ | $0.45^y$ | $0.43^z$ |

Mean values superscribed by common letters do not differ significantly at 5% level.

TABLE 4. AVERAGE RANK VALUE OF *CHHANA* AND *RASAGOLLA* MADE FROM DIFFERENT COMBINATIONS

| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ | $T_7$ | $T_8$ | $T_9$ | $T_{10}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| *Chhana* | 9.54 | 9.00 | 4.00 | 6.79 | 7.69 | 1.47 | 6.46 | 1.94 | 4.74 | 2.90 |
| *Rasagolla* | 9.08 | 7.83 | 5.00 | 6.25 | 9.83 | 1.16 | 4.16 | 2.00 | 6.75 | 2.91 |

For details of $T_1$ $T_2$................$T_{10}$ see text.

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# Triacylglycerol Composition of Cow Milk Fat

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**The triacylglycerols (TG) of cow milk fat were separated first into high molecular weight TGs (HMT), medium molecular weight TGs (MMT), and low molecular weight TGs (LMT). These were further resolved into saturated *trans* monoene, diene and polyene TGs using argentation thin layer chromatography (TLC). The HMT contained mostly the long chain fatty acids whereas most of the short chain acids of the milk fat were concentrated in LMT. The TG species separated on the basis of their degree of unsaturation had differing combination of fatty acids.**

There are a few reports on the triacylglycerol composition of cow milk fat.[1-6] The triacylglycerol (TG) composition of buffalo milk fat has recently been reported.[7] Many factors such as species, breed, feed and climatic conditions are known to alter the TG composition of the milk fat considerably. Recent studies have demonstrated how the altered TG composition of the fat affects its overall physical characteristics.[8,9] The present investigation was undertaken to obtain comparative data on the TG composition of Indian cow and buffalo milk fats under identical conditions of feeding and management.

## Materials and Methods

Cow milk samples were collected from cows (Tharparkar breed) maintained at the Institute. The animals selected were in their middle lactation (between 120 and 160 days after parturition).

*Fractionation of triacylglycerols according to molecular weight:* Isolation of TGs from milk and their fractionation were carried out as described by Breckenridge and Kuksis[2].

The total TGs were first fractionated on the basis of molecular weight of their component fatty acids. Approximately, 10-15 mg of TGs were applied as a thin layer (0.5 mm) of silica gel H (E. Merck, Darmstadt, Germany). The plates were developed in heptane-isopropyl ether-acetic acid (60:40:4, V/V/V). The TG bands were located by spraying the plate with 0.05 per cent solution of 2,7-dichlorofluorescein in 50 per cent methanol and viewing under UV light. The fastest and the slowest bands were designated high molecular weight triacylglycerols (HMT) and low molecular weight triacylglycerols (LMT) respectively. The faint band between HMT and LMT was designated as medium molecular weight triacylglycerols (MMT).

*Fractionation of triacylglycerols on the basis of their degree of unsaturation:* Ten milligram of the TG fraction was applied to a thin layer of silica gel G(0.5 mm) impregnated with 20 per cent (W/W) silver nitrate. The plates were developed in chloroform: methanol (100:1, V/V) and the TG bands were located as described above. The TG bands were identified by fatty acid (FA) analysis and by reference to standard TGs. Thus, from each TG fraction (HMT, MMT and LMT), five TG classes were obtained viz. saturated, *trans*-monoene, *cis*-monoene, diene and polyene TGs in order of decreasing mobility.

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*Estimation of TG/TG fractions:* Silica gels of the corresponding TG bands were scraped off the plates and extracted with 5 per cent methanol in diethyl ether. The relative proportions of these TG fractions and TG classes were determined by gas liquid chromatography (GLC) as their constituent methyl esters using methyl heptadecanoate (Applied Science Laboratories, USA) as an internal standard.

*Analysis of fatty acids:* The TGs were trans-esterified by the sealed tube method of de Man[10]. The chromatographic separations were carried out in a stainless steel column (5 ft × ⅛") packed with 100-120 Gaschrom-P coated with 10 per cent EGSS-X (Applied Science Laboratories, USA). The sample was injected at an initial temperature of 90°C. After emergence of 4:0, the oven temperature was programmed at the rate of 4°C/min to 180°C. The temperatures of the injection port and detector were 280°C and 250°C respectively. Nitrogen (90 ml/min) was used as the carrier gas. Weight response factors ($F_w$) for methyl esters were determined by chromatography of a standard mixture of methyl esters of FAs (Applied Science Laboratories, USA) as described by Smith[11], and peak areas were measured by triangulation.

**Results and Discussion**

*TG classes based on molecular weight:* The total TG of cow milk fat was separated into HMT, MMT and LMT. The mean values of three trials showed that cow milk fat contained 52.9 per cent of HMT, 18.9 per cent of MMT and 28.2 per cent of LMT. The corresponding values reported for buffalo milk fat[7] were 42.4, 17.1 and 40.5 per cent respectively, which indicated that cow milk fat contained higher proportion of HMT and lower level of LMT than buffalo milk fat.

Table 1 shows the fatty acid (FA) composition of whole cow milk fat and its TG fractions. The major fatty acids were 14:0, 16:0, 18:0 and 18:1 in HMT; 6:0, 14:0, 18:0 and 18:1 in MMT and 4:0, 14:0, 16:0 and 18:1 in LMT. The results indicate that certain fatty acids tended to concentrate in specific TG fractions: 18:1 in HMT, 6:0 in MMT and 4:0 in LMT. A comparison

TABLE 1. FATTY ACID COMPOSITION (WT. %) OF COW MILK FAT AND ITS TRIACYLGLYCEROL FRACTIONS OF DIFFERING MOLECULAR WEIGHTS

| Fatty acid | Whole fat | HMT | MMT | LMT |
|---|---|---|---|---|
| 4:0 | 3.7 | — | 1.2 | 19.9 |
| 6:0 | 2.6 | 0.2 | 11.5 | 5.5 |
| 8:0 | 2.3 | 0.4 | 4.7 | 2.9 |
| 10:0 | 3.5 | 2.1 | 6.6 | 3.3 |
| 10:1 | 0.3 | 0.2 | 1.2 | 0.2 |
| 12:0 | 3.3 | 2.5 | 4.3 | 3.5 |
| 14:0 | 12.3 | 12.2 | 11.4 | 12.9 |
| 14:1 | 0.7 | 1.0 | 1.2 | 0.8 |
| 15:0 | 0.8 | 1.3 | 1.6 | 1.5 |
| 16:0 | 28.5 | 29.0 | 28.3 | 27.4 |
| 16:1 | 1.4 | 1.1 | 0.6 | 0.7 |
| 18:0 | 13.2 | 16.2 | 8.4 | 6.2 |
| 18:1 | 25.3 | 30.8 | 17.1 | 14.0 |
| 18:2 | 1.2 | 1.7 | 1.1 | 0.8 |
| 18:3 | 0.9 | 1.3 | 0.8 | 0.5 |

HMT: High molecular wt triacylglycerol
MMT: Medium molecular wt triacylglycerol
LMT: Low molecular wt triacylglycerol

TABLE 2. PROPORTIONS (WT %) OF TRIACYGLYCEROL CLASSES OF DIFFERING LEVELS OF UNSATURATION PREPARED FROM HIGH, MEDIUM AND LOW MOLECULAR WEIGHT TRIACYLGLYCEROLS OF COW MILK FAT

| TG Classes | High molecular wt-TG | | Medium molecular wt-TG | | Low molecular wt-TG | | Whole TG |
|---|---|---|---|---|---|---|---|
| | Wt % in TG fraction | Wt % in whole milk fat | Wt % in TG fraction | Wt % in whole milk fat | Wt % in TG fraction | Wt % in whole milk fat | Wt % in whole milk fat |
| | 100.0 | 52.9 | 100.0 | 18.9 | 100.0 | 28.2 | 100 |
| Saturated | 23.2 | 12.3 | 35.8 | 6.8 | 38.9 | 11.0 | 30.1 |
| *Trans*-monoene | 9.3 | 4.9 | 8.2 | 1.5 | 7.6 | 2.1 | 8.5 |
| *Cis*-monoene | 33.0 | 17.5 | 33.8 | 6.4 | 33.7 | 9.5 | 33.4 |
| Diene | 26.1 | 13.8 | 17.7 | 3.3 | 14.6 | 4.1 | 21.2 |
| Polyene | 8.4 | 4.4 | 4.5 | 0.9 | 5.2 | 1.5 | 6.8 |

of the fatty acid composition of the TG fractions of cow milk fat in the present study with that of the buffalo milk fat[7] would reveal that the differences in the proportions of HMT and LMT fractions could be ascribed to 4:0, 18:0 and 18:1. Hence, the lower content of LMT in cow milk fat observed in this study compared to buffalo milk fat[7] could be due to lower amount of 4:0 in cow milk fat. Similarly, the higher content of HMT in cow milk fat reported here as compared to buffalo milk fat[7] can be attributed to the higher proportion of 18:1.

*TG classes based on unsaturation:* HMT, MMT and LMT of cow milk fat were fractionated by argentation TLC and the relative proportions of the TG classes of differing levels of unsaturation are given in Table 2. The results indicate that saturated, *Cis*-monoene, and diene TGs were the major classes of all the fractions, whereas *trans*-monoene and polyene TGs were minor constituents. Saturated TGs increased and the unsaturated TGs decreased with the decrease in the molecular weight of the TG fractions.

Buffalo milk fat prepared under identical conditions[7] showed that all the TG fractions had higher concentrations of saturated TGs and lower levels of the other TG species than those reported here for cow milk fat. In a comparable study on cow milk fat, Breckenridge and Kuksis[2-4] reported the proportions of saturated, monoene, diene, triene, and polyene TGs as 16.5, 36.7, 27.7, 12.9 and 6.2 per cent respectively in long chain TGs; 38.8, 38.3, 14.4, 8.7 and 6 per cent respectively in medium chain TGs and 45.0, 38.1, 11.7, 5.6 and 0 per cent, respectively in short chain TGs. Values for saturated TGs of HMT reported in this study (Table 2) are significantly higher and for unsaturated TGs are lower than the above values. Taylor and Hawke[5] also obtained higher values for unsaturated and lower values for saturated TG species. These variations could be attributed to the lower level of 18:1 and higher proportions of saturated fatty acids of the Indian cow milk fat.

Data in Table 3 present the distribution of fatty acids among various TG classes. The saturated TGs of HMT were composed mostly of 14:0, 16:0 and 18:0. Whereas the saturated TGs of MMT contained all the saturated fatty acids of milk fat in fair proportions, the saturated TGs of LMT had 4:0, 14:0 16:0 and 18:0 as the major acids. Both *trans* and *cis*-monoene TGs from the respective TG fractions had identical fatty acid composition. The major fatty acids in the diene TGs of HMT were 14:0, 16:0 and 18:1 suggesting that the TGs were mostly of two 18:1 in combination with either 16:0 or 14:0. In the MMT, the diene TGs were made up of two 18:1 in association with a medium chain or a short chain acid. Most of the diene TGs of LMT were composed of 4:0, 16:0, 18:1 and 18:2. In contrast to both

TABLE 3. FATTY ACID COMPOSITION (WT. %) OF TRIACYLGLYCEROL CLASSES OF DIFFERING LEVELS OF UNSATURATION PREPARED FROM DIFFERENT FRACTIONS OF COW MILK FAT

| Fatty acid | S | TM | CM | D | P |
|---|---|---|---|---|---|
| **High moleculat weight TGs** | | | | | |
| 4:0 | — | — | — | — | — |
| 6:0 | 0.7 | 0.6 | 0.5 | 0.6 | 0.3 |
| 8:0 | 2.5 | 2.2 | 1.6 | 0.8 | 1.2 |
| 10:0 | 5.7 | 4.9 | 2.6 | 1.8 | 2.0 |
| 10:1 | — | — | — | 0.3 | 1.0 |
| 12:0 | 5.8 | 5.2 | 3.2 | 1.5 | 2.4 |
| 14:0 | 19.8 | 13.2 | 12.8 | 6.8 | 7.6 |
| 14:1 | — | 0.5 | 1.1 | 2.3 | 2.8 |
| 15:0 | 2.2 | 1.5 | 1.4 | 1.5 | 1.2 |
| 16:0 | 39.0 | 29.5 | 30.8 | 18.8 | 18.4 |
| 16:1 | — | 0.3 | 0.9 | 2.1 | 1.9 |
| 18:0 | 24.3 | 14.2 | 15.5 | 8.6 | 6.4 |
| 18:1 | — | 27.7 | 29.6 | 54.9 | 41.9 |
| 18:2 | — | — | — | — | 7.4 |
| 18:3 | — | — | — | — | 5.5 |
| **Medium molecular weight TGs** | | | | | |
| 4:0 | 1.3 | 1.6 | 1.8 | 1.6 | 0.8 |
| 6:0 | 13.6 | 9.4 | 11.2 | 8.1 | 3.6 |
| 8:0 | 6.7 | 4.7 | 6.8 | 5.5 | 4.9 |
| 10:0 | 8.4 | 6.3 | 6.8 | 5.4 | 3.9 |
| 10:1 | — | — | 0.6 | 6.4 | 3.9 |
| 12:0 | 6.2 | 5.7 | 3.9 | 3.2 | 3.6 |
| 14:0 | 15.8 | 10.3 | 10.0 | 7.5 | 10.5 |
| 14:1 | — | 1.3 | 2.6 | 3.4 | 3.2 |
| 15:0 | 1.6 | 1.3 | 1.1 | 1.0 | 1.2 |
| 16:0 | 35.6 | 21.2 | 20.6 | 12.0 | 16.6 |
| 16:1 | — | 0.9 | 1.2 | 1.4 | 1.9 |
| 18:0 | 10.8 | 7.9 | 5.5 | 2.4 | 2.9 |
| 18:1 | — | 29.4 | 27.5 | 39.2 | 29.0 |
| 18:2 | — | — | — | 2.9 | 7.8 |
| 18:3 | — | — | — | — | 6.0 |
| **Low molecular weight TGs** | | | | | |
| 4:0 | 19.5 | 17.7 | 18.6 | 15.7 | 13.5 |
| 6:0 | 5.7 | 4.2 | 5.9 | 5.2 | 3.2 |
| 8:0 | 1.3 | 2.8 | 1.6 | 1.3 | 1.6 |
| 10:0 | 2.4 | 3.0 | 2.3 | 2.1 | 1.8 |
| 10:1 | — | 0.1 | 0.3 | 1.9 | 1.4 |
| 12:0 | 3.6 | 3.1 | 2.8 | 1.3 | 2.0 |
| 14:0 | 17.2 | 9.3 | 8.3 | 3.5 | 6.6 |
| 14:1 | — | 1.5 | 1.8 | 2.4 | 1.4 |
| 15:0 | 1.9 | 1.9 | 1.2 | 0.9 | 0.9 |
| 16:0 | 34.8 | 21.4 | 20.5 | 9.1 | 15.2 |
| 16:1 | — | 1.2 | 1.4 | 3.8 | 0.5 |
| 18:0 | 13.6 | 5.7 | 4.5 | 4.2 | 4.0 |
| 18:1 | — | 28.2 | 30.8 | 43.3 | 27.6 |
| 18:2 | — | — | — | 5.3 | 10.3 |
| 18:3 | — | — | — | — | 10.0 |

S: Saturated TG; TM: *Trans*-monoene TG; CM: *Cis*-monoene TG; D: Diene TG; P: Polyene TG

HMT and MMT, 18:2 and 18:3 emerged as an important constituent of diene and polyene TGs of LMT. Polyene TGs of the HMT and MMT fractions contained 14:0, 16:0, 18:1 and 18:2 as the major acids, whereas polyene TGs of LMT were composed of 4:0, 16:0, 18:1, 18:2 and 18:3.

The distribution of fatty acids among the various TG classes (Table 3) suggests that an increase in unsaturation corresponded with an increase in chain length of the fatty acids and a decrease in short chain acids of the TG classes. This reverse relation reflects that the unsaturated acids are synonymous with long chain acids. This was particularly apparent in the TG classes of LMT fraction, where one long chain fatty acid was in combination with two short chain or medium chain acid in saturated TGs. In TGs of greater unsaturation namely, diene and polyene TGs, two unsaturated long chain acids were in association with one medium or short chain acid. If this pattern was any different in HMT, it was because this fraction carried very small proportion of short chain acids and only 14:0, 16:0 and 18:0 were available for combination with 18:1 and 18:2.

Despite the variations in the fatty acid composition and the proportion of various TG classes between the Indian cow milk fat reported here and those for Western cows[2,3] and buffalo[7], the general trend in the association of fatty acids among the TG classes appears to be similar.

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# Single Cell Protein Production from Wheat Straw and Its Fractions by *Myrothecium verrucaria*


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**Wheat straw consists of 68% holocellulose (25% hemicellulose and 43% cellulose).** *Myrothecium verrucaria* **was grown on whole straw and its cellulosic fractions such as holocellulose and cellulose for single cell protein (SCP) production. Five days incubation period with 0.05 g N-ammonium dihydrogen ortho-phosphate/1, with pH of 4.5 and cellulose as the sole source of carbon proved optimum for maximum (30.5%) SCP production. Amino acid analysis of** *M. verrucaria* **biomass showed the presence of all essential amino acids except the sulphur containing amino acids.**


About 35 million tonnes of wheat is produced every year in India and an equal amount of wheat straw[1]. Wheat straw contains cellulose, hemicellulose and lignin in large quantities but it is poor in protein content.

Efforts have been made to produce protein rich animal feed by microbial fermentation of cellulosic substrates, but association of lignin reduces the enzymatic accessibility[3]. Thus, lignocellulosic substrates require physical or chemical pretreatment before fermentation. Several methods of pretreatments have been tried by earlier

workers[3–7]. These methods, however, have disadvantages: either they do not yield products of acceptable food value or there is loss of hemicellulose during delignification. Pretreatment with sodium chlorite and acetic acid has advantage over other methods as they remove only lignin. In the present investigation, efforts have been made to compare single cell protein (SCP) production by *i*) wheat straw as such, *ii*) with straw (holocellulose) delignified by sodium chlorite, and *iii*) cellulose isolated from holocellulose.

## Materials and Methods

*Chemical analysis:* Wheat straw was analysed for holocellulose (cellulose+hemicellulose), cellulose[8], water soluble sugars[9], crude protein[10], lignin, crude fat and total mineral contents[11].

*Medium:* The basal synthetic medium as reported by Chahal *et al.*[12] was used for SCP production. Fifty ml of the basal medium was placed in 250 ml Erlenmeyer flask containing 500 mg of substrate (60-mesh) which comprised of wheat straw, holocellulose, and cellulose used separately. The flasks were autoclaved at 121°C for 20 min.

*Preparation of inoculum:* The basal synthetic medium was fortified with 15 g glucose/l. Flasks were inoculated with actively growing culture of *Myrothecium verrucaria* and incubated for 5 days at 28±1°C on a rotary shaker. The mycelium was washed and macerated in 100 ml sterile distilled water with Virtis homogenizer for 20 sec. Each flask was inoculated with 2 ml of this macerated suspension.

*SCP production and analysis: Myrothecium verrucaria*, a cellulolytic fungus, was obtained from the culture collection of the department of Microbiology. After the incubation period, the contents of flask were filtered through Whatman No. 1 filter paper and the weight of the biomass (mycelium + undigested substrate) was determined. The filter paper along with biomass was dried at 60°C till constant weight. The dried biomass was ground to 40 mesh and was analysed for its nitrogen content by the method of McKenzie and Wallace[10]. Amino acid of the protein in biomass was estimated by using a Beckman Model 116 amino acid analyser after hydrolysing the biomass with 6 N HCl at 110°C for 24 hr under vacuum.

## Results and Discussion

*Chemical treatments:* Chemical analysis of wheat straw showed that it contains 68 per cent holocellulose (43 per cent cellulose+25 per cent hemicellulose), 1.35 per cent water soluble sugars, 2 per cent crude protein, 0.61 per cent crude fat, 13.7 per cent lignin and 11.9 per cent total minerals. Hemicellulose which is usually lost during alkali treatment along with lignin[13], was retained with cellulose while treating the straw with sodium chlorite and acetic acid. Therefore, this method of delignification had significant advantage over alkali treatment.

*Production of SCP: Myrothecium verrucaria*, a cellulolytic non-toxic fungus was grown on different substrates for 5 days. The total protein production was 132 mg/g of holocellulose, 166 mg/g of cellulose and 52 mg/g of wheat straw. The protein was 18.2, 26.9, and 5.9 per cent for holocellulose, cellulose and wheat straw respectively. When lignin was removed from wheat straw, protein increased more than three times. The protein production was less on holocellulose as compared to cellulose. This might be due to the presence of pentoses (xylose and arabinose) in hemicellulose which were poorly utilized

TABLE 1. EFFECT OF INCUBATION PERIOD ON SCP PRODUCTION BY *M. VERRUCARIA* ON DIFFERENT SUBSTRATES

| Days | Wheat straw | | Holocellulose | | Cellulose | |
|---|---|---|---|---|---|---|
| | Protein* (%) | Total protein (mg/g substr) | Protein* (%) | Total protein (mg/g substr) | Protein* (%) | Total protein (mg/g substr) |
| 2 | 3.1 | 30 | 5.3 | 48 | 8.1 | 72 |
| 3 | 3.8 | 36 | 9.8 | 84 | 13.8 | 110 |
| 4 | 4.0 | 44 | 14.4 | 114 | 19.8 | 144 |
| 5 | 6.1 | 54 | 18.2 | 132 | 27.3 | 172 |
| 6 | 7.5 | 64 | 21.8 | 144 | 27.3 | 172 |
| 7 | 9.4 | 78 | 21.7 | 143 | 27.1 | 170 |
| 8 | 9.4 | 78 | 21.7 | 142 | 27.0 | 170 |

Based on three replicates. *On dry wt basis; N×6.25

TABLE 2. EFFECT OF NITROGEN SOURCES ON SCP PRODUCTION BY *M. VERRUCARIA* ON VARIOUS SUBSTRATES

| Nitrogen source | Wheat straw | | Holocellulose | | Cellulose | |
|---|---|---|---|---|---|---|
| | Protein* (%) | Total protein (mg/g substr) | Protein* (%) | Total protein (mg/g substr) | Protein* (%) | Total protein (mg/g substr) |
| Ammonium chloride | 4.9 | 44 | 11.5 | 82 | 15.0 | 108 |
| Ammonium dihydrogen orthophosphate | 9.7 | 82 | 23.0 | 148 | 28.1 | 178 |
| Ammonium nitrate | 4.6 | 40 | 11.4 | 84 | 13.3 | 100 |
| Ammonium sulphate | 8.0 | 68 | 15.6 | 114 | 24.7 | 156 |
| Potassium nitrate | 9.3 | 76 | 21.5 | 142 | 27.0 | 170 |
| Sodium nitrate | 4.4 | 40 | 10.1 | 82 | 16.3 | 116 |
| Urea | 7.4 | 64 | 21.4 | 138 | 26.3 | 164 |

Based on three replicates.
Incubation period 7 days; *On dry wt basis, N×6.25

by most of the fungi[14,15]. Some of the sugars which were also in methylated form[16] might resist the enzymatic degradation. Similarly Mandels and Reese[17], and Moo-Young *et al*[15], reported that increased methylation of carboxymethyl cellulose imparts resistance to enzymatic hydrolysis.

*Effect of incubation period:* Both total protein and per cent protein were maximum after 5,6 and 7 days of incubation on cellulose, holocellulose and wheat straw respectively (Table 1). From this, it is evident that not only the total and per cent protein increased on delignified straw, but also there was decrease in incubation period which have immense economic importance in production of SCP on industrial scale.

*Effect of nitrogen sources:* Seven nitrogen sources viz., ammonium chloride, ammonium dihydrogen orthophosphate, ammonium nitrate, ammonium sulphate, potassium nitrate, sodium nitrate and urea were tried to find out the most suitable nitrogen source for SCP production. Each nitrogen source was added in amount equivalent to 400 mg N/l of the medium. Among these, ammonium dihydrogen orthophosphate showed maxi-

TABLE 3. EFFECT OF HYDROGEN ION CONCENTRATION ON SCP PRODUCTION BY *M. VERRUCARIA* ON VARIOUS SUBSTRATES

| pH | Wheat straw | | Holocellulose | | Cellulose | |
|---|---|---|---|---|---|---|
| | Protein* (%) | Total protein (mg/g substr) | Protein* (%) | Total protein (mg/g substr) | Protein* (%) | Total protein (mg/g substr) |
| 3.0 | 6.1 | 44 | 9.1 | 54 | 11.0 | 78 |
| 3.5 | 7.3 | 64 | 17.0 | 104 | 19.1 | 118 |
| 4.0 | 9.3 | 76 | 19.2 | 122 | 25.8 | 148 |
| 4.5 | 10.2 | 88 | 22.1 | 152 | 28.5 | 180 |
| 5.0 | 9.4 | 78 | 21.5 | 146 | 27.9 | 176 |
| 5.5 | 9.1 | 76 | 18.3 | 112 | 24.9 | 136 |
| 6.0 | 6.4 | 42 | 14.2 | 84 | 19.8 | 106 |

Based on three replicates.
Incubation period 7 days; *On dry wt basis N×6.25

TABLE 4. SCP PRODUCTION BY *M. VERRUCARIA* UNDER OPTIMUM* CULTURE CONDITIONS OF VARIOUS SUBSTRATES

| Substrate | Protein (%) | Total protein (mg/g substr) | Protein** (folds increase) | Protein (mg/g of original substr) |
|---|---|---|---|---|
| Wheat straw | 11.2 | 96 | 5.1 | 96.0 |
| Holocellulose | 26.8 | 170 | 13.4 | 115.6 |
| Cellulose | 30.5 | 192 | 15.1 | 81.6 |

**As compared to unfermented wheat straw. *pH 4.5 and 0.5 g N/l of ammonium dihydrogen orthophosphate
Based on three replicates.

mum protein production on all the three substrates and it was closely followed by potassium nitrate (Table 2). Wheat straw proved to be the poorest substrate compared to holocellulose and cellulose. These findings are corroborative of the observations of Miller and Johnson[18]. However, Sinden *et al.*[19] have reported that the rate of breakdown of cellulose by *M. verrucaria* was higher when ammonical N and nitrate N were employed in the ratio of 1:24 than when ammonical N alone was used as the sole source of nitrogen.

*Effect of different concentrations of ammonium dihydrogen orthophosphate:* Ammonium dihyrodgen orthophosphate which was adjudged the best N source in the previous experiment was added to give 0.3, 0.4, 0.5 and 0.69 N/l of the basal medium. Results showed that the concentration of 0.5 g N/l of the medium proved to be optimum for SCP production which is 186 mg/g of cellulose and 86 mg/g of wheat straw. Singh and Kalra[20] reported that addition of (0.4 g N/l of the medium) potassium nitrate produced maximum SCP when *M. verrucaria* was grown on corncobs.

*Effect of hydrogen ion concentration:* All the previous experiments were conducted at pH 5.0, but in this experiment different pH were tried (3,3.5,4,4.5,5.5 and 6). The results given in Table 3, show that pH 4.5 proved to be optimum for SCP production when *M. verrucaria* was grown in all the three media. pH, higher or lower than optimum had adverse effect on SCP production. Further, it was observed that the initial pH of 4.5 increased to 6.7, and 7.5 after 7 days fermentation of the media containing wheat straw, holocellulose and cellulose, respectively. Singh and Kalra[20] also recorded similar observations while employing corn cobs as carbon source.

*SCP production under optimum conditions:* When *M. verrucaria* was grown under optimum conditions (pH 4.5 and 0.5 g N/l as ammonium dihydrogen orthophosphate) on wheat straw, holocellulose and cellulose for 7,6 and 5 days respectively, the SCP production increased by 5.1 fold on wheat straw, 13.4 fold on holocellulose and 15.1 fold on cellulose as compared to the protein content of wheat straw. However, from economic point of view, maximum protein of 115.6 mg/g of the substrate was produced from holocellulose (Table 4).

*Amino acid analysis:* Amino acid analysis of the biomass of *M. verrucaria* showed that it was rich in essential amino acids in comparison to FAO reference standard[21] except for methionine (Table 5). Earlier workers also observed this when *M. verrucaria* was grown on corn cobs[20] and sugarbeet pulp[22].

It can be concluded that pretreatment of lignocellulosic material is important to enhance SCP production. Further, SCP from *M. verrucaria* has good nutritional quality.

TABLE 5. AMINO ACID PROFILE OF *M. VERRUCARIA* BIOMASS GROWN UNDER OPTIMUM CONDITIONS

| Amino acid | *M. verrucaria* (g/16 gN) | FAO[19] (g/16 gN) |
|---|---|---|
| Isoleucine | 5.8 | 4.2 |
| Leucine | 10.0 | 4.8 |
| Phenylalanine | 3.5 | 2.8 |
| Tyrosine | 1.5 | — |
| Threonine | 4.2 | 2.6 |
| Tryptophan | N.D. | 1.4 |
| Valine | 5.9 | 4.2 |
| Arginine | 4.2 | — |
| Histidine | 2.1 | — |
| Lysine | 4.6 | 4.2 |
| Methionine | 1.0 | 2.2 |
| Cystine | N.D. | 2.0 |

N.D.—Not determined
Data based on three replicates.

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# RESEARCH NOTES

## A NOTE ON SCREENING THE INDIAN SORGHUM COLLECTION FOR POPPING QUALITY

**The Indian Sorghum germplasm collection (3682 accessions) maintained at the ICRISAT Center was screened for superior pop sorghums and 36 accessions exhibiting more than 80% popped grains per sample were identified. In general, the pop sorghums possessed a small grain size, medium thick pericarp, hard endosperm and a very low germ/endosperm size ratio.**

Sorghum (*Sorghum bicclor* L. Moench) grain is consumed in India mostly in three different forms, namely, unleavened bread (*roti*), dumpling (*sangati*, *mudde*) and boiled rice-like product (*annam*). In addition, some sorghums have been used traditionally by farming families specifically for popping, and popped sorghum is used in several snack foods[1]. Considerable variation has been reported for popping quality among recently developed sorghums[2]. The popping quality of sorghum grain could be improved through technological methods[3]. However, identification of cultivars which possess inherent superior popping quality[4] is preferred by breeders for development of high yielding cultivars suitable for popping. The world collection of sorghum

TABLE 1. GRAIN QUALITY CHARACTERS OF POP SORGHUMS FROM INDIA

| S.No. | IS.No. | Place of Origin | Corne$^a$—ousness | Pericarp$^b$ thickness | Weight (g/100) | Breaking$^c$ strength (kg) | % Popping |
|---|---|---|---|---|---|---|---|
| 1 | IS-1192 | Andhra Pradesh | 1 | Thin | 2.77 | 7.9 | 80 |
| 2 | IS-1199 | Tamil Nadu | 1 | Thick | 2.11 | 8.5 | 80 |
| 3 | IS-2185 | Maharashtra | 3 | Medium | 2.63 | 7.6 | 92 |
| 4 | IS-2205 | Uttar Pradesh | 3 | Medium | 2.38 | 5.6 | 80 |
| 5 | IS-4596 | Maharashtra | 1 | Medium | 1.66 | 6.6 | 82 |
| 6 | IS-4803 | Gujarat | 3 | Medium | 2.97 | 7.0 | 90 |
| 7 | IS-4939 | Maharashtra | 2 | Medium | 3.02 | 7.4 | 82 |
| 8 | IS-5111 | Maharashtra | 1 | Medium | 1.87 | 8.2 | 91 |
| 9 | IS-5112 | Maharashtra | 1 | Medium | 1.80 | 5.2 | 87 |
| 10 | IS-5113 | Andhra Pradesh | 1 | Medium | 1.75 | 6.4 | 87 |
| 11 | IS-5115 | Andhra Pradesh | 2 | Medium | 1.49 | 5.5 | 87 |
| 12 | IS-5116 | Andhra Pradesh | 2 | Medium | 1.96 | 7.4 | 87 |
| 13 | IS-5285 | Andhra Pradesh | 2 | Medium | 2.25 | 6.1 | 91 |
| 14 | IS-5418 | Tamil Nadu | 1 | Thin | 1.89 | 6.9 | 85 |
| 15 | IS-5484 | Karnataka | 2 | Thin | 2.37 | 6.0 | 82 |
| 16 | IS-5566 | Karnataka | 3 | Medium | 2.43 | 6.1 | 90 |
| 17 | IS-5604 | Karnataka | 2 | Medium | 2.46 | 5.6 | 98 |
| 18 | IS-5638 | Karnataka | 2 | Medium | 2.19 | 6.9 | 97 |
| 19 | IS-5646 | Karnataka | 3 | Medium | 2.27 | 4.2 | 94 |
| 20 | IS-5648 | Karnataka | 3 | Thin | 2.27 | 5.3 | 85 |
| 21 | IS-5651 | Karnataka | 2 | Medium | 2.80 | 7.0 | 96 |
| 22 | IS-5653 | Karnataka | 2 | Medium | 2.38 | 7.5 | 96 |
| 23 | IS-5655 | Karnataka | 2 | Thin | 2.40 | 5.8 | 96 |
| 24 | IS-5665 | Karnataka | 3 | Thin | 2.51 | 7.0 | 90 |
| 25 | IS-5726 | Bihar | 1 | Medium | 1.36 | 7.1 | 85 |
| 26 | IS-5732 | Bihar | 1 | Medium | 1.71 | 7.5 | 82 |
| 27 | IS-5741 | Bihar | 1 | Thick | 1.72 | 8.7 | 90 |
| 28 | IS-5849 | Madhya Pradesh | 1 | Thick | 1.74 | 7.9 | 86 |
| 29 | IS-5910 | Madhya Pradesh | 1 | Medium | 1.99 | 8.2 | 82 |
| 30 | IS-6243 | West Bengal | 1 | Thick | 1.80 | 6.3 | 80 |
| 31 | IS-6248 | West Bengal | 2 | Medium | 2.63 | 8.6 | 92 |
| 32 | IS-8347 | Maharashtra | 1 | Medium | 2.47 | 7.8 | 88 |
| 33 | IS-17860 | Andhra Pradesh | 2 | Medium | 2.17 | 6.4 | 85 |
| 34 | IS-17903 | Andhra Pradesh | 1 | Medium | 2.01 | 6.1 | 80 |
| 35 | IS-18363 | Maharashtra | 2 | Thin | 3.08 | 7.3 | 80 |
| 36 | IS-18488 | Andhra Pradesh | 2 | Thin | 3.31 | 8.5 | 82 |

*a.* Corneousness of the grain was scored on a scale of 1 to 5 where 1 represented very hard endosperm and 5 represented completely floury endosperm

*b.* Pericarp thickness was scored visually

*c.* Breaking strength was measured with a KIYA rice grain hardness tester

germplasm maintained by the Genetic Resources Unit of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) contains 17,986 accessions. Out of these, 3682 originate from India. It is not known how many, and which of these possess superior popping quality, although colloquial names of a few accessions indicate their use for popping. Viraktamath and Desikachar[2,4] considered an extensive program of screening of cultivars suitable for such food products as popped sorghum useful. With such a screening objective, a systematic assessment of all the sorghum cultivars originating from India for their popping quality was attempted.

Grain samples chosen for popping studies were drawn from a permanent cold store (regularly maintained at 4°C and 40 per cent RH). They had a moisture content of 9 to 10 per cent. A 10g sample of the grain was placed on an open steel pan maintained at 300 to 325°C and stirred briskly. The grain samples were not subjected to any pretreatment. The number of completely puffed grains per sample were recorded after 1½ min and expressed as "per cent popping". Preliminary observations indicated that traditional pop sorghums can readily be identified by the instant and complete puffing of 90 per cent or more of the grains in the sample with a synchronous hissing noise. Normal sorghums, however, exhibit a much lower percentage of puffed grains per sample (0 to 50 per cent) and the individual grains frequently show delayed and partial puffing. In order to rapidly screen large numbers of cultivars, a 10g grain sample from each of the 3682 Indian sorghums maintained at ICRISAT was scored for per cent popping on a scale of 1 to 5 where, 1=81 to 100 per cent, 2=61 to 80 per cent, 3=41 to 60 per cent, 4=21 to 40 per cent and 5=0 to 20 per cent. Those accessions which obtained a score of 1 or 2 were reexamined several times using larger samples. Thirty six accessions showed 80 per cent or more popping. The identity and physical grain quality characters of the superior pop sorghums are presented in Table 1. Most of these exhibited small grain size, white colour, medium thick pericarp, a breaking strength of about 7 kg and a hard endosperm. This is in conformity with the comments made by earlier workers[1], that several cultivars did not show a satisfactory popping quality inspite of possessing all these characters. It was also observed visually that pop sorghums have a very low germ/endosperm size ratio and the germ is located at a corner of the hilar region. Volume of the puffed product was not measured in this study, since variability for this trait in the cultivars selected was observed to be limited. Puffed product volume appeared to be mostly dependent on the grain size. The availability of sorghum cultivars whose grains exhibit superior popping quality without any pretreatment, should be of significance to food technologists and breeders. Utilization of these pop sorghums in breeding programs aiming at improved popping quality might be rewarding.

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## EFFECT OF CALCIUM AND BORON ON THE INCIDENCE OF INTERNAL BREAKDOWN IN ALPHONSO MANGO (*MANGIFERA INDICA* LINN).

**Influence of calcium and boron on the internal breakdown was studied. Preharvest sprays of Alphonso mango trees with calcium (5000 ppm) and boron (500 ppm) either alone or in combination, and post-harvest dip treatment with calcium did not reduce the incidence of internal breakdown, and enhanced the calcium level of the fruit.**

Internal breakdown of 'Alphonso' mango is a serious physiological disorder noticed during ripening and storage. The incidence varies from 1 to 88 per cent in different 'Alphonso' growing regions.[1] Causative factors for the onset of this disorder are not known. It was thought that it may be due to deficiency of minerals, especially calcium as it has been shown to play a role in physiological disorders of fruits[2,3] and plants[4]. Preliminary study[1] has indicated low level of calcium in the affected pulp. Hence, a study was made during 1977 and 1978 to find out the effect of preharvest sprays of calcium and boron and post-harvest treatment with calcium on the incidence of breakdown.

Sixteen mango trees for each treatment with 4 replications were used. Drench preharvest sprays of (*i*) calcium chlorde at 5000 ppm, (*ii*) boric acid at 500 ppm, and (*iii*) a combination of the two were used, 3 times

Contribution No. 1113.

at monthly intervals commencing from fruit set. Two hundred and fifty mature fruits were collected from each tree, ripened at 28°C and observed for the incidence of breakdown.

Post-harvest treatments consisted of: (*i*) fruits with preharvest spray alone, (*ii*) Preharvest spray combined with post-harvest dip at 2500 ppm and 5000 ppm of $CaCl_2$ at 28°C for 5 min. Fruits with specific gravity more than 1.02 were dipped in (*a*) $CaCl_2$ solution of 5000 ppm concentration at 28°C for 5 min and (*b*) fruit temperature was raised to 35-37°C by dipping in hot water at 52°C for 5 min and then dipped in $CaCl_2$ solution (5000 ppm) at 10-12°C for 5 min. This was supposed to enhance the calcium uptake by creating reduced internal gas pressure. One hundred fruits were used for each treatment and one per cent of Tween-80 was used as a surfactant. Calcium was estimated using flame photometer.

The mean incidence of breakdown was 17 per cent during 1977 and 29 per cent in 1978. There were no significant differences due to preharvest sprays in the percentage breakdown, as compared to the control and also among the treatments. Post-harvest treatments either alone or combined with preharvest spray did not reduce the incidence of internal breakdown in mangoes. In fruits with specific gravity more than 1.02, overall incidence was around 41 per cent indicating that these fruits are more susceptible to breakdown as reported earlier[5]. There was no reduction in breakdown, when these were given a post-harvest dip with $CaCl_2$. Calcium level of the fruit varied from 280 to 350 ppm (dry weight), but this did not increase significantly due to pre-or post-harvest treatments with calcium. A report on controlling the development of this disorder in mango by pre-and post-harvest treatment has appeared[6]. But our observations in the present study are contradictory to this.

It can be concluded that this disorder of mango has not been affected by calcium treatment. Further detailed study is necessary to understand this problem.

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# STORAGE BEHAVIOUR OF A LOW COST FOOD SUPPLEMENT IN FLEXIBLE CONSUMER PACKAGES

**Moisture sorption and storage studies were carried out on a food blend based on corn, sugar and fat. The product had initial and critical moisture contents (dry weight) of 5.4% and 8.2% respectively. The food blend packed (500 g) in low and high density polyethylene pouches were stored for 4½ months and analysed for moisture, free fatty acids and peroxide values. The product had a shelf life of 1½ months at 38°C and 90% RH, whereas at 27°C and 65% RH, the shelf-life was 4½ months in LDPE pouches. Shelf-life was longer (4½ months) in HDPE pouches under both conditions of storage.**

A high calorie, low cost food blend containing ground corn, sugar (jaggery) and fat was stored in unit consumer packages. The product was intended for distribution in areas having varying temperature and relative humidity conditions. The food blend had an initial moisture content of 5.1 per cent (as is basis) and the composition of the product was reported[1] as proteins–15.4 per cent, ether extractives–18.4 per cent and ash–4.9 per cent.

*Moisture sorption studies:* The food blend was mixed in a blender and subjected to moisture adosrption–desorption studies by exposing 6-8 g of the sample to relative humidities varying between 11 and 92 per cent under saturated salt solution at 27±1°C[3]. The samples were weighed at regular intervals to find out the changes in moisture and physical conditions like caking, darkening and onset of mould growth. The food blend had an initial moisture content of 5.4 per cent on dry weight basis and the relation between the moisture content and relative humidity is shown in Table 1. The safe critical moisture content for free flowing and acceptable taste was 7.8 per cent (8.2 per cent on dry weight basis) with an equilibrium relative humidity of 56 per cent. The moisture sorption isotherm at 27°C, when plotted showed a well defined sigmoid shape indicating that the carbo-

TABLE 1. MOISTURE SORPTION STUDIES OF FOOD BLEND AT 27°C.

| Relative humidity (%) | Equilibrium moisture content (%) | | Quality |
|---|---|---|---|
| | Wet basis | Dry wt basis | |
| 11 | 3.5 | 3.7 | Free flowing |
| 22 | 4.3 | 4.6 | ,, |
| 32 | 5.0 | 5.3 | ,, |
| 44 | 6.0 | 6.4 | ,, |
| 56 | 7.8 | 8.2 | Acceptable |
| 64 | 9.2 | 9.7 | Slight off odour. |
| 75 | 14.3 | 15.1 | Lumpy and rancid odour |
| 86 | 22.4 | 23.7 | Mould growth |
| 92 | 26.8 | 28.3 | ,, |

hydrates and proteins present in the product are mainly responsible for the water binding property.

*Storage studies:* The food blend (500 g) was packed in LDPE and HDPE flat pouches of size 15×20 cm made of 50 microns thick films having water-vapour transmission rates of 8.7 and 2.0 g/m$^2$ d at 38°C and 90 per cent R.H. differential when tested according to standard procedure[2]. The samples were stored for 4½ months at 27°C±1°C (room temperature) with 65±2 per cent R.H. and 38±1°C with 90±2 per cent R.H. and the changes in the total weight of the package, free fatty acid and peroxide values were determined at regular intervals by standard methods[4].

The results presented in Table 2 indicate that the product stored at room temperature (27°C) had picked up 2.1 per cent additional moisture in LDPE pouches and only 0.7 per cent in HDPE pouches at the end of 4½ months storage. After 3 month's storage seepage of fat was observed in LDPE pouches and after 4½ months in the case of HDPE pouches. The initial free fatty acid (FFA) value of 0.87 per cent (as oleic acid) increased only to 0.9 per cent at the end of 4½ months storage in both types of polyethylene pouches. The initial peroxide value (m.eq. per kg of fat) of 2.31 had risen to 5.71 in LDPE and only 4.26 in HDPE pouches at the end of the storage period.

Storage studies carried out at 38°C showed that the product had attained the critical moisture level of 7.8 per cent (wet basis) after 1½ months in LDPE pouches, whereas the shelf life was 4½ months in HDPE film pouches. In LDPE pouches after one month of storage, slight seepage of fat was observed but it was more pronounced after 2 months. The product had developed a bitter taste after 3 months of storage when the FFA was 1.2 per cent. The product packed in HDPE pouches attained the critical level at 4½ months. The FFA values increased steadily in both LDPE and HDPE pouches to 1.55 and 1.1 per cent (as oleic acid) respectively after 4 months. The rise in FFA was more in LDPE probably due to the higher transmission to water vapour. The peroxide value reached maximum after 3 months and then declined, which could be attributed to the depletion of head-space oxygen as well as the degradation of per-

TABLE 2. STORAGE STUDIES OF FOOD BLEND PACKED IN LOW AND HIGH DENSITY POLYETHYLENE POUCHES

| Storage condition | | Storage period (months) | Low density polyethlene | | | High density polyethylene | | |
|---|---|---|---|---|---|---|---|---|
| Temp (°C) | R.H. (%) | | Moisture (%) | FFA (%) | PV | Moisture (%) | FFA (%) | PV |
| 27 | 65 | Initial | 5.1 | 0.87 | 2.31 | 5.1 | 0.87 | 2.31 |
| ,, | ,, | 1½ | 5.9 | 0.87 | 3.86 | 5.3 | 0.87 | 3.08 |
| ,, | ,, | 3 | 6.7 | 0.87 | 5.36 | 5.6 | 0.90 | 4.58 |
| ,, | ,, | 4½ | 7.2 | 0.90 | 5.71 | 5.8 | 0.90 | 4.26 |
| 38 | 92 | 1 | 7.2 | 0.94 | 3.38 | 5.8 | 0.86 | 3.04 |
| ,, | ,, | 2 | 9.3* | 1.01 | 5.76 | 6.5 | 0.86 | 3.38 |
| ,, | ,, | 3 | 10.2 | 1.18 | 9.37 | 7.1 | 0.88 | 4.58 |
| ,, | ,, | 4 | 12.1 | 1.55 | 3.30 | 7.7 | 1.09 | 3.50 |
| ,, | ,, | 4½ | — | — | — | 7.8** | — | — |

Moisture is on wet basis:

FFA: Free fatty acids, as % oleic acid.

*P.V.: Perioxide value, as milli equivalents of peroxides per kg of fat.

**Critical moisture content.

oxides into ketones, aldehydes and other ingredients. Since the oxygen transmission rates of HDPE and LDPE as reported[5] were 500-1000 and 6000-8000 cc/m$^2$/day for 25 microns film at NTP conditions, the PV for LDPE was almost double compared to that of HDPE pouch at 38°C at the end of 3 months. Thus, it was concluded that at the accelerated condition (38°C) of storage, the shelf life of the product was $1\frac{1}{2}$ and $4\frac{1}{2}$ months in LDPE and HDPE pouches respectively, whereas at room temperature (27°C) the shelf life was nearly $4\frac{1}{2}$ months in LDPE pouches; HDPE offered a longer shelf-life for the product.

Each empty pouch weighs about 3.8 g and the cost works out nearly Re 0.10 per pouch which is not high for a low cost food blend. If it is envisaged to bulk package (25-50 kg) the product, inclusion of an inner liner of 200 gauge HDPE film in hessian sacks would provide better protection as the surface area exposed to unit weight of the product will be less than that of a 500 g unit package. Further, the advantages of retarded seepage of fat, good protection against development of off flavour and better resistance to the penetration by insects could be attained by using HDPE film liners.

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## DETERMINATION OF TIN COATING WEIGHT IN DIFFERENTIAL TINPLATE BY CLARKE'S TEST

**Clarke's test has been slightly modified to determine the thickness of tin coating in differential tinplate. Tin coating weight on both the sides can be determined separately by this method.**

Differential tinplate has different thickness of tin coating on the two sides of the base plate. By the gravimetric method suggested by Clarke[1], tin coating weight is normally obtained as combined weight on both the faces of the tinplate. There is a volumetric method[2] of determining total tin coating weight utilised for reference purposes, which can also be used for determining tin coating weight in differential tinplates. Since in this method the entire sample is dissolved, two specimens must be used if tin coating weight on both faces is separately required. Moreover, this method is cumbersome for routine analysis. Tin coating determination in differential tinplate by coulometric method[3] requires sophisticated apparatus. Hence attempts were made to adopt Clarke's test for this purpose. The results of this investigation are reported in this note. The method is simple, reproduciable and useful in the quality control laboratories and in food canning factories.

Tin plate specimen with a convenient area of 35 cm$^2$ to 100 cm$^2$ is degreased with carbon tetrachloride, dried and weighed to the nearest milligram or less. One surface of the specimen is coated with bees wax. The first coating is applied at about 90°C both vertically and horizontally to ensure perfect coating and second coating was applied at 75°C in the same way with the help of a match stick rolled with cotton at one end. The other surface of the specimen is degreased with a piece of cotton dipped in carbon tetrachloride. The specimen is immersed in antimony trichloride solution in such a way that the wax coated surface is at the bottom and tin surface facing upwards until about 1 min after all gas evaluation has ceased. It is then removed, washed immediately in running water and loosely adherent deposit of antimony removed by mopping with a soft cotton wool swab. The wax coating is removed by melting and rubbing with cotton and thoroughly degreased with carbon tetrachloride. The specimen is dried and weighed again. To find the coating thickness on the other surface, wax is coated to the surface on which tin coating has been removed and the above procedure is repeated. From the weight differences, tin coating weight is calculated and expressed as g/m$^2$ or lb/base box. A correction is made for the amount of iron in the iron-tin alloy layer. The correction is minus 0.35 g/m$^2$ or 0.0156 lb/base box on each side.

If the tinplate can is lithographed or lacquered, the tincoating weight is determined on the plain surface by coating wax to the lithographed or lacquered side first. Subsequently the lithograph or lacquer is removed by any one of the following procedures and the tin coating weight on that surface is determined in similar way. To remove lithograph or lacquer the specimen is boiled for 25-30 min in dimethyl formamide (for some type of lacquers or lithographs it takes more time). Then it is washed in running cold water rubbing with a cotton wool swab. Boiling in dimethyl formamide will not affect tin coating

TABLE 1. TIN COATING WEIGHT IN DIFFERENTIAL TINPLATE

| Sample No. | Inside exposed to $SbCl_3$ solution (outside wax coated) | | | Outside exposed to $SbCl_3$ solution (inside wax coated) | | |
|---|---|---|---|---|---|---|
| | Weight diff. (g) | Total coating wt* (g/m2) | Tin coating wt.** (g/m2) | Weight diff. (g) | Total coating wt.* (g/m2) | Tin coating wt.** (g/m2) |
| 1 | 0'0402 | 11.48 | 11.13 | 0.0200 | 5.71 | 5.36 |
| 2 | 0.0413 | 11.80 | 11.45 | 0.0205 | 5.86 | 5.51 |
| 3 | 0.0442 | 12.63 | 12.28 | 0.0220 | 6.28 | 5.93 |
| 4 | 0.0453 | 12.94 | 12.59 | 0.0224 | 6.39 | 6.04 |

*Includes alloyed tin wt. **After substracting correction factor Area taken is 35 $cm^2$

weight. Delacquering reagent is also prepared by dissolving 30 g of sodium hydroxide pellets or flakes in 375 ml water and 125 ml of ethylene glycolmonoethylether (high boiling point). The solution is boiled and the tin plate is dipped for ½-2 min and the sample removed with the help of a magnet (round magnetic needle connected to rubber tube which will help the removal of the sample from the boiling solution) and washed with water. A few types of lacquers may be removed by rubbing with a cotton swab dipped in acetone.

Removal of lithograph or lacquer may also quickly be done by dipping and shaking in the boiling solution of a mixture of 1 part of aniline and 10 parts of 12 per cent ammonia for 1 to 2 min.

Tin coating weights on each surface determined have been given in Table 1. The total tin coating weight on both the surfaces by Clark's test compares well with the combined weight of the two surfaces carried out separately by wax coating method suggested in these studies. Wax does not interfere in the reaction. Bees wax is found to be more suitable as compared to the other waxes.

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## CHEMICAL COMPOSITION OF *BRASSICA* SEEDS

**Four varieties, each of *Brassica campestris* and *Brassica juncea* were studied for their chemical composition. Oil content and mineral content of the two species showed little variations. *B. campestris* had higher protein, crude fibre and lower content of allylisothiocyanates than *B. juncea*. Lysine and methioine were more in *B. campestris* as compared to *B. juncea*. Thus *B. campestris* is found to be nutritionally superior to *B. juncea*.**

The information on the chemical composition including the amino acid make up of *Brassica* species grown in India is limited. In an earlier communication, we reported the fatty acid composition of *Brassica juncea*[1] (Raya) and *Brassica campestris*[2] (Toria). In the present study some promising strains from the *Brassica* species namely *Brassica juncea* L. Cess var. 'Raya' and *Brassica campestris* L. Var. 'Toria' were selected and evaluated for chemical constituents with special reference to amino acid composition.

Representative samples of *Brassica* species having brown coloured seeds, were collected from the crop sown in rabi 1977 at Punjab Agricultural University, Ludhiana. The oil content, protein, crude fibre, allylisothiocyanates and mineral content were estimated by AOAC methods[3]. The quantitative analysis of amino acids was carried out on an amino acid analyser (Beckman-Model 120C) as described earlier[4].

The proximate composition of the two *Brassica* species is given in Table 1. The oil content varied from 40.00 to 44.75 per cent in the two *Brassica* species with mean values of 41.68 per cent in *B. campestris* and 41.17 per cent in *B. juncea*. Thus, there was only a small variation in oil content of the two types. Bechyne and Kondra[5] reported a range of oil content of brown seeds from 38.4 to 46.2 per cent in summer turnip rape. However, the variation of oil content in *B. campestris* was greater than in *B. juncea*. Recently Labana *et al*[1,6] reported oil

TABLE 1. PROXIMATE COMPOSITION OF *BRASSICA* SPECIES

| *Brassica* species | Oil (%) | Protein (%) | Minerals (%) | Crude fibre (%) | Allyliso thiocyanates (%) |
|---|---|---|---|---|---|
| *B. campestris* | | | | | |
| M 3 | 40.00 | 22.82 | 4.08 | 13.35 | 0.26 |
| TL 5 | 44.75 | 29.05 | 4.16 | 10.20 | 0.22 |
| Shamgarh | 41.05 | 27.87 | 3.94 | 8.90 | 0.23 |
| ITSA | 40.92 | 23.23 | 4.50 | 7.90 | 0.29 |
| Mean | 41.68 | 25.77 | 4.17 | 10.09 | 0.25 |
| CD at 5% | 3.28 | 1.34 | 0.38 | 3.76 | 0.06 |
| CD at 1% | 6.02 | 2.45 | 0.70 | 6.89 | 0.12 |
| *B. juncea* | | | | | |
| RLM 29/25 | 40.97 | 34.06 | 4.38 | 7.45 | 0.52 |
| RLM 198 | 41.62 | 27.87 | 4.16 | 8.45 | 0.55 |
| RLM 188 | 42.07 | 33.52 | 4.32 | 11.76 | 0.41 |
| RL 18 | 40.02 | 28.87 | 4.14 | 7.60 | 0.36 |
| Mean | 41.17 | 31.08 | 4.25 | 8.82 | 0.46 |
| CD at 5% | 1.43 | 3.78 | 0.32 | 3.21 | 0.32 |
| CD at 1% | 2.63 | 6.83 | 0.58 | 5.90 | 0.58 |

CD - Critical difference.

content values varying from 36.0 to 42.7 per cent in *B. juncea*. The protein content ranged from 22.82 to 33.52 per cent with a mean value of 25.77 per cent in *B. campestris* and 31.0 per cent in *B. juncea*, which indicated that *B. juncea* contained significantly higher protein. Labana *et al.*[6] observed a protein content of 26.2 to 31.5 per cent in *Brassica juncea* mutants.

*B. campestris* had higher content of crude fibre (10.90 per cent), but lower content of allylisothiocyanates (0.25 per cent) as compared with *B. juncea*. The differences in allylisothiocyanates contents were found to be significant. Much variation was not found in the mineral content of the two species. Loof and Appelqvist[8] reported 10.5 per cent crude fibre in brown seeds and 7.2 per cent in yellow seeds of *B. juncea*. Labana *et al.*[6] also reported the range of crude fibre from 8.3 to 13.3 per cent in *B. juncea* mutants. The correlation studies among all these biochemical constituents revealed no significant correlations.

The amino acid composition of *Brassica* species is given in Table 2. The mean amino acid content was quite comparable to that reported by Josefsson[9]. Among the amino acids, glutamic acid was highest in both the species. Aspartic acid and leucine were also present in

TABLE 2. AMINO ACID COMPOSITION OF *BRASSICA* SPECIES (g/16 gN)

| Amino acid | *Brassica campestris* | | | | | | | *Brassica juncea* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M 3 | TL 5 | S. Garh | ITSA | Mean | CD at 5% | CD at 1% | RLM 29/25 | RLM 198 | RLM 188 | RL 18 | Mean | CD at 5% | CD at 1% |
| **Essential** | | | | | | | | | | | | | | |
| Lysine | 7.14 | 6.17 | 5.67 | 7.76 | 6.75 | 4.24 | 7.78 | 6.79 | 5.41 | 5.47 | 5.53 | 5.80 | 2.98 | 5.47 |
| Methionine | 2.13 | 1.54 | 1.42 | 1.65 | 1.69 | 1.40 | 2.57 | 1.49 | 1.42 | 1.53 | 1.12 | 1.39 | 0.84 | 1.53 |
| Valine | 6.96 | 6.03 | 5.51 | 6.55 | 6.25 | 2.83 | 5.20 | 6.67 | 4.94 | 5.37 | 5.46 | 5.61 | 3.34 | 6.13 |
| Isoleucine | 4.83 | 4.04 | 4.42 | 4.68 | 4.43 | 1.56 | 2.86 | 4.75 | 3.61 | 3.96 | 4.99 | 4.31 | 2.93 | 5.33 |
| Leucine | 7.91 | 8.10 | 7.99 | 8.46 | 8.10 | 1.09 | 2.01 | 8.20 | 7.71 | 7.46 | 7.00 | 7.59 | 2.25 | 4.13 |
| Tyrosine | 2.69 | 2.55 | 2.68 | 2.99 | 2.73 | 0.84 | 1.54 | 2.66 | 2.25 | 2.43 | 2.36 | 2.42 | 0.78 | 1.43 |
| Phenylalanine | 4.50 | 4.22 | 4.11 | 4.18 | 4.25 | 0.77 | 1.41 | 4.48 | 4.07 | 4.22 | 4.28 | 4.26 | 0.76 | 1.40 |
| Threonine | 4.28 | 3.05 | 3.47 | 3.78 | 3.65 | 2.33 | 4.28 | 3.26 | 3.05 | 3.18 | 3.10 | 3.15 | 0.41 | 0.76 |
| **Nonessential** | | | | | | | | | | | | | | |
| Histidine | 3.07 | 2.71 | 2.93 | 3.91 | 3.15 | 2.36 | 4.33 | 2.97 | 2.77 | 3.50 | 2.44 | 2.92 | 2.00 | 3.67 |
| Aspartic acid | 7.45 | 7.23 | 7.20 | 7.91 | 7.45 | 1.48 | 2.71 | 7.84 | 6.46 | 7.23 | 7.13 | 7.17 | 2.54 | 4.67 |
| Arginine | 6.95 | 8.27 | 7.12 | 8.23 | 7.64 | 3.17 | 5.82 | 6.16 | 6.11 | 6.97 | 6.12 | 6.34 | 1.89 | 3.47 |
| Serine | 2.22 | 2.91 | 3.97 | 4.04 | 3.29 | 3.95 | 7.25 | 3.26 | 3.61 | 4.10 | 3.11 | 3.52 | 1.98 | 3.63 |
| Glutamic acid | 17.25 | 19.02 | 21.59 | 15.95 | 18.45 | 10.98 | 20.16 | 23.25 | 22.35 | 21.87 | 21.73 | 22.30 | 3.09 | 5.67 |
| Proline | 5.86 | 4.93 | 5.48 | 5.27 | 5.41 | 1.64 | 3.02 | 5.56 | 4.81 | 4.97 | 5.36 | 5.17 | 1.55 | 2.85 |
| Glysine | 6.58 | 5.29 | 5.73 | 6.99 | 6.15 | 3.49 | 6.41 | 5.94 | 5.28 | 5.77 | 5.89 | 5.72 | 1.36 | 2.49 |
| Alanine | 4.42 | 3.49 | 3.41 | 3.67 | 3.75 | 2.08 | 3.81 | 3.70 | 3.31 | 3.20 | 3.77 | 3.43 | 1.27 | 2.33 |

CD - Critical difference.

TABLE 3. CORRELATION COEFFICIENTS AMONG ESSENTIAL AMINO ACIDS OF DIFFERENT *BRASSICA*

| | Valine | Methionine | Isoleucine | Leucine | Phenylalanine | Threonine | Histidine | Arginine |
|---|---|---|---|---|---|---|---|---|
| Lysine | 0.902** | 0.644 | 0.842** | 0.754* | 0.482 | 0.738* | 0.585 | 0.487 |
| Valine | | 0.698 | 0.902** | 0.590 | 0.784* | 0.716* | 0.348 | 0.330 |
| Methionine | | | 0.560 | 0.502 | 0.497 | 0.841** | 0.445 | 0.348 |
| Isoleucine | | | | 0.533 | 0.691 | 0.765* | 0.377 | 0.202 |
| Leucine | | | | | 0.042 | 0.410 | 0.541 | 0.634 |
| Phenylalanine | | | | | | 0.473 | 0.037 | 0.206 |
| Threonine | | | | | | | 0.456 | 0.256 |
| Histidine | | | | | | | | 0.506 |

Significant at* P - .05; **P - .01

considerable amounts. Lysine commonly observed as the first limiting amino acid in vegetables was found to be higher (6.75 per cent) in *B. campestris* as compared to that in *B. juncea* (5.80 per cent). Methionine was also present in higher amounts in *B. campestris* (1.49 per cent). *B. juncea* showed higher content of only glutamic acid in comparision with *B. campestris*. Appelqvist and Nair[10] compared the amino acid composition of *B. campestris* with that of *B. napus* and reported *B. campestris* to be higher in amount with respect to several amino acids. Abidi and Tripathi[11] observed a range of 0.56 to 1.92 per cent for methionine in *B. juncea*.

Correlation coefficients among the essential amino acids are given in Table 3. Lysine was found to have a positive and significant correlation with valine, isoleucine, leucine, and threonine. Valine was positively and significantly correlated with isoleucine, phenylalanine and threonine. Threonine was also positively and significantly correlated with methionine and isoleucine. The significant and positive association of threonine with lysine and methionine is of particular interest as these are the three amino acids which are generally found most deficient in vegetables.

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**REVIEW PAPER**

# Pyrrolizidine Alkaloids: A Review

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**The review covers the occurrence of pyrrolizidine alkaloids in the *Senecio* spp; the hepatotoxic nature of chemical, their separation and chemical assay methods. Attempts have been made to relate the toxicity of the compound to chemical nature and structure. The toxic effects observed in livestock have been delineated and the $LD_{50}$ values for various pyrrolizidine alkaloids have been discussed.**

## Introduction

Naturally occurring toxic alkaloids in foods and feeds have been a topic of considerable attention in recent years[1-3]. Among poisonous plants that are harmful to live stock, the *Senecio* species are prominent as a result of their hepatotoxic properties. A large number of organic compounds responsible for the chronic disease. Pyrrolizidine alkaloidosis, in grazing animals have been isolated from various species of *Senecio*. Although the toxic alkaloids are found in many other genera such as *Crotalaria*, *Heliotropium*, *Amsinckia*, *Echium*, *Cynoglossum*, and *Trichodesma*, they are conventionally referred to the genus *Senecio*, since it was the first to attract attention in most countries. Consumption of *Senecio* plants during shortage of other feeds have caused serious problems and occasionally heavy losses in farm animals. In man, the reported cases of poisoning resulted from contaminated cereal foods and from the use of the *Senecio* plants in medicine. The transfer of the pyrrolizidine alkaloids from *Senecio jacoboea* (tansy ragwort) into the milk of lactating cows and goats[4] could conceivably exert their effect in human population in areas where the plant is extensively growing. A considerable amount of research effort has been put in towards the pyrrolizidine alkaloids in view of their role in liver and lung damage. Consequently, several reports on chemical[5-9], biological and pathological[9-10], biochemical[11-12], and toxicological[13-16] aspects of the pyrrolizidine alkaloids have been published. Since the plants containing pyrrolizidine alkaloids are involved one way or other in animal feed or human foods, it is the intention of this article to pool the relevant information.

## Occurrence, separation, and assay

The pyrrolizidine alkaloids are found mostly in three botanical families of which genus *Senecio* (family, compositae) comprises of the largest alkaloid-bearing species. A number of species of *Crotalaria* (Leguminosae), *Heliotropium* (Boraginaceae), and *Cynoglossum* (Boraginaceae) are a good source of the alkaloids. The common names of some of the plant species containing pyrrolizidine alkaloids are listed in Table 1. More than a hundred

Table 1. Common names of some pyrrolizidine alkaloid-bearing plant species

| Botanical name | Common names | Ref. No. |
|---|---|---|
| *Amsinckia intermedia* | Fiddleneck | 47 |
| | Tarweed | 47 |
| | Fireweed | 47 |
| | Yellow forget-me-not | 48 |
| *Crotalaria* | Rattle box | 47 |
| | Rattle pod | 48 |
| *Crotalaria dura* } | Wild lucerne | 49 |
| *Crotalaria globifera* } | Jaagsiektebossie | 49 |
| *Crotalaria fulva* | Whiteback | 48 |
| *Crotalaria mucronata* | Streaked rattle pod | 48 |
| *Crotalaria retusa* | Wedge-leaved rattlepod | |
| | Earring plant | 49 |
| *Echium plantagineum* | Viper's bugloss | 47 |
| | Salvation Jane | 48 |
| *Hetiotropium europaeum* | Common heliotrope | 48 |
| | Caterpillar weed | 48 |
| | Potato weed | 47 |
| *Senecio jacoboea* | Common ragwort | 48 |
| | Stinking Willie | 47 |
| *Senecio latifolius* | Dan's cabbage | 49 |
| | Groundsel | 49 |
| | Ragwort | 49 |
| | Rhodesia ragwort | 49 |
| *Senecio retrorsus* | Dan's cabbage | 49 |
| | Ragwort | 49 |
| *Senecio ridelli* | Woolly groundsel | 47 |
| | Thread-leaf groundsel | 47 |
| | Riddell's groundsel | 47 |
| *Senecio spartioides* | Broom groundsel | 47 |
| *Senecio vulgaris* | Common groundsel | 47 |

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alkaloids have been isolated from various plant species and structurally identified as pyrrolizidine derivatives.

Most naturally occurring pyrrolizidine alkaloids behave as a mixture of tertiary base and N-oxide. The latter may be revealed by an additional spot on paper chromatogram[17]. The N-oxide form being highly soluble in water cannot be extracted from an aqueous medium by chloroform. In order to include total alkaloids, Koekemoer and Warren[18] and Culvenor and Smith[19] reduced N-oxides to free bases prior to their extraction with organic solvent. Also, the procedure of Culvenor and Smith[19] eliminated the possible decomposition of the alkaloids, during drying of plant material, alkali hydrolysis, reaction with chloride ions or halogenated solvents. However, there is no general procedure for isolation of pyrrolizidine N-oxides from plant material. The alkaloid mixture may be obtained in a relatively pure form by adsorbing an aqueous alcoholic plant extract on a sulfonic acid resin and subsequent elution with ammonium hydroxide[20].

Chalmers *et al.*[21]. while characterizing the alkaloids found that paper, thin layer, and gas chromatography are satisfactory for their resolution on the basis of their different partition-adsorption, adsorption-base strength, and volatility respectively. The pyrrolizidine alkaloids appeared on paper chromatogram as distinctively coloured spots when developed by ascending flow of an organic phase of a 50 per cent mixture of butanol in 5 per cent acetic acid. An alkali-treated silica gel G plate held for at least a day and developed with methanol was useful for reproducible mobilities of the alkaloids relative to the solvent front. Retention time data were important in arriving at structural features of new alkaloids or derivatives in the series of ester alkaloids.

A quantitative assessment of the alkaloid can be made by the procedure of Culvenor and Smith[19] which involves titration against 0.01 N p-toluene-sulfonic acid in chloroform with dimethyl yellow as an indicator. Although pyrrolizidine alkaloids have no characteristic UV absorption or fluorescence useful for detection, Mattocks[22] made spectrophotometric determination possible by converting them into a pyrrole which produced a red colour with the modified Ehrlich reagent (4-dimethylaminobenzaldehyde and boront rifluoride in methanol).

## Chemical structure and toxicity

The Senecio alkaloids form a structurally distinct group of compounds characterized by the presence of a pyrrolizidine nucleus (Fig. 1). This system usually contains a double bond at 1.2 position and generally exists as monoester, diester or cyclic diester of amino alcohol (Table 2) at positions 1 and/or 7 The nature of esterifying acid, particularly important in structure elucidation and toxicity, is indicated by catalytic hydrogenation and hydrolytic reactions.

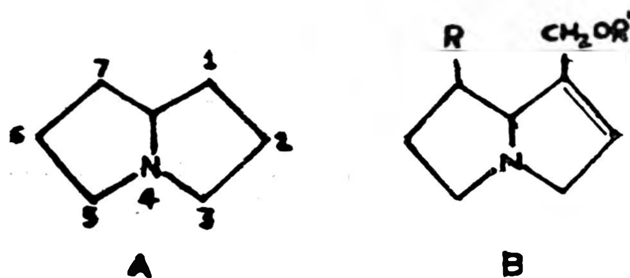


Fig. 1. Nucleus and skeleton of pyrrolizidine alkaloids. (A) Pyrrolizidine; (B) Amino-alcohol(s): Heliotridine, (R - OH, R' - H); Retronecine, (R = OH, R' - H); and Supinidine, (R, R' - H).

Several workers have discussed hepatotoxic effects of a number of alkaloids in relation to their chemical structure[9,11,13-15,23-25]. Thus the toxic action was attributed to the presence of a double bond in the 1,2 position and esterified primary hydroxyl group in the pyrrolizidine ring. This was also evident from the fact that non hepatotoxic alkaloids showed no double bond, modified pyrrolizidine ring with a partial N-C8 bond or a lack of ester function[9]. The nature of substituent at position 7, which may occur as H, $\alpha$ -or $\beta$-OH, $\alpha$ -or $\beta$-O-acyl, has no control over hepatotoxicity of pyrrolizidines. However, a branched alkyl chain of esterifying acid at position 1 is an essential requirement for hepatotoxicity. The degree of esterification showed profound influence on toxicity especially when the alkaloids occurred as cyclic diesters. The allylic ester structure makes the active alkaloid a potential alkylating agent capable of bringing about changes in nucleic acids or enzymes which lead to inhibition of cell division and mutation[9]. Also, low water solubility and low base strength are associated with acute hepatotoxicity.

Mattocks[11] gave several reasons including route of administration of the alkaloid, metabolizing enzyme system, resistance of some species of animals or organisms, toxicity of synthetic derivatives, and chemical reactivity of the alkaloids which indicated that the alkaloid toxicity is due to metabolites. Non cytotoxic effects of certain alkaloids[25] as well as lower toxicity of pyrrolizidine N-oxides than the parent alkaloids[26] have suggested that hydrolysis and N-oxidation may be involved in detoxification processes. Relatively less toxicity of N-oxides can be explained on the basis of their high solubility in water which makes a suitable medium for rapid excretion of the metabolities. However, the high toxicity of retrorsine N-oxide in male rats injested orally resulted from the alkaloid base formed by reduction in the gut[11]. Jago *et al.*[26] examined sheep urine after a dose of heliotrine and found a metabolite which lacked

TABLE 2. THE ESTERIFYING R AND/OR R' GROUPS OF SOME PYRROLIZIDINE ALKALOIDS[9]

| Amino-alcohol | Alkaloid | Esterifying groups, (acids) |
|---|---|---|
| Heliotridine | 7-Angelylheliotridine (Rivularine) | R = $CH_3CH{:}C(CH_3)COO-$, (Angelic acid)<br>R' = H |
| | Echinatine | R = OH<br>R' = $-COC(i\text{-}pr)OHCH(CH_3)OH$, (Viridifloric acid) |
| | Europine | R = OH<br>R' = $-COC[COH(CH_3)_2]OHCH(CH_3)OCH_3$, (Lasiocarpic acid) |
| | Heliosupine | R = $CH_3CH{:}C(CH_3)COO-$, (Angelic acid)<br>R' = $-COC[COH(CH_3)_2]OHCH(CH_3)OH$, (Echimidinic acid) |
| | Heliotrine | R = OH<br>R' = $-COC(i\text{-}pr)OHCH(CH_3)OCH_3$, (Heliotric acid) |
| | Heliotrine-N-oxide (N—→O bond) | R and R' same as Heliotrine |
| | Lasiocarpine | R = $CH_3CH{:}C(CH_3)COO-$, (Angelic acid)<br>R' = $-COC[COH(CH_3)_2]OHCH(CH_3)OCH_3$, (Lasiocarpic acid) |
| | Lasiocarpine-N-oxide (N—→O bond) | R and R' same as Lasiocarpine |
| Retronecine | Echimidine | R = $CH_3CH{:}C(CH_3)COO-$, (Angelic acid)<br>R' = $-COC[COH(CH_3)_2]OHCH(CH_3)OH$, (Echimidinic acid) |
| | Jacobine[a] | O (epoxide bridging CH and C)<br>$CH_3CH - CCH_2CH(CH_3)C(CH_3)OH$, ('Jacobinecic' acid)<br>COO- = R ; -OC = R' |
| | Jaconine[a] | $CH_3CH(Cl)COHCH_2CH(CH_3)C(CH_3)OH$, ('Jaconinecic' acid)<br>COO- = R ; -OC = R' |
| | Latifoline | R = $CH_3CH{:}C(CH_3)COO-$, (Angelic acid)<br>CO / \ $CH_3CH$ O<br>R' = $-COC$——$CHCH_3$. (Latifolic acid)<br>OH |
| | Monocrotaline[a] | $CH_3CHCOH(CH_3)C(CH_3)OH$, (Monocrotalic acid)<br>COO- = R ; -OC = R' |
| | Retrorsine[a] | $CH_3CH{:}CCH_2CH(CH_3)COHCH_2OH$, (Isatinecic acid)<br>COO- = R ; -OC = R' |
| | Senecionine[a] | $CH_3CH{:}CCH_2CH(CH_3)C(CH_3)OH$, (Senecic acid)<br>COO- = R ; -OC = R' |
| | Seneciphylline[a] | $CH_2$ (=, double bond)<br>$CH_3CH{:}CCH_2CC(CH_3)OH$, (Seneciphyllic acid)<br>COO- = R ; -OC = R' |

TABLE 2. CONTINUED......

| Amino-alcohol | Alkaloid | Esterifying groups, (Acids) |
|---|---|---|
| | Spectabiline[a] | $CH_3CHC(OCOCH_3)$ $(CH_3)C(CH_3)OH$, (β-Acetylmonocrotalic acid)<br>COO– (=R) –OC (R′–) |
| Supinidine | Cynaustine | R = H<br>R′ = $-COC(i\text{-}pr)OHCH(CH_3)OH$, (Viridifloric acid) |
| | Heleurine | R = H<br>R′ = $-COC(i\text{-}pr)\ OHCH(CH_3)OCH_3$, (Heliotric acid) |
| | Supinine | R = H<br>R′ = $-COC(i\text{-}pr)OHCH(CH_3)OH$, (Trachelanthic acid) |

[a]The R and R′ groups are involved in the formation of cyclic diester.

a methyl group in its ester side chain· This reaction is also known to reduce toxicity. Another reaction that converts heliotrine to non-toxic bases in the sheep rumen is due to the reduction by a hydride ion from NADH[27-28]. The products, 7 α -hydroxy-1 methylene-8 α -pyrrolizidine and 7 α -hydroxy-1β-methyl-8 α -pyrrolizidine' lost their toxicity due to the absence of allylic ester group. Although epoxides have been suspected to be more toxic than the parent alkaloids[9,29], the experimental evidence obtained with two epoxides of monocrotaline has eliminated such a possibility[30-31]. Mattocks[11] also failed to demonstrate the conversion of epoxides to metabolic pyrroles which are responsible for some or all of the toxic effects of the alkaloids. A pyrrole derivative because of its conjugated nitrogen ring system, activates an ester group for a nucleophilic attack by such groups as amines or thiols and forms relatively stable alkylation product. Thus cell damage or death could occur from alkylation of cell components by extremely active pyrrolic esters[11]. According to Butler *et al.*[32], pyrrole derivatives can produce toxic effects similar to that caused by pyrrolizidine alkaloid, but at much lower dose level. Further support for this was obtained by the hepatotoxic nature of pyrroline diester[33]. Non-toxic action of rosamarinine resulted from the stable structure of its metabolic pyrrole[34-35] which contains remaining ester group unconjugated with the nitrogen.

The formation of pyrrolic metabolites has been shown to occur in the rat liver microsomes[35] as well as in human embryo liver[36]. Subsequently, the metabolites may transfer to other organs and cause damage[37-38]. Although the liver microsomal fraction is capable of converting alkaloids to N-oxides[39], the resultant products do not serve as intermediates in the formation of the metabolic pyrroles[35,40]. Mattocks[11] described the formation of the pyrroles as metabolic mistake, since such reactions as N-and C-hydroxylation, N-oxidation, and N-and O-dealkylation tend to form detoxified water soluble derivaties which can be rapidly eliminated from the body. It is the bound pyrroles which are associated with the cellular components for a longer period cause toxic effects.

**Pharmacology and toxicology**

Poisoning in animals from plants containing pyrrolizidine alkaloids has been known throughout the world under different names[9,15]. General symptoms of poisoning in livestock are loss in weight, severe diarrhea, inability to stand, coma, and rapid death within about a month. However, there is no definite disorder in farm animals resulting from consumption of these poisonous plants. This is mainly because of the chemical composition of the plants. The alkaloids have produced liver lesions in poultry, sheep, horses, pigs, and dogs; and lung lesions in sheep, poultry, and pigs. Apart from the liver damage, horses have been adversely affected by neurological problems; and sheep by haemolytic disorder. Schoental[14] claimed that some of the pyrrolizidine alkaloids are carcinogenic, however this has been considered doubtful by many workers.

The toxic symptoms caused by the poisonous plants could also be produced in experimental animals with a suitable dose of a specific alkaloid. The pattern of toxicity notably peracute, acute, cytotoxic, and chronic, depends much upon animal species, age, structure of the alkaloid, and the mode of alkaloid injestion[11]. Hyperacute or peracute deaths are caused by a dose larger than acute type, generally preceded by convulsions which take place within a short time. Acute intoxication has been marked by haemorrhagic necrosis of the liver cells[37,41] which may lead to death within a week. The $LD_{50}$ values for various pyrrolizidine alkaloids given to rats intraperitoneally showed a wide range from 34 to 5000 mg/kg based on their chemical structure and physical properties (Table 3). A number of workers have investigated

TABLE 3. $LD_{50}$ VALUES[a] OF VARIOUS PYRROLIZIDINE ALKALOIDS GIVEN TO RATS BY INTRAPERITONEAL INJECTION

| Amino-alcohol | Alkaloid | Sex | $LD_{50}$(mg/kg) | Ref. No. |
|---|---|---|---|---|
| Heliotridine | 7-Angelylheliotridine | M | 260[b] | 9 |
| | Echinatine | M | 350 | |
| | Europine | M | 1000 | |
| | Heliosupine | M | 60 | |
| | Heliotrine | M | 300 | |
| | | F | 340[b] | |
| | | M | 280 | 11 |
| | | MF | 274.2 | 50 |
| | Heliotrine-N-oxide | M | 5000 | 9 |
| | | F | 2500 | |
| | Lasiocarpine | M | 72 | 9 |
| | | F | 79 | |
| | | M | 77 | 11 |
| | | MF | 88.1 | 50 |
| | Lasiocarpine-N-oxide | M | 547 | 9 |
| | | F | 181 | |
| Retronecine | Echimidine | M | 200 | 9 |
| | Jacobine | F | 138 | |
| | Jaconine | F | 168 | |
| | Latifoline | M | 125 | |
| | Monocrotaline | M | 175 | |
| | | F | 189 | |
| | | MF | 91.7 | 50 |
| | | M | 109 | 11 |
| | | F | 230 | |
| | Rerorsine | MF | 38.4 | 50 |
| | | M | 34 | 11 |
| | | F | 150 | |
| | | M | 35 | 14 |
| | Senecionine | M | 85 | 9 |
| | | M | 50 | 11 |
| | Seneciphylline | M | 77 | 9 |
| | | F | 83 | |
| | | MF | 80 | 50 |
| | Spectabiline | M | 220 | 9 |
| Supinidine | Cynaustine | M | 260 | 9 |
| | Heleurine | M | 140 | |
| | Supinine | M | 450[b] | |
| | | MF | 211.9 | 50 |

[a]Based on survivors up to 3 days[9,14], 4 days[11], and 7 days[50].
[b]All deaths occurred within 1 hour.

the development of chronic lesions in the rat liver within a few weeks to years after repeated administration of very small doses or a single dose[42-44]. The lesion consisting of grossly enlarged liver cells (megalocytes) may lead to death as a result of liver failure from almost fully occupied giant cells. A chronic lung lesion has also been shown to be fatal[38]. In general, toxic action of pyrrolizidine alkaloids are more pronounced in young rats than adult ones[29,45]. The toxic effects of pyrrolizidine alkaloids in man as observed mostly in West Indies[46] were of acute nature and were recognized as a veno-occulsive disease. More recently, Culvenor[16] has summarized preventive measures of the alkaloid poisoning in animals and human beings.

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**FORM IV**

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## BOOK REVIEWS

*Advances in Nutritional Research: Vol.* 3, Ed. by Harold H. Draper, Plenum Press, New York—London, 1980, Pp. 365, Price £ 32.50.

The present volume continues the tradition of publishing in-depth reviews on topics of contemporary interest. Three articles are devoted to lipids. The first by H.O. Bang and J. Dyerberg gives an account of the diet composition, plasma lipid patterns and hemostatic variables of Greenland Eskimos amongst whom ischemic heart diseases or thrombotic disorders are conspicuously absent. The second by Claudio Galli discusses 'Dietary influences on prostaglandin synthesis' including conversion of polyunsaturated fatty acids into prostaglandins, effects of dietary constituents on accumulation in tissues of polyunsaturated fatty acids, and on formation of prostaglandins and the metabolic effects thereof. The third article by Frank D. Sauer and John K. G. Kramer entitled 'The metabolism of long-chain monoenoic fatty acids on heart muscle and their cardiopathogenic implications' discusses at length the recent controversial topic on the relationship of erucic and related fatty acids on cardiac health.

Three articles relate to trace elements in nutrition. The first of these by C.E. Casey and K. M. Hambidge covers 'Trace element deficiencies in man' including Zn, Cu, Cr, Se, Mn, Co, Mo, F, Ni, V, Si, Sn and As. The second one describes 'Stable isotope method for bioavailability assessment of dietary minerals in humans' (M. Janghorbani and Vernon R. Young). The third article by Forrest H. Nielson summarises the 'Evidence of the essentiality of As, Ni and V and discusses their possible nutritional significance'.

The article 'Protein in the nutrition of the preterm infant: biochemical and nutritional considerations' by Niels C. R. Ratha traces the development of enzymes involved in protein synthesis and degradation in the foetus and discusses the implications in the infant nutrition. An article which may prove to be of special interest to food scientists is 'current concepts of i.v. hyperalimentation' by M. Deitel and Linda D. Macdonald. Finally, Yoshio Ueno surveys information on mycology, chemistry and toxicology of tricothecene mycotoxins.

The volume will no doubt prove of interest to research scientists in the field of nutrition, food science and medical sciences.

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*Introduction to Fishery Byproducts:* by M. Windsor and S. Barlow, Fishing News Book Ltd., 1981 pp. 187, Price £ 13.50.

The eight chapters of the book deal with Introduction; Fish meal production; Use of fish meal in animal nutrition; Fish oil production and use in foods; Fish silage; Hydrolysed fish products; Fish protein concentrates (FPC) and other methods of utilizing fishery byproducts.

The book includes methods of analyses and provides list of organizations which supply information on processes and/or products. The book is well illustrated with clear diagrams.

However, the book cannot be called an exhaustive one as considerable amount of work done on different aspects of fishery by products in various countries has not been included.

Chitin/chitosan are two valuable byproducts of shrimp processing inaustry which could have been covered in detail as they are getting more importance currently.

M. N. MOORJANI
C.F.T.R.I., MYSORE.

*Autooxidation in Food and Biological Systems:* By (Eds.) Michael G. Simic and Marcus Karel, Plenum Press, New York and London 1979, pp. 659, Price 65 £.

This is a very useful collection of papers by leading workers in the area of autooxidations and antioxidants in biological systems. These contributions were presented at a workshop organised jointly by the US Army Research and Development Command and Massachusetts Institute of Technology and held at Natick, October, 29-31, 1979. A few contributions were presented at the IFT Basic Symposium on "Food lipids in St. Louis, Missouri on June 8-9, 1979".

The contributions have been grouped under five sections namely (1) mechanisms of autooxidations; (2) food and model systems; (3) antioxidants; (4) biochemical systems and (5) biological systems.

In the first section namely, mechanisms of autooxidation, are included (*a*) involvement of radicals in the initiation of autooxidation of linoleate or other PuFA, (*b*) formation of nitrous acid by hydrogen abstraction during interaction between $NO_2$ and PuFA, (*c*) inhibition of photooxidative deterioration of vegetable oils

by $\beta$-carotene and protective action of tocopherols against autooxidation, (*d*) formation of lipid radicals via. the action of superoxide on hydroxyl radical of poly unsaturated lipids in microsomal preparations, (*e*) direct oxidation of organic substances through the formation of dioxygen complexes with possible involvement of some metals as catalysts and (*f*) oxidation of cholesterol to a myriad of alcohol, ketone, aldehyde, epoxide, etc. through the formation of hydroperoxide in the $\beta$ ring and side chain by the oxygen of air.

The second section, on food and model systems begins with a brief sketch of the activities of FDA Division of Nutrition regarding cholesterol oxides. This is followed by papers dealing with (*a*) analytical methods used in the study of autooxidation processes, (*b*) secondary reactions of lipid oxidations and water activity of foods as an antioxidant, (*e*) oxidative behaviour of unsaturated fatty acids at relatively high temperature such as those employed in frying, baking, etc., (*d*) lipid hydroperoxide induced oxidation of cystine in peptides, (*e*) l-tryptophan degradation by peroxidising lipids and (*f*) the biological significance of a new type of cross linking of phenolic esters of polysaccharides.

Under the section 'Antioxidants', papers dealing with (*a*) natural antioxidants particularly of soybeans and other oilseeds, (*b*) antioxidant effect of Millard reaction intermediates, (*c*) ascrobic acid and $\alpha$-tocopherol as blocking agents to prevent the formation of human carcinogens and (*d*) synergism in protecting unsaturated fatty acids from spontaneous, non-enzymatic oxidation between $\alpha$-tocopherol, ascorbic acid and lecithin are included.

The fourth section on biochemical systems includes papers dealing with (*a*) peroxidation of lipids in model systems and in membranes, (*b*) a model membrane system to interpret and predict the reactions occurring in membranes, (*c*) prevention of the attack of superoxide and $H_2O_2$ on DNA by dismutases and catalases, (*d*) the pathways by which glyceride lipid is converted to oxygeneted fatty acids, (*e*) enzyme catalysed lipid oxidation in muscle membranes, (*f*) lipoxygenases from different sources and (*g*) the the role of hepatic FAD monooxygenase.

The last section deals with (*a*) intracellular mechanisms for lipid peroxide decomposition, (*b*) biological effects of some products of cholesterol oxidation, (*c*) oxidised lipids in the cause of atherosclerosis, (*d*) arterial cell wall injury caused by cholesterol and its oxidation products and (*e*) formation of malonaldehyde from unsaturated fatty acids by the action of $H_2O_2$ which alkalyates with DNA.

The entire book is a very useful compilation to workers in a wide area of lipid oxidations and their implications on human health with latest analytical methods included. This book proves to be a very good asset to any library.

V. SREENIVASA MURTHY
C.F.T.R.I., MYSORE.

*Toxicological Data Profile on Vanillin:* Data Collection and Compilation by R. R. Khan and Gomathy Iyer, and evaluated by C. R. Krishnamurti and S. K. Khanna, Industrial Toxicology Research Centre, Lucknow, India, 1981, pp. 43.

This is a very useful compilation of valuable information on vanillin, the aromatic aldehyde being used as a food additive (flavouring agent) and in industries for various purposes. The current data has been presented briefly under different headings and sub-headings which cover the physico-chemical properties, use, production processes, the present day demand for the compound, metabolic pathways studied *in vivo* and *in vitro* in man and animals, toxicity of the compound (animal and plant), biodegradation and legal status.

Under the toxicity studies, different toxicity tests like acute, subacute, chronic and other studies conducted on various animals (dog, rabbit, rat, mouse, hamster, guinea pig, chicken, fish, seaurchen, mosquito etc.) are covered. Carcinogenicity, mutagenicity and teratogenicity test reports are included under special toxicity. Absence of major occupational health problem with vanillin has been mentioned under epidemiological studies. Effects on plant growth are also mentioned under phytotoxicity.

Finally a conclusion is added. The book contains 132 references and bibliography (249 references) on various aspects of vanillin.

H. P. RAMESH
C.F.T.R.I., MYSORE.

# SILVER JUBILEE OF THE ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS (INDIA) (1957 – 1982)

The Association of Food Scientists and Technologists (India) is celebrating its SILVER JUBILEE this year. The Association was established in June 1957 under the leadership of the late Dr. V. Subramanyam, Director, Central Food Technological Research Institute, Mysore, with about 150 members mostly drawn from the Central Food Technological Research Institute, Mysore. Today it has grown to a membership of about 2000, distributed in 13 chapters located in the principal cities in India, besides its head quarters at Mysore. There are also about 100 members from other parts of the world. The Association of Food Scientists and Technologists (India) is an affiliate of Institute of Food Technologists (IFT), USA and a member of International Union of Food Scientists and Technologists (IUFoST).

The Association was started with two main objectives: (*a*) to stimulate research on various aspects of food science and technology; (*b*) to provide a forum for exchange, discussion and dissemination of current developments in the field of food science and technology. This has been achieved by symposia, seminars, food conventions, special lectures and so on. Besides, the Association has also instituted several awards to encourage outstanding achievements in food science and technology. This year, it has instituted a new International Award in Food Science and Technology. To start off the Silver Jubilee year, an International Food Conference–AHARA '82 is slated to be held at Bangalore in May 1982.

One of the principal activities of the Association is the publication of the bimonthly *Journal of Food Science and Technology*, which is in its 19th year of publication. A new journal, *Indian Food Industry*, will commence publication during this Silver Jubilee year.

Success of the Association has come from the support of its members, its staff at the chapters and at head quarters, the conscientious work of the publication staff, the labours of the AFST(I) secretariat, Mysore and the support received from the host institution, the Central Food Technological Research Institute, Mysore. To all of them, we express our gratitude as we enter our Silver Jubilee year, 1982.

**P. Narasimham**
*Hony. Exec. Secretary, A F.S.T. (I)*

**Ludhiana Chapter**

Dr. K. Kirpal Singh, Director of Food Technology, Processing and Marketing, Punjab Agriculutral University, Ludhiana, and President of Ludhiana Chapter, has represented Government of India in the Technical Consultation Meeting for the Promotion of an Asian Network for Technology Transfer in Agro-Industries held at Bangkok from August 24 to 29, 1981.

Eleven countries from South-East Asia participated in these consultation meetings. Research and development work on post-harvest technology being carried out at various institutions in the South-East Asian region was reviewed. It was decided to establish a net-work and also sub-network on the basis of important commodities. CFTRI, Mysore has been recognised as a centre and the Directorate of Food Technology, Processing and Marketing, PAU, Ludhiana, has been identified as one of the sub-centres for carrying out post-harvest technology work in India. The programme envisages to promote agro-industries in this region, so that at least part of the primary produce could be taken up for processing. Promotion of agro-industries dealing with the marketing and processing of surplus primary agri-horticultural commodities will ensure more profitable marketing, better utilization and better economic returns to the farmers and will also generate more employment opportunities in the rural sector.

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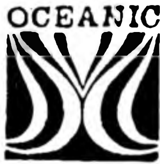


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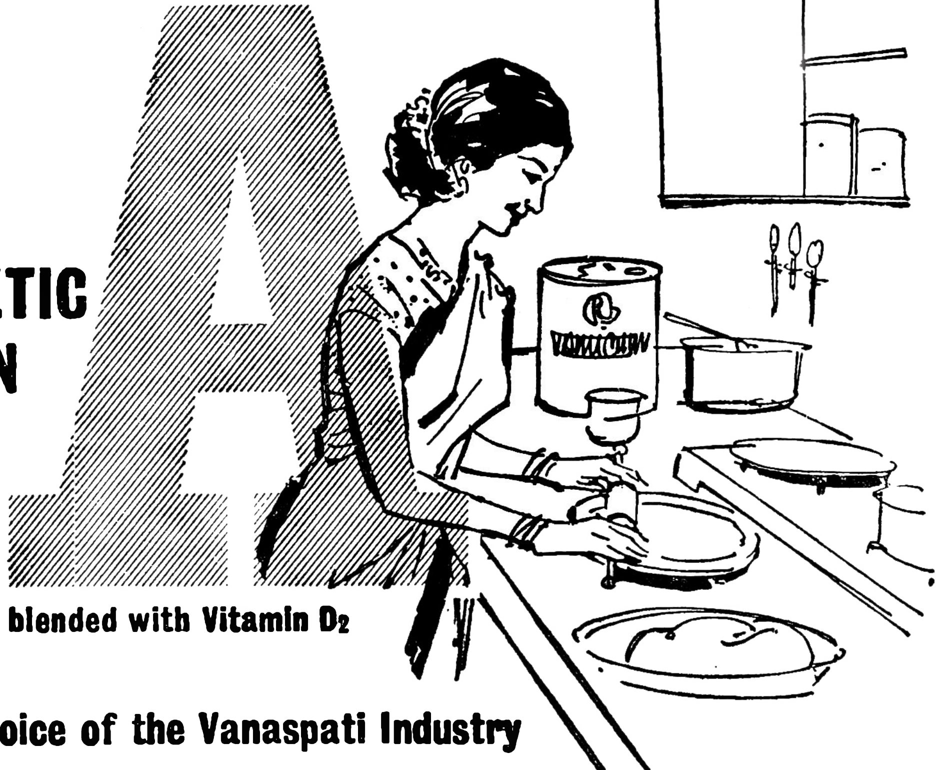

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1. Manuscripts of papers (*in triplicate*) should be typewritten in double space on one side of bond paper. The manuscripts should be complete and in final form, since only minor corrections are allowed at the proof stage. The paper submitted should not have been published or data communicated for publication anywhere else. Review papers are also accepted.

2. Short communications in the nature of Research Notes should clearly indicate the scope of the investigation and the salient features of the results.

3. Names of chemical compounds and not their formulae should be used in the text. Superscript and subscripts should be legibly and carefully placed. Foot notes especially for text should be avoided as far as possible.

4. **Abstract:** The abstract should indicate the principal findings of the paper. It should be about 200 words. It should be in such a form that abstracting periodicals can readily use it.

5. **Tables:** Tables as well as graphs, both representing the same set of data, should be avoided. Tables and figures should be numbered consecutively in Arabic numerals and should have brief titles. They should be typed on separate paper. Nil results should be indicated and distinguished clearly from absence of data, which is indicated by '—' sign. Tables should not have more than nine columns.

6. **Illustrations:** Graphs and other line drawings should be drawn in *Indian ink* on tracing paper or white drawing paper preferably art paper. The lettering should be in double the size of printed letters. For satisfactory reproduction, graphs and line drawings should be at least twice the printed size 16cms (ox axis) × 20cms (oy axis); photographs must be on glossy paper and must have good contrast; *three copies* should be sent.

7. Abbreviations of the titles of all scientific periodicals should strictly conform to those cited in the World List of Scientific Periodicals, Butterworths Scientific Publication, London, 1962.

8. **References:** Names of all the authors along with title of the paper should be cited completely in each reference. Abbreviations such as *et al.*, *ibid. idem*, should be avoided.

   The list of references should be included at the end of the article in serial order and the respective serial number should be indicated in the text as superscript.

   Citation of references in the list should be in the following manner:

   (*a*) *Research Paper:* Jadhav, S. S. and Kulkarni, P. R., Presser amines in foods, *J. Fd Sci. Technol.*, 1981, **18**, 156.

   (*b*) *Book:* Venkataraman, K., The Chemistry of Synthetic Dyes, Academic Press, Inc., New York, 1952, Vol. II, 966.

   (*c*) *References to article in a book:* Joshi, S. V., in the Chemistry of Synthetic Dyes, by Venkataraman, K., Academic Press, Inc., New York, 1952, Vol. II, 966.

   (*d*) *Proceedings, Conferences and Symposia Papers:* Nambudiri, E. S. and Lewis, Y. S., Cocoa in confectionery, *Proceedings of the Symposium on the Status and Prospects of the Confectionery Industry in India*, Mysore, May 1979, 27.

   (*e*) *Thesis:* Sathyanarayan, Y., Phytosociological Studies on the Calcicolous Plants of Bombay, 1953, Ph.D. Thesis, Bombay University.

   (*f*) *Unpublished Work:* Rao, G., unpublished, Central Food Technological Research Institute Mysore, India.

9. Consult the latest copy of the *Journal* for guidance.

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