RESULTS & DISCUSSION

Chapter IV

RESULTS AND DISCUSSION

The results are discussed in this chapter in relation to each objective which are restated here.

I. 1. Restandardisation of optimum conditions for steeping and germination for the preparation of ARF from cereals and millets;

2 .Standardisation of optimum conditions for steeping and germination for the preparation of ARF from legumes.

II. To study the effect of addition of ARF at graded levels on viscosity reduction.

III. To study the effect of temperature on the activity of ARF.

IV. 1. To study the effect of the period of germination on the hydrocyanic acid (HCN) content of ARF;

2. Its distribution in the grains and the sprouts;

3. Effect of heat treatment on the HCN content.

V. To study the microbiological quality of uncooked and cooked gruels prepared using ARF that had been stored for 1 to 6 months under ambient conditions.

VI. To study the effect of addition of jaggery and salt on viscosity and viscosity reduction power of various ARFs on gruels prepared from -

1) Cereal and pulse mixes
2) Donated foods
3) Commercial weaning foods.

VII. To compare the in vitro digestibility of gruels with ARF versus gruels without ARF.

I.1. Restandardisation of optimum conditions for steeping and germination for the preparation of ARF from cereals and millets

Steeping :

Our previous studies have shown that an ARF with amylase activity of 788- 800 maltose units brought about a similar reduction in viscosity as that of an ARF with amylase activity of 3000 maltose units under similar experimental conditions. This implies that an abundance of enzyme has no additional beneficial effect in terms of rate of reduction in viscosity and total viscosity reduction. Therefore, the conditions employed for the preparation of ARF were restandardised. The grains used were market variety of wheat, corn (white), pearlmillet and sorghum. Steeping and germination was carried out under ambient conditions of temperature (32-35°C) and relative humidity (40-50%). Germinating activity of the grains steeped for 6, 12, 24 and 48 hours was measured in terms of percentage germination. The steeping time required to obtain highest percentage germination was determined for each grain. The results are summarised in Table 17.

Table 17 : Effect of steeping period on percentage germination in wheat, corn, pearlmillet and sorghum

Grain1	Percentage germination at the end of			
	6 hours	12 hours	24 hours	48 hours
Corn	2.0 (1-3)	92.0 (91-93)	93.0 (91-95)	94.0 (92-94)
Wheat	46.6 (45-48)	94.0 (90-94)	96.0 (90-98)	97.6 (96-98)
Sorghum	67.3 (65-69)	96.6 (95-99)	97.3 (96-99)	97.6 (96-98)
Pearlmillet	94.6 (92-99)	96.6 (95-99)	96.0 (94-98)	96.0 (94-98)

1 - Grains have been arranged in descending order of size Values reported are mean of three observations; Figures in parantheses denote range.

A steeping period of 12 hours was found to result in maximum percentage germination in wheat, corn and sorghum. A steeping period of 6 hours was adequate to yield highest germination activity in pearlmillet. The germination of grains is dependent on rate and degree of hydration of the endosperm which is influenced by the temperature and physico-chemical characteristics of the grain. The optimum temperature for a good yield of sprouts has been reported to be between 15-31°C for wheat and between 32-35°C for corn

(Mayer and Poljakoff Mayber 1963). The ambient condition of temperature prevalent was in the favourable range for all the grains.

As mentioned previously the physico-chemical properties of the grain viz. seed size, seed coat permeability, and endosperm components also influence the water uptake by seeds. As is obvious from the data presented in Table 17, the smaller seeds like pearlmillet and sorghum required a shorter steeping period as compared to corn and wheat. Wheat, pearlmillet and certain varieties of sorghum have a less hard pericarp which facilitates easy uptake of water. Among the endosperm components, proteins are responsible for water absorption and therefore, seeds with higher protein content viz. wheat, pearlmillet and sorghum showed higher percentage germination even at the end of 6 hours as compared to corn. Corn, besides having a large seed size has a tough seed coat and endosperm rich in starch and low in protein (Chavan and Kadam 1989).

Lasekan (1991) has reported a high correlation between germinative capacity of grain and percent moisture content. Sorghum was found to have a germinative capacity of 98% at the end of the sixth day of germination. Soaking at 55 ± 0.1°C for 6 hours was found to be adequate for optimum germination of corn. The higher temperature employed for soaking was conducive for optimum phytase activity and the resultant germinated product had low phytate levels (Sattar et al 1985). A progressive increase in moisture content during steeping of corn, wheat, sorghum, pearl millet, finger millet, kodo millet, foxtail millet and barnyard millet was observed. Most of the grains attained a saturation moisture content within 24 hours at 25°C except corn which re quired 30 hours (Malleshi and Desikachar 1986). Kneen (1944) reported a steeping period of 24 hours at `room temperature' (temperature not specified) for wheat, barley, rye, oats, corn,

sorghum and rice. A steeping time of 2 hours for pearlmillet and 12 hours for wheat, sorghum and corn was required for maximum germinative activity (Gandhi 1985, Deshpande 1987, Kapoor 1986, Chaudhary 1986).

Based on the observations of the current study, a steeping period of 6 hours for pearlmillet and that of 12 hours for wheat, sorghum and corn was deduced to be most favourable for the devel opment of ARF.

Germination

Pearlmillet grains were soaked for 6 hours and wheat, sorghum and corn were subjected to 12 hours of soaking. At the end of the respective soaking period, grains were drained and spread on absorbent paper to remove excess moisture. These grains were then subjected to 24, 48, 72 and 96 hours of germination. Germination was arrested by drying at 50°C. The milled flour was studied for its liquifying power using a pure starch system as described in Chapter III.

The data on the viscosity reduction power of ARF prepared from wheat, sorghum, pearlmillet and corn at the end of 24, 48, 72 and 96 hours of germination, are set out in Table 18.

Table 18 : **Effect of period of germination on the liquifying power of ARF prepared from wheat, sorghum, pearl millet and corn**

ARF Source	Percentage reduction in viscosity at			
	24 hours	48 hours	72 hours	96 hours
Wheat	48	69	72	81
Sorghum	98	98	99	99
Pearlmillet	97	99	99	99
Corn	86	91	97	97

– Values reported are mean of three observations.

The liquifying power of each ARF was found to increase with progressive germination due to increased α-amylase content. Pearlmillet, sorghum and corn ARF lowered the viscosity by 97, 98 and 80% respectively even at the end of 24 hours. Beyond 24 hours of germination a marginal increase in liquifying power of the above grains was observed. In case of wheat ARF the development of liquifying enzyme was slow initially i.e. during the first 24 hours. Optimum viscosity reduction was observed at 48 hours. Extending the germination period beyond 48 hours resulted in a marginal increase in the amylolytic activity which was of little significance when observed visually. Wheat ARF had comparatively lower liquifying power at each stage of germination as compared to the ARF from sorghum, pearlmillet and corn.

Pal et al (1976) have reported the absence of α-amylase in ungerminated pearlmillet malt. α-amylase activity progressively increased in malt prepared from 2 and 3 day germinated seeds. The low amylase activity observed could be due to the kilning of germinated grains at 80°C-90°C. α- amylase is a heat labile enzyme. Corder and Henry (1989) have reported a 69% reduction in a-amylase activity in germinated wheat on air drying at 30°C as against freeze drying. Kneen (1944) have reported a high β-amylase activity in ungerminated wheat. An increase in α-amylase activity coincident with the period of germination was observed in wheat, corn and sorghum. Malleshi and Desikachar (1986) studied the malting characteristics of cereals and millets and reported a maxima for a-amylase activity at the end of 24 hours of germination. At the end of 48 hours pearlmillet showed higher activity than triticale or wheat. In vivo starch degradation by α-amylase was accompanied by an increase in free sugars and damaged starch of flours from germinated wheat and pearlmillet grain. The wheat starch granules were not severely attacked even though a-amylase activity increased the most in wheat. On the other hand the extent of attack of millet starch granules appeared to parallel the increase in α-amylase activity during germination. The authors conclude that the amylase of germinated millet was the most active enzyme on the basis of starch granule degradation during germination (Lineback et al 1977). The present study confirms the above finding in vitro. It is also observed that most of the studies report total amylase activity and therefore an increase in the same fails to show a parallel increase in viscosity reduction.

At the end of this experiment a germination period of 24 hours was chosen for the preparation of ARF from corn, sorghum and pearlmillet and that of 48 hours for the wheat ARF. The germinated grains were dried at 50°C and milled to yield a flour of + 60 BSS. This restandardised procedure was followed through-out the study.

On restandardisation a **shorter germination period (24-48 hours)** was found to yield an ARF of optimum amylolytic power as against the previously reported germination periods (48-96 hours) for the various grains (Deshpande 1987, Chaudhary 1986, Kapoor 1986, Gandhi 1985).

I.2. Standardisation of optimum conditions for steeping and germination for preparing ARF from greengram (Phaseolus aureus Roxb.), Bengalgram (Cicer arietinum) and soyabean (Glycine max mer.)

A similar procedure as for cereals was followed and percentage germination in seeds soaked for 6, 12 and 24 hours was studied. Table 19 gives data on the same.

Table 19 : Effect of period of steeping on percentage germination in greengram, Bengalgram and soyabean

Grain	Percentage germination at		
	6 hours	12 hours	24 hours
Bengalgram	–	66.6 (62-69)	90.6 (88-94)
Greengram	52.0 (50-54)	91.0 (90-92)	94.0 (92-96)
Soyabean	–	31.6 (21-38)	42.3 (38-49)

- Figures in parantheses denote range.
- Values reported are mean of three observations.

Bengalgram was found to require a longer steeping period (24 hours) to reach stable moisture content as indicated by the percentage germination. King and Puwastein (1987) have reported a germination rate of 19% and 96% for legumes at the end of 48 and 120 hours respectively, using the `between paper' technique. A steeping period of 12 hours was found to be adequate for greengram and soyabean in the present study.

Soyabean was often found to develop sliminess and fungal growth and utmost care had to be taken to remove excess surface moisture prior to germination. Despite the best efforts a low germination capacity was observed in soyabean. This is probably due to poor seed viability and loss in viability and vigour on storage (personal communication, Pulse Research Station, Baroda). Macleod (1967) discussed various factors believed to be responsible for dormancy in barley in relation to the occurrance of these conditions in other seeds. Structural features of the grain, physiological factors such as inadequate availability of oxygen and possibly the presence of inhibitors in the embryo have been identified as the important factors.

Bengalgram was steeped for 24 hours; greengram and soyabean were steeped for 12 hours. The excess water was drained and the grains were spread on absorbent paper to remove surface moisture. The grains were then subjected to a germination period of 24, 48, 72 and 96 hours as mentioned in Chapter III. Samples were analysed for amylase activity and viscosity reduction property at each stage of germination and Table 20 summarises the results.

As can be seen from the data bengalgram and greengram have negligible amylase content at the end of 24 hours of germination which increases (3-4 times) with progressive germination. The amylase content was however inadequate to bring about any appreciable reduction in viscosity even when incorporated at 10% solids level. An extensive review of literature on important starch hydrolysing enzymes namely, the α-amylases, suggests that the source of the enzyme can be conveniently classified into four groups: (a) the higher starch containing plants, (b) mammals, (c) bacteria and (d) fungi.

Table 20 : Effect of period of germination on amylase activity and liquifying power of legume ARFs

Source of ARF	Period of Germination							
	24 hours		48 hours		72 hours		96 hours	
	Amylase activity	Visco -sity	Amylase activity	Visco -sity	Amylase activity	Visco -sity	Amylase activity	Visco -sity
Control*	–	8700	–	8700	–	8700	–	8700
Bengalgram	58	8600	128	8000	130	7600	132	7600
Greengram	22	8600	135	8500	140	7700	145	7700
Soyabean	500	8000	1129	7800	1539	7000	1949	6800

* Sample without ARF

– Values reported are mean of three observations

Kulkarni et al (1990) have reported an amylase activity of 72°L and proteolytic activity of 213 mgN/100 g for germinated greengram. Since legumes are a rich source of protein with low starch content, it follows that they are not a potential source for α-amylases.

Ban and Dabry (1979) prepared protein fractions from soyabean by soaking at 50°C for 3 hours and germination for 3 days. Proteolytic activity increased on germination and the trypsin inhibition activity was lowered. Germination of winged beans (Psopocarpus tetragonolobus) led to an increase in the essential amino acids viz. cysteine, aspartic acid and histidine; a decrease in the lipoxygenase activity and reduction in the oligosaccharide content. Oxidative degradation of unsaturated fatty acids has been suggested which gives rise to off flavour in the flour from germinated winged bean (King and Puwastein 1987).

β-amylase of soyabean, pea and mung beans has been studied extensively. The enzyme is not located in the same cellular compartment as is particulate starch. Therefore, the role of β-amylase in starch degradation is uncertain (Lizotte et al 1989). Nayak (1980) has reported an insignificant role of malted legumes in viscosity reduction in a malted cereal and legume based weaning mixes. The viscosity reduction obtained was predominantly due to the malted cereal component. The results of the experiment on germinated legumes ruled out the possibility of utilising the same as a source for developing Amylase-Rich Food on account of its poor liquifying capacity. Therefore, ARF from wheat, sorghum, pearlmillet and corn was utilised in the subsequent experiments.

II. Effect of addition of ARF at graded levels (1-5% of total solids) and viscosity reduction

The effect of addition of ARF at graded levels viz. 1-5% of total solids, on viscosity reduction was studied in a pure starch system as described in Chapter III.

ARF prepared from wheat, pearlmillet, sorghum and corn using standardised conditions was incorporated at 1-5% of total solid contents in a pregelatinised purified corn starch slurry. Addition of ARF in the ungelatinised slurry was found to have negligible amylolysis. A number of investigators have found that some amylases do degrade ungelatinised starch granules, although the action is slow (Robyt and Whelan 1968).

The viscosity reduction brought about by various ARFs incorporated at 1-5% of total solid contents in a 10% starch slurry is presented in Table 21 and Figure 8.

The hydrolysis curve of various ARFs shows a positive correlation between the concentration and viscosity reduction. The starch liquification is higher with increase in the amount of ARF incorporated. All the curves follow a similar trend i.e. a steep slope which flattens out. The rate of hydrolysis of gelatinised starch (as measured by a reduction in viscosity) is gradual at lower concentrations of ARF in case of corn and wheat ARF. The rate of reduction in viscosity was rapid when the ARFs were incorporated at a level of 4%. Beyond 4% level the increment in viscosity reduction was negligible.

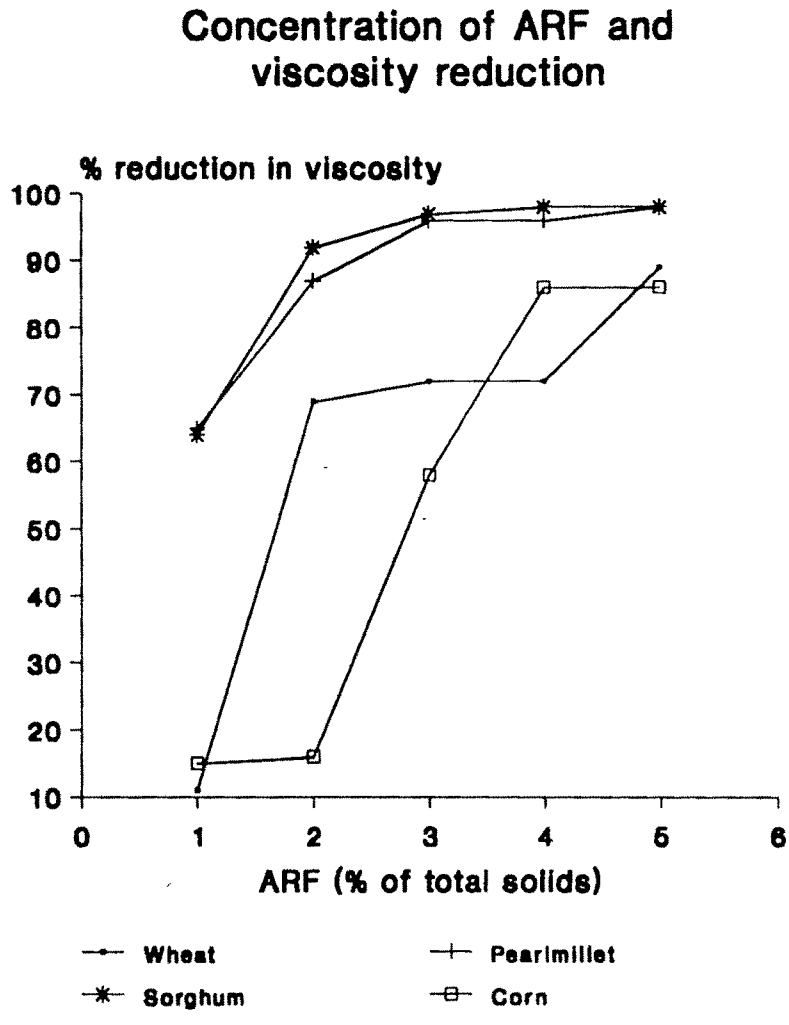
Concentration of ARF and viscosity reduction
% reduction in viscosity
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
ARF (% of total solids)
Wheat
Pearlmillet
Sorghum
Corn

Figure: 8

Table 21 : Effect of concentration of ARFs on viscosity reduction

ARF used	% Viscosity reduction on addition of ARF at				
	1	2	3	4	5*
Wheat	11	69	72	72	89
Pearlmillet	65	87	96	96	98
Sorghum	64	92	97	98	98
Corn	15	16	58	86	86

* – % of total solids

Reed and Underkofler (1966) have reported the comparative percentage starch conversion as a function of concentration of a and β-amylase from wheat on starch slurries during baking of bread. The data are presented in Table 22.

The data show that starch conversion is primarily affected by the α-amylase and increases with an increase in the enzyme concentration. Acharya (1934) has reported a similar curvilinear relationship between enzyme concentration and liquifying power in barley and sorghum malt.

Table 22 : Effect of wheat alpha and beta-amylase on starch slurries during bread dough baking

α-amylase per g starch	β-amylase per g starch	Starch conversion (%)
6.1	0	6.1
112.5	0	36.5
5.6	11.2	16.2
5.6	22.4	26.7
0	23.6	9.0

The curves are steep at the earlier stages but soon tend to flatten out i.e. small amounts of enzyme are able to produce relatively larger percentage of hydrolysis as measured by the reduction in viscosity than larger amounts of enzyme. Bird and Hopkins (1954) studied the action pattern of α-amylases and drew attention to a relatively neglected aspect, namely, of the differences that occur with a single species of α-amylase when the enzyme substrate ratio is altered. In the late stages of hydrolysis of amylose glucose, maltose and maltotriose are elaborated. The maltotriose could be converted completely into glucose only by increasing enzyme concentration (Bines 1958).

It is also interesting to note that in the initial stages of starch hydrolysis a greater number of reducing groups are produced. A smaller increase is observed

in the second phase. This slow increase which is a characteristic of the second phase is due to the slow degradation of maltotriose, the intermediate product of hydrolysis. It has further been shown that barley malt α-amylase had a very low affinity for maltohexaose as compared with amylose, and could degrade it only one-sixth as fast as could human salivary amylase (Robyt and Whelan 1968). Thus the differences observed in the rate of hydrolysis of starch by amylases can be explained on the basis of their affinity or lack of it for the substrate and the intermediate products formed. This also explains the trend of hydrolysis curve observed.

In industrial starch conversions the levels of starch used are much higher (about 30%) and the commercial amylase preparations vary in their action pattern with respect to their end products (Windish and Mhatre 1965).

Thus, the enzyme substrate ratio is crucial and determined by the desired end product. In the current study incorporation of various ARFs at 4% level was found to give desirable viscosity reduction and ARF was therefore incorporated at the same level in the further experiments.

III. Effect of temperature on the activity of ARF

Temperature plays an important role in the enzyme catalysed reaction. Most of the enzymes are active within a particular temperature range and have a temperature optima. Keeping the temperature optimum for the particular enzyme system is essential to obtain the desirable rate of reaction and the esired end products. (Howling 1989)

Therefore, the effect of temperature on ARF activity was studied in a purified corn starch system where ARF was incorporated at 4% level to pre-gelatinised starch slurries held at 80°C, 70°C, 60°C and 50°C and the viscosity reduction measured as described in Chapter III.

The data obtained are presented in Figure 9.

Amylase from each ARF source exhibits a characteristic amylolytic action pattern at various temperatures. Wheat and pearlmillet ARF show significant reduction in viscosity at 50°C (45% and 63% respectively). While it is negligible in case of sorghum and corn ARF (2-3%). Lower percentage reduction in viscosity is observed at 60°C and 70°C in case of wheat ARF and pearlmillet ARF. Corn and sorghum ARF show subsequent reduction in viscosity with increase in temperature from 50-70°C. Most interestingly all the **ARFs show peak liquifying activity at a temperature of 80°C.**

This is in contradiction to most of the studies which report a temperature optima between 45-66°C for α-amylases of plant origin. Table 23 summarises the data from a few selected studies.

Effect of temperature on the amlolytic activity of various ARFs

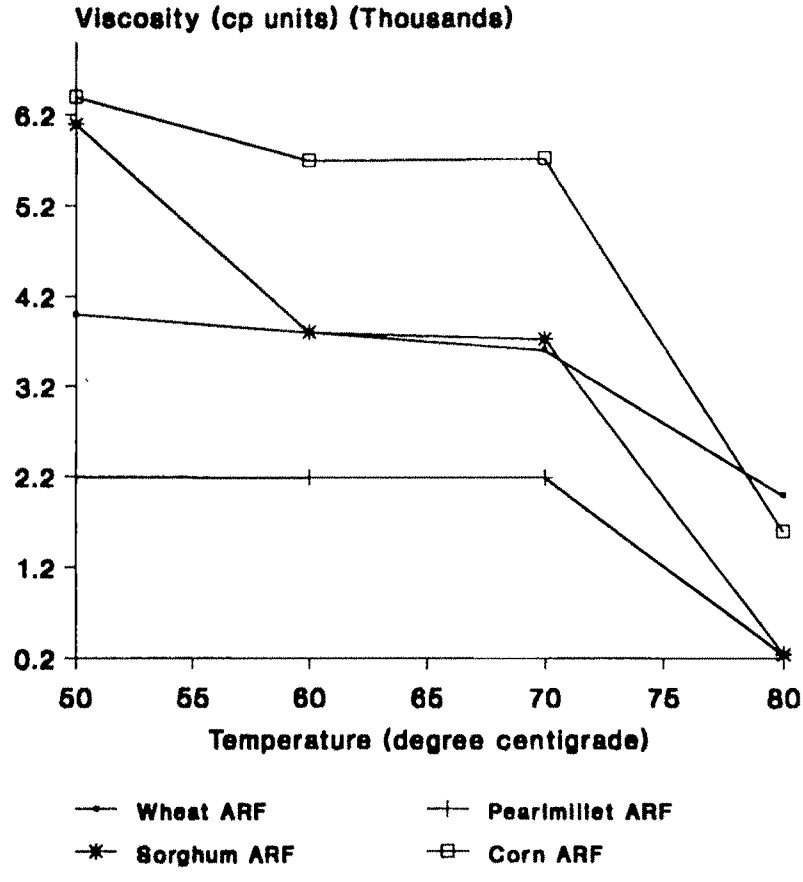

Figure : 9

Table 23 : Temperature optima for malt alpha-amylases from various cereal sources

Optimum temp. °C	Barley	Pearl-millet	Wheat	Sorghum	Ragi	Rice
1.	51-60°C	–	60-66°C	–	–	45-50°C
2.	35°C	–	–	–	–	–
3.	45°C	–	–	50°C	–	–
4.	–	60 x C	–	–	–	–
5.	48-55°C	–	49°C	≥ 40°C	–	–
6.	–	–	–	–	60°C	–

Source : 1. Amylase Research Society of Japan (1985)

2. Robyt and Whelan (1968)

3. Acharya (1934)

4. Chandrasekhar and Swaminathan (1956)

5. Greenwood and Milue (1968)

6. Chandrasekhar and Swaminathan (1953)

Hough (1985) observed most rapid reactions at a temperature of 70°C in barley malt α-amylases. The heat stability of amylases varies with the source. The enzyme sources in order of decreasing heat stability are bacterial, cereal and fungal. At 60°C all three retained 100% of their activity while at 80°C 92%, 25% and 1% of residual activity was observed in bacterial, cereal and fungal enzymes

respectively. Addition of flour improved heat stability of the enzymes (Reed and Underkoffler 1966).

The differences observed in the data from various studies and the present study could be due to the difference in methodology used. Most of the studies have essentially reported the residual activity of the enzyme extracts treated at various temperatures or measure the viscosity reduction in a system where enzyme is added to ungelatinised substrate and heated together to desired temperature. In the present study enzyme (in the form of ARF) was added to a pregelatinised starch slurry held at a specific temperature. In the former a major portion of the enzyme gets destroyed prior to starch gelatinisation with lower resultant viscosity reduction. While in the latter rapid hydrolysis occurs on addition of enzyme to the pregelatinised starch which is fully susceptible to enzyme action.

Thus the results obtained so far indicate the possibility of developing ARF using a short period of steeping (12 hours) and germination (24-48 hours) from cereals and millets. Further, the ARF thus obtained can withstand a cooking temperature of 80°C and is required in small amounts i.e. 4% of total solids level for desirable viscosity reduction.

IV. To study the effect of period of germination on -

1. elaboration of hydrocyanic acid (HCN) in various grains;
2. its distribution in the grain and sprout; and
3. the effect of heat treatment on the HCN content

IV.1. Elaboration of Hydrocyanic acid (HCN) in various grains.

Several grains are reported to contain cyanogenic glycosides which

dissociate into the constituent sugar moiety and free hydrocyanic acid (HCN) on germination. The rate and quantity of HCN liberated depends on the cyanogenic glycoside content and the temperature and period of germination. Therefore, the effect of period of germination (24 hours to 120 hours) on elaboration of HCN (at ambient temperature of 30 ± 1°C) was studied using market variety of wheat, corn, pearlmillet, sorghum and sorghum GJ35 and GJ36, a high tannin and low tannin cultivar respectively, grown in the State of Gujarat. ARF was prepared from all the grain samples and analysed for the HCN content as described in Chapter III. On preliminary screening wheat, corn and pearlmillet were found to contain negligible amount of HCN which was below detectable limits, therefore the three varieties of sorghum were used for further studies. The data pertaining to the change in HCN content with progressive germination are recorded in Table 24.

All the three varieties of sorghum were found to contain low levels of HCN in the ungerminated state. The same was found to increase with progressive germination. The GJ35 variety had highest HCN content in the ungerminated state (28.33 ppm) as well as at the end of 120 hours (608.33 ppm) followed by the GJ36 variety (12.33 and 383.33 ppm respectively) and the market varie ty of sorghum (10.66 and 179.66 ppm respectively). The difference in HCN content at the end of each germination period was significant as indicated by the F ratio and the critical difference.

Panasiuk and Bills (1984) reported a linear increase in the potential HCN content of sorghum sprouts grown at 25°C. The three cultivars of sweet sorghum with traces of HCN were found to yield sprouts with dangerously high level of

Table 24 : Effect of period of germination on HCN content (ppm) of ARF with sprouts from various cultivars of sorghum

Period of germination (hours)	Variety		
	GJ 35	GJ 36	Market variety
0	28.33 ± 1.07^{a}	12.33 ± 0.74^{a}	10.66 ± 0.94^{a}
24	102.67 ± 2.36^{b}	91.00 ± 1.29^{b}	40.00 ± 1.63^{b}
48	196.66 ± 4.27^{c}	150.16 ± 1.77^{c}	99.16 ± 2.79^{c}
72	371.16 ± 7.36^{d}	269.00 ± 6.74^{d}	184.33 ± 4.74^{d}
96	504.66 ± 9.26^{e}	412.50 ± 6.23^{e}	254.66 ± 7.03^{e}
120	608.33 ± 8.59^{f}	383.80 ± 3.84^{f}	179.33 ± 4.42^{f}
Cal F	7451.43	7514.77	3352.30
Tab F	3.70	3.70	3.70
CD	13.95	9.70	8.48

Unidentical superscripts denote significant difference within the group at 5%.

HCN (695-1030 ppm) at the end of 3 days of germination. The sprouts from grain sorghum had comparatively lower levels of HCN when grown under similar exper imental conditions (258-508 ppm). Dada and Dendy (1987) have reported similar trends wherein, traces of HCN were detected in unsprouted sorghum (19 varieties), pearlmillet and fingermillet. The HCN content of pearlmillet and fingermillet remained below detectable range on steeping the grains for 24 hours at 20°C and germinating them for 2 days at 30°C and 95% relative humidity. The HCN content of various sorghum samples ranged from 74 - 658 ppm.

The HCN elaboration capacity was found to be strongly affected by the variety of sorghum used. Table 25 presents data on varietal influence on HCN content of the ARF prepared from sorghum.

The data clearly indicate a significantly higher HCN content in ARF prepared from GJ 35 variety followed by GJ 36 and the market variety. The GJ 35 variety which had highest HCN potential was also found to contain high levels of tannin (9%) followed by the GJ36 (1%) and the market variety of sorghum (0.8%) (the values on tannin content are based on unpublished data from sorghum research station Surat). However, comparative studies on 19 varieties of sorghum have failed to establish a correlation between the tannin content and the HCN generating capacity of the grain (Dada and Dandy 1987). Thus, it is evident that the HCN potential of the sorghum grain is influenced by the cultivar used though, the specific factor(s) or constituent affecting the same has not been identified yet. The extensive review of cyanogenic glycoside focuses on examining the physical and genetic factors which determine the level of cyanogenic glycoside in the seed (Conn 1969, Eyjolfsson 1970, Conn 1978, 1979A, 1979B, and Seigler 1975 and 1976).

Table 25 : HCN content (ppm) of ARF[1] with vegetative portion from various cultivars of sorghum

Source of ARF	HCN Content (ppm)
GJ 35 variety	102.66 ± 2.35^{a}
GJ 36 variety	91.00 ± 1.29^{b}
Market variety	40.00 ± 1.63^{c}
Cal F	8.31
Tab F	6.36
CD	30.00

1 - ARF prepared using standardised conditions.

Unidentical superscripts denote significant difference at 5%.

It is also interesting to note the rate of change in the HCN content in the three varieties of sorghum viz. GJ 35, GJ 36 and the market variety which is depicted in Figure 10. As is evident from the graph the HCN content of GJ 36 and market variety of sorghum increased rapidly during the first 24 hours of germination (21 times and 17 times respectively); the rate of HCN elaboration was slow between 72 to 96 hours and a sharp decrease was observed between 96 and 120 hours of germination. In the GJ 35 variety the HCN content increased rapidly and peaked at 72 hours of germination beyond which the increment was slow. Rate of elaboration of HCN has been studied as a function of temperature

Rate of elaboration of HCN (ppm) in sorghum with progressive germination

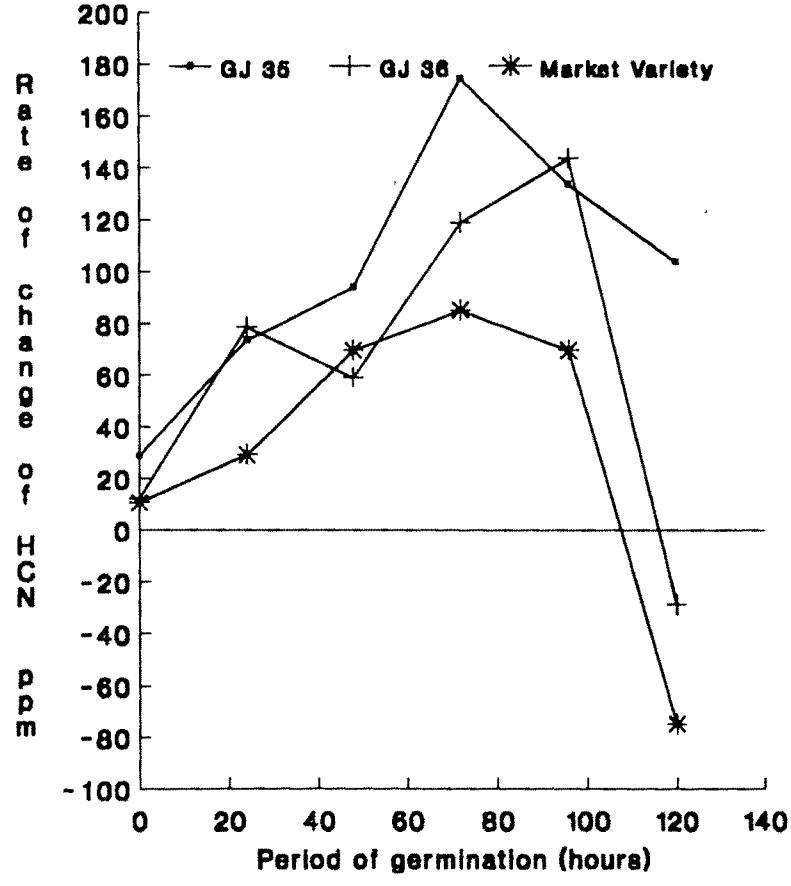

Figure : 10

of germination in Bird-a-boo cultivar of grain sorghum. The rate of elaboration of HCN in grain sorghum germinated at 25°C, 30°C and 35°C was reported to peak at 48 hours of germination. The HCN content continued to increase at a slow rate in the grains germi nated at 25°C while a decrease in rate of elaboration of HCN was observed in the sprouts grown at 30°C and 35°C beyond 48 hours of germination. The average HCN potential of sprouts grown at 35°C was half as much as that of those grown at 30°C (230 vs 554 ppm).

The above discussion suggests a definite relationship between the variety of the grain and its HCN potential. It is also evident that physical factors like temperature too have a profound influence on the quantity and rate of elaboration of HCN. Therefore, the variety and conditions for germination must be selected carefully in order to obtain a product low in HCN content. The HCN content of GJ 35 variety, at the end of 96 hours of germination, was found to exceed the limit of 200 ppm - the maximum level recommended in the several countries for HCN in lima beans (Panasiuk and Bills 1984). Since the ARF prepared using standardised conditions utilises a germination period of 24-48 hours and is incorporated at 4-5% level i.e. 8-10 g/meal, the product can be considered safe for consumption. However, we cannot overlook the goitrogenic effects of ingestion of sublethal doses of HCN over a long period.

IV.2. Distribution of HCN in the grain and vegetative portion at the end of 24-120 hours of germination

Preliminary studies on the effect of various treatments on HCN content of sorghum sprouts have shown a marked reduction (10 times) in the cyanide content of devegetated grains dried at 50°C and milled to a fine powder (Dada

and Dendy 1987). Therefore, the distribution of HCN in grain and vegetative portion at the end of 24, 48, 72, 96 and 120 hours of germination was studied systemat ically in three cultivars of sorghum viz. GJ 35, GJ 36 and the market variety. Hundred g batches of each sample were germinated for 24, 48, 72, 96 and 120 hours and dried at 50°C. The vegeta tive portion was removed carefully with hand abrasion and per centage yield of vegetative portion (on dry weight basis of ungerminated seeds) was calculated. The data are recorded in Appendix 4. The devegetated grains and the vegetative growth were milled separately and used for analysis. The results are presented graphically in Figure 11 and 12 and summarised in Table 26 and 27. As can be seen from the data and the graph, the HCN content of vegetative growth was less than that of the grain during the initial period of germination (20-24 hours) and increased rapidly thereafter in all the samples. The HCN content of roots and shoots was 3 - 3.5 times higher than that of the grain at all stages of germination in various cultivars. In the GJ 36 and market variety the HCN content of vegetative growth decreased slightly between 96 hours and 120 hours of germination. The cyanide content of devegetated grains from the three cultivars was similar at respective stages of germination. Thus, the variation observed in the HCN content of ARF from whole grain from various cultivars can be attributed to the difference in HCN content of the growth. About 77-85% of the total HCN was concentrated in the roots and shoots which formed only 2-7% of the grain (depending on the grain and stage of germination) calculated as dry weight of ungerminated seeds. The HCN content of deveg etated grain and growth increased 3-5 times and 6-10 times respectively with progressive germination. However, the HCN content of grains remained well within the stipulated limit of 200 ppm in the three sorghum cultivars studied. Due to lack of data from parallel studies it is difficult to corroborate the findings of the present study. However, it can certainly be concluded here that

Effect of period of germination on distribution of HCN (ppm) in grain

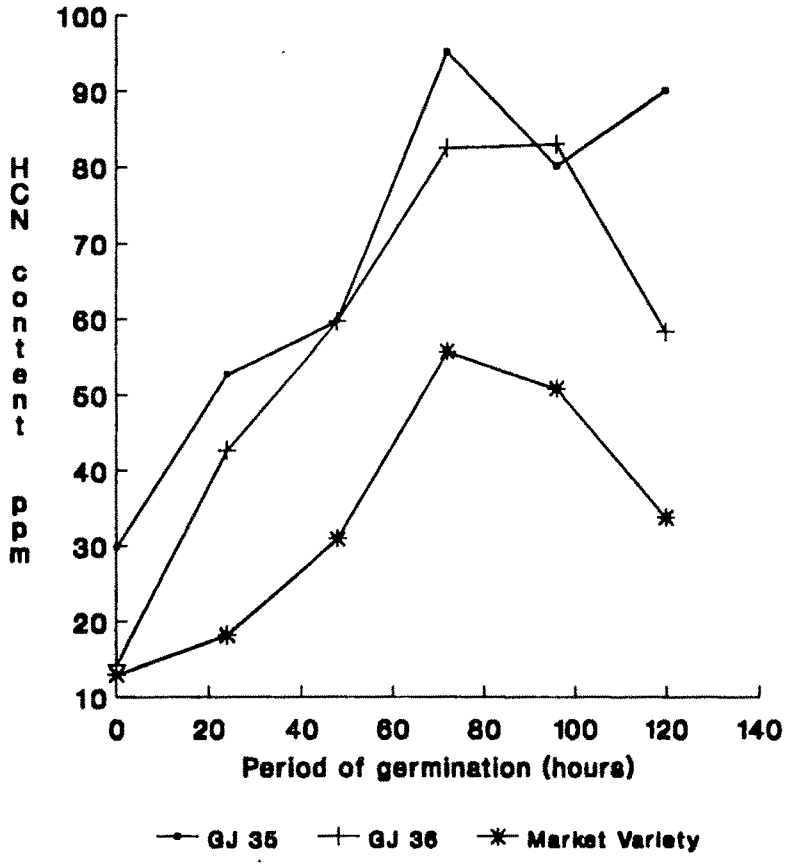

Figure : 11

Effect of period of germination on distribution of HCN in sprouts

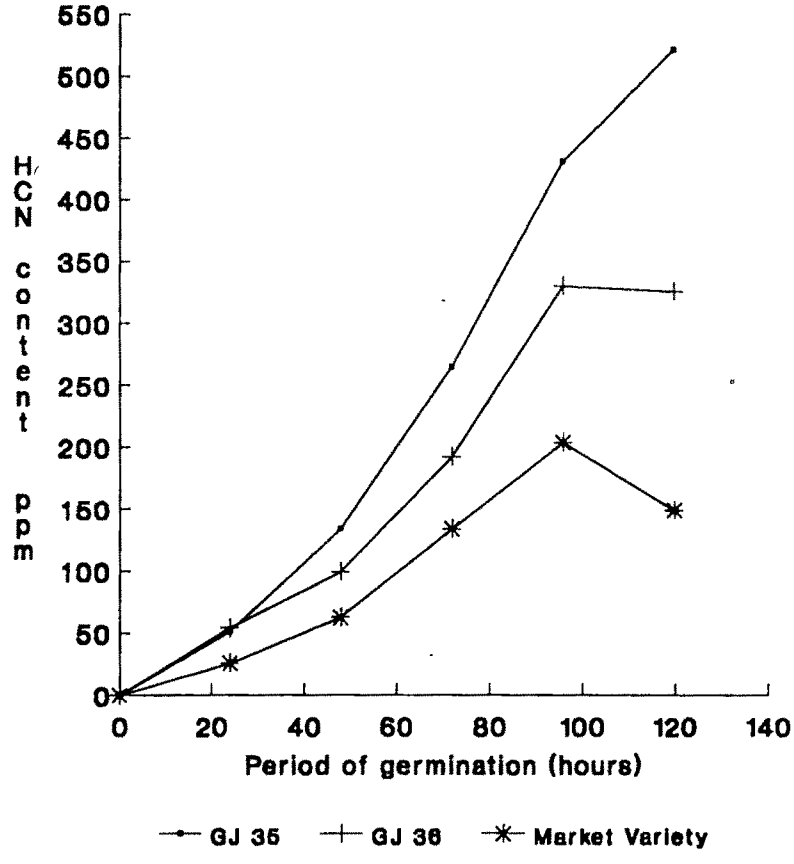

Figure : 12

the removal of roots and shoots from germinated, dried sorghum prior to milling will remove 77-85% of the toxin. The ARF prepared by using previously standardised procedure i.e. 12 hours of steeping and 24 hours of germination would contain on an average 80 ppm of HCN of which 61-68 ppm would be

Table 26 : Effect of period of germination on distribution of HCN (ppm) in (a) the grain from various cultivars of sorghum

Period of germination (hours)	Variety		
	GJ 35	GJ 36	Market variety
0	29.83 ± 0.68^{a}	14.16 ± 1.06^{a}	13.00 ± 0.57^{a}
24	52.66 ± 1.88^{b}	42.66 ± 1.37^{b}	18.16 ± 1.06^{a}
48	59.83 ± 1.06^{b}	59.66 ± 1.37^{c}	31.00 ± 1.15^{b}
72	95.33 ± 1.59^{c}	82.50 ± 1.89^{d}	55.66 ± 1.59^{c}
96	80.16 ± 1.06^{d}	83.00 ± 1.63^{e}	50.83 ± 1.06^{d}
120	90.16 ± 1.06^{e}	58.33 ± 1.69^{f}	33.83 ± 1.06^{e}
Cal F	1887.00	1441.19	1147.00
Tab F	3.70	3.70	3.70
CD	8.02	8.58	8.63

Unidentical superscripts denote significant difference within the group at 5%.

Table 27 : Effect of period of germination on the distribution of HCN (ppm) in (b) the vegetative portion from various cultivars of sorghum

Period of germination (hours)	Variety		
	GJ 35	GJ 36	Market variety
0	–	–	–
24	51.33 ± 1.88^{a}	54.16 ± 2.11^{a}	25.83 ± 1.06^{a}
48	134.33 ± 4.02^{b}	99.66 ± 1.24^{b}	62.66 ± 1.10^{b}
72	265.00 ± 4.86^{c}	192.33 ± 2.13^{c}	134.33 ± 2.35^{c}
96	431.00 ± 1.63^{d}	330.16 ± 1.77^{d}	204.00 ± 2.94^{d}
120	521.00 ± 2.06^{e}	325.66 ± 3.63^{d}	149.33 ± 2.28^{e}
Cal F	15.07	13.82	13.55
Tab F	4.18	4.18	4.18
CD	56.00	40.15	40.74

Unidentical superscripts denote significant difference within the group at 5%.

removed on discarding the growth. When this ARF is utilised in small quantities (8 g/200 ml feed) only 1-2mcg of HCN will be ingested by the young infant/toddler per meal depending on the variety of sorghum utilised. This establishes that removal of roots and shoots is an essential step in the preparation of ARF from sorghum and the ARF thus prepared is safe for use in infant feeding.

IV.3 Effect of heat treatment on HCN content of ARF from whole and devegetated grains

ARF is subjected to heat treatment when it is cooked alongwith the gruel or alternately, when added to a hot cooked gruel. The temperature to which ARF is subjected to is likely to vary in above instances. Therefore, it was thought worthwhile to study the change in HCN content on treating the ARF at 50°C, 60°C, 70°C, 80°C and 90°C. The above range of temperature was selected based on the fact that the amylase activity is negligible below 50°C and the enzyme is inactivated beyond 90°C. ARFs were prepared from sorghum GJ 35, GJ 36 and market variety by utilising 24, 48, 72, 96 and 120 hours of germination. One half of each sample was devegetated prior to milling while the other half was milled whole. The ARF thus prepared was heat treated and analysed for the cyanide content as mentioned in Chapter III. The results are depicted graphically in Figure 13 and 14 and summarised in table 28 and 29.

Effect of heat treatment on HCN content of ARF with sprouts

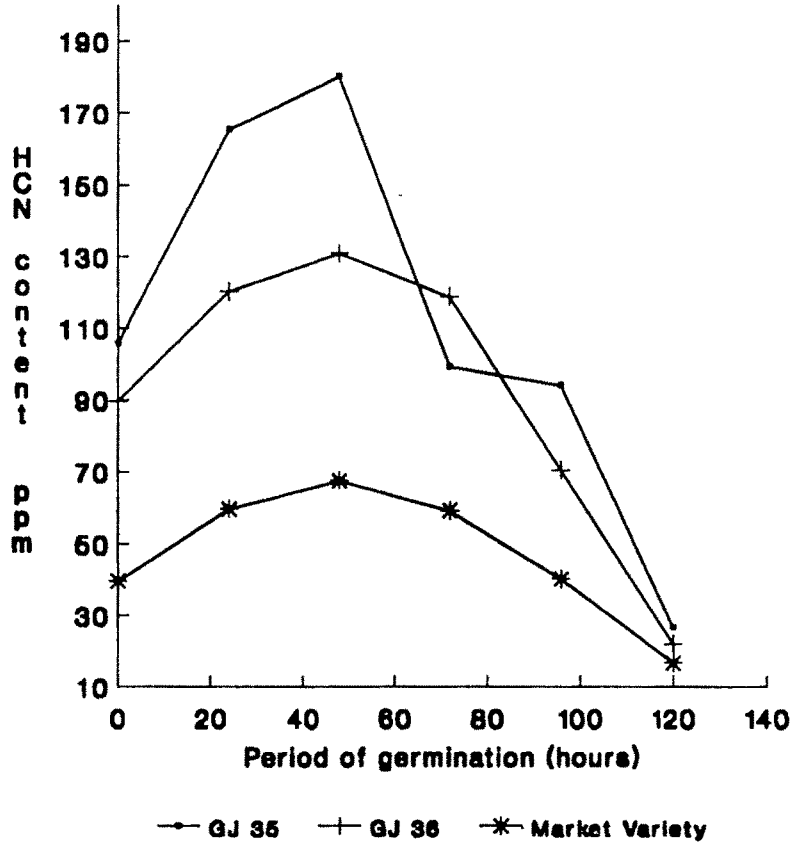

Figure : 13

Effect of heat treatment on HCN content of ARF without sprouts

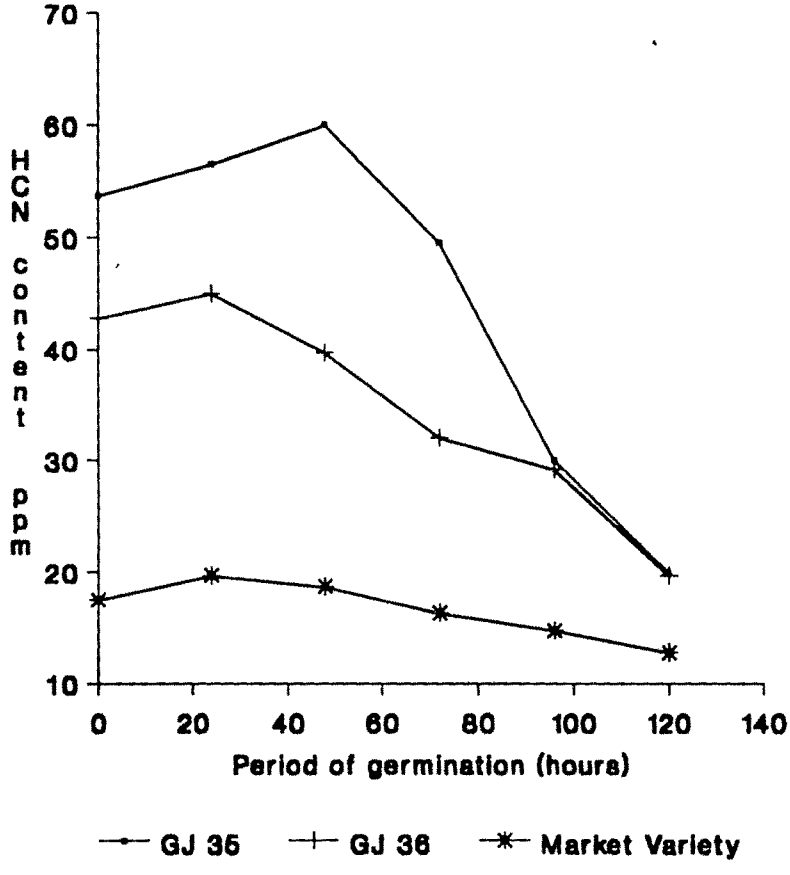

Figure : 14

Table 28 : Effect of heat treatment on HCN content (ppm) of ARF[1] with vegetative portion from various cultivars of sorghum

Temperature (°C)	Variety		
	GJ 35	GJ 36	Market variety
0	105.83 ± 1.47a	89.66 ± 1.03a	39.66 ± 1.21a
50	165.50 ± 3.56b	120.33 ± 1.03b	59.66 ± 1.03b
60	180.16 ± 1.60c	131.00 ± 1.26c	67.66 ± 1.50c
70	99.33 ± 0.81a	118.83 ± 1.47d	59.16 ± 1.47d
80	94.16 ± 1.60a	70.50 ± 1.51e	40.33 ± 1.03c
90	26.66 ± 1.50d	22.00 ± 1.38f	16.66 ± 1.03f
Cal F	635.08	600.80	1783.00
Tab F	3.70	3.70	3.70
CD	11.56	2.72	2.32

1 _ ARF prepared using standardised conditions.

Unidentical superscripts denote significant difference within the group at 5%.

Table 29 : Effect of heat treatment on HCN content (ppm) of ARF[1] without vegetative portion from various cultivars of sorghum

Temperature (°C)	Variety		
	GJ 35	GJ 36	Market variety
0	53.66 ± 1.06^{a}	42.83 ± 1.60^{a}	17.50 ± 1.04^{a}
50	56.50 ± 0.96^{a}	45.00 ± 1.41^{a}	19.66 ± 1.03^{a}
60	60.00 ± 1.02^{a}	39.66 ± 1.03^{a}	18.66 ± 1.36^{a}
70	49.50 ± 0.84^{a}	32.00 ± 1.26^{a}	16.33 ± 1.03^{a}
80	30.00 ± 1.99^{b}	29.16 ± 0.98^{b}	14.66 ± 2.01^{b}
90	19.88 ± 1.83^{c}	19.66 ± 1.03^{c}	12.83 ± 0.98^{c}
Cal F	11.99	359.07	40.93
Tab F	3.70	3.70	3.70
CD	21.00	12.65	2.34

1 – ARF prepared using standardised conditions.

Unidentical superscripts denote significant difference within the group at 5%.

As can be seen from the graph and the table, heating at 50-60°C left the HCN content unaltered. Heating at 70°C and beyond lowered the HCN content drastically (40-50 times) irrespective of the cultivar and the method of preparation used. Dada and Dendy (1987) studied the effect of various household technologies on recovery of HCN from food products. The investigators reported no significant change in HCN content of sprouted sorghum dried at 50°C and milled. This endorses the conclusion of Panasiuk and Bills (1984). Since HCN has a boiling point of 25.7°C the observation that drying at 50°C did not reduce the amount of cyanide in sprouted grains suggests that cyanide exists largely in the form of heat stable nonvolatile cyanogenic glycoside. A simple qualitative test detected HCN in the atmosphere over dried sorghum meal (dried and milled germinated sorghum) that had been held at - 8°C for a few weeks. These observations further indicate that the endogenous autolytic enzymes viz. β-glucosidase and hydroxynitrile lyase, which are responsible for the breakdown of the cyanogenic glycoside, remained partially active on drying the sorghum sprouts at 50°C. It is important to exercise caution in handling such a product. Allowing the product to stand for some time before cooking or eating could lead to elaboration of a considerable amount of potentially available HCN. It thus rules out the feasibility of adding ARF to a previously cooked hot gruel ($\leq$ 70°C).

Heating at 70°C and beyond for 5 minutes lowered the HCN content substantially (40-50 times). Boiling at 90°C for 5 minutes left traces of HCN (14-26 ppm) in the various samples studied.

This confirms the findings of Panasiuk and Bills (1984) and Montgomery (1965). The authors have reported complete destruction of the autolytic enzymes in the vicinity of 100°C and thereby a reduction in the HCN content of the food stuff. Dada and Dendy (1987) studied the effect of various `household techniques' on the HCN content of sorghum meal prepared by germinating sorghum at 30°C for 2 days at 95% relative humidity and drying at 50°C prior to milling. Toasting the dried meal at 100°C was found to lower the HCN slightly while at a temperature of 180°C 90% of the HCN was removed. Toasting the wet meal at 100°C and 180°C was more effective since it reduced the cyanide content by 83% and 96.5% respectively. Boiling the dried meal (temperature and duration not mentioned) left only traces of HCN. However, in absence of relevant data from other studies it is difficult to corroborate the findings of present study.

Considering the fact that ARF is utilised in small quantities (around 8 g/feed) only 1-2mcg of cyanide will be consumed per feed depending on the variety of sorghum used for preparing ARF using pre-standardised conditions. The results of this investigation strongly suggest that **sorghum ARF can be safely used for food purpose provided care is taken to remove the vegetative growth; the ARF is incorporated prior to cooking and the gruel is cooked for atleast five minutes.**

V. To study the microbiological quality of uncooked and cooked gruels prepared using ARF stored for 0-6 months under ambient conditions

Preliminary studies in this department have shown that the ARFs prepared from wheat, sorghum, corn and pearlmillet using previously standardised procedure had a shelf life of 3-6 months (Gopaldas 1988). However,

utilising microbially safe ARF cannot assure the microbial safety of the gruel mainly because ARF is incorporated in small quantities (8 g/feed). The microbial load of the flour, water and utensils used are more important in determining the microbial safety of the gruel. Further, in a typical rural or urban slum setting due to lack of fuel and time, food is cooked in bulk once or at the most twice a day; it is stored under ambient conditions (30-40°C) and consumed over a period of 8 hours. For the aforesaid reasons it is at times subject only perfunctory boiling. These in turn decide the microbial quality of the weaning food (Rowland et al 1978). Therefore, it was thought worthwhile to study the :

(1) microbial quality of each ingredient utilised in the preparation of gruels;

(2) effect of addition of ARF stored for 0-6 months on the microbial profile of the raw and cooked gruels with and without ARF;

(3) microbial quality of cooked gruels with and without ARF stored under ambient conditions for 0-8 hours.

The microbial quality was studied using following parameters namely total plate count, E.coli and yeast and mould count.

V.1 Microbial quality of ingredients utilised in the preparation of gruel

The total plate count, yeast and mould and *E.coli* count of raw wheat flour, ARF, jaggery and tap water was determined and the data are presented in Table 30.

As evident from the data each raw ingredient contributed to the total microbial load. The total viable count and yeast and mould count of ARF and wheat flour was similar. Water was the main source of *E.coli*. Though water is used in the preparation of ARF, E.coli count of the same was nil. This could probably be due to the heat treatment (at 50°C) given in the last stage of preparation of the gruel. Jaggery was the major source of yeast and mould. However; wheat, ARF, jaggery and water are present in the gruel in the following proportion i.e.: 16.2 : 0.8 : 20 : 80 respectively.

Table 30 : Microbial load contributed by various constituents of the gruel

Constituents	Total viable count[1]	E.coli[1]	Yeast and mould[1]
Wheat flour[2]	32±10	_	3±1
ARF[2]	30±12	_	3±1
Jaggery[2]	10± 4	6±2	_
Water[3]	20±10	_	6±4

1. Colony forming units (cfu) x 10^3/g
2. Average value obtained from sample stored for 0-6 months
3. Average of 6 samples of tap water collected during various seasons.

Thus, in the finished product, water is the main source of contamination followed by wheat flour, jaggery and least being the ARF.

Rowland et al (1978) determined the points at which bacterial contamination occurred during the process of preparation and storage. The millet

flour was found to contribute upto 10^9 total organisms/g and 10^2 *E. coli*/g of food. The water was heavily contaminated with highest counts being recorded in the wet season of 10^5 total organisms/ml and 10^2 E. coli/ml.

To infer it can be said that the microbial quality of wheat flour, jaggery and water which are the macro constituents of the gruel, is of primary importance and concern followed by the microbial quality of ARF which is a micro constituent.

V.2 Effect of addition of ARF stored for 0-6 months on the microbial profile of the raw and cooked gruels

Gruels were prepared using a standardised method with ARF at 4% of total solids. ARF stored for 0-6 months in a heat sealed HDPE bag stored in glass jar was utilised. A control gruel without ARF was also prepared. Each lot was divided into two. One half was left uncooked and the other half was cooked in a water bath at 90-92°C for five minutes and microbial profile was determined. Results are tabulated in Table 31.

The data presented in Table 31 show that the microbial load of all the uncooked samples was similar. This indicates that addition of ARF did not alter the microbial load of the sample and period of storage of ARF did not change the same. Cooking was found to effectively destroy all the vegetative cells. Though the uncooked samples were found to have acceptable total viable plate count (<50,000/g of food) the E.coli count was higher than the specified limit of 20^2/g for special dietary foods as determined by the International Commission on Microbiological Specifications for Foods (ICMSF 1974). However, all the cooked samples were microbiologically safe.

Table 31 : Total viable count, E.coli and yeast and mould count of raw and cooked gruels with ARF stored for 0-6 months

Type of gruel	ARF used	Total viable count[1]	E.coli form[1]	Yeast and mould[1]
Raw	-	48 ± 11	17 ± 6	2 ± 1
Cooked	-	-	-	-
Raw	0 month	42 ± 11	15 ± 4	2 ± 1
Cooked	0 month	-	-	-
Raw	1 month old	48 ± 10	12 ± 5	2 ± 1
Cooked	1 month old	-	-	-
Raw	2 months old	45 ± 20	13 ± 5	5 ± 2
Cooked	2 months old	-	-	-
Raw	3 months old	37 ± 14	13 ± 6	3 ± 1
Cooked	3 months old	-	-	-
Raw	4 months old	35 ± 14	14 ± 6	4 ± 2
Cooked	4 months old	-	-	-
Raw	5 months old	47 ± 9	12 ± 5	2 ± 1
Cooked	5 months old	-	-	-
Raw	6 months old	35 ± 10	12 ± 4	5 ± 2
Cooked	6 months old	-	-	-

[1] Colony forming units (cfu) x 10^3/g of food.

Mathur and Reddy (1983) found 60% and 15% of the weaning food samples collected from LIG and HIG families respectively to be unacceptable as indicated by the high *E. coli* and *Staph. aureus* count. These samples were collected from the feeding bowl at the time of feeding under appropriate aseptic

conditions. *E. coli* and *Staph. aureus* were present in the hand washings from mothers of LIG and HIG and the counts were same in both. This can add to the microbial load especially when food is handled carelessly. Further, under normal food handling conditions in the household, contamination via unclean utensils or hands is known to introduce a number of pathogens around 10^3 per g of food (Svanberg et al 1992).

During brief cooking of traditional weaning gruels the simmering mixture has been reported to contain 10^4 total microorganisms/g of food which is poured into the feeding bowls which are scrupulously clean and have a total viable counts of the order of 10^5 - 10^6 organisms per empty bowl (Rowland et al 1978). Therefore, the high level of contamination observed in other studies could have been due to contamination from utensils and hands. It is evident from the above discussion that addition of ARF either fresh or that stored for 0 to 6 months does not alter the microbial safety of gruels and further, freshly cooked gruels are absolutely safe for consumption irrespective of the type of ARF (i.e. fresh or stored for 1-6 months) used.

V.3 Microbial quality of cooked gruels with and without ARF stored under ambient conditions for 0-8 hours

The gruels were prepared either with or without the addition of ARF and stored under ambient conditions covered in the cooking pot. The study covered a period of six months where the temperature and relative humidity ranged between 30-40°C and 60-95% respectively. The samples were withdrawn aseptically at 0 hour and 1 hour interval thereupon and analysed as mentioned in details in Chapter III. Table 32 summarises the results.

The data indicate that cooking at 90-92°C for 5 minutes reduced the microbial load of freshly cooked samples with and without ARF completely. At the end of four hours of storage the total viable count increased beyond the stipulated limit of 50 X 10^3 cfu/g of food. Similarly, the *E.coli* count exceeded the safe limit of 2 X 10^2 cfu/g of food (IS 1969, ICMSF 1974). The microbial profile and load of gruels with and without ARF was similar indicating no beneficial or harmful effect of addition of ARF on the microflora of the cooked gruels during storage under ambient conditions. Wahed et al (1992) studied the multiplication of *S. flexneri* and enterotoxigenic *E. coli* added at 10^3 cfu/ml in rice porridges with and without wheat ARF. The growth of both the enteropathogens was similar in gruels with and without ARF. Thus, addition of ARF neither inhibited nor accelerated the growth of added pathogens. The increase in total viable count and *E.coli* on storage of cooked gruels indicates spore formation during cooking at 90- 92°C for 5 minutes. The spores under favourable conditions of temperature regenerate into vegetative cells over a period of time. A high total plate count and coliform count of a product can not always render the product unsafe. However, the presence of *E. coli* of fecal origin is regarded has to be regarded as a risk factor. It is also known that enterotoxigenic *E. coli* require > 10^5 viable cells per g of food and a sufficient time to result in the formation of exterotoxin to cause morbidity (Frank 1988).

Table 32 : Change in microbial profile of cooked gruels with and without ARF during storage

Sample	Period of storage (hours)	Total viable count[1]	E.coli[1]	Yeast and mould[1]
Control gruel	0	–	–	–
Experimental gruel	0	–	–	–
Control gruel	1	20 ± 8	–	–
Experimental gruel	1	22 ± 10	–	–
Control gruel	2	32 ± 10	–	–
Experimental gruel	2	30 ± 10	–	–
Control gruel	3	48 ± 11	–	–
Experimental gruel	3	45 ± 12	–	–
Control gruel	4	58 ± 11	10 ± 4	1.0 ± 1
Experimental gruel	4	57 ± 12	10 ± 6	1.2 ± 1
Control gruel	5	62 ± 12	10 ± 5	3.1 ± 1
Experimental gruel	5	64 ± 12	11 ± 5	3.7 ± 1
Control gruel	6	70 ± 17	12 ± 5	3.7 ± 1
Experimental gruel	6	71 ± 13	12 ± 4	4.0 ± 2
Control gruel	7	70 ± 18	14 ± 3	4.1 ± 2
Experimental gruel	7	72 ± 20	13 ± 4	4.2 ± 2
Control gruel	8	79 ± 30	14 ± 5	5.0 ± 2
Experimental gruel	8	79 ± 32	14 ± 6	5.7 ± 2

[1] Colony forming units (cfu) x 10^3/g of food

Control gruel without ARF

Experimental gruel with ARF

Studies from Bangladesh (Black and Brown 1989, Black et al 1984), India (Mathur and Reddy 1983), Kenya (Van Steenberg 1983) and Gambia (Rowland et al 1978) have shown evidence of heavy microbial load even in freshly cooked samples which increased further on storage. These studies also report heavy contamination of the utensils used for storage and feeding. Higher contamination of weaning foods has been reported in samples stored in a clay pot or a glass bottle which are highly adsorbent surfaces. Products stored in cooking pot were less contaminated as against those stored in other containers (Simango 1988). In most of these studies there is a lack of parallel data on surveillance on morbidity wherever the data on microbial contamination is supported by surveillance of population for diarrhoeal morbidity a low incidence of diarrhoea (0.54 attacks per child per year) is reported (Pertlet et al 1988).

Thus the following important points emerge from above discussion :

- The freshly cooked gruels with and without ARF have a zero microbial load and remain fit for consumption upto 3 hours of storage under ambient conditions.

- The change in microbial profile of cooked gruels with and without ARF is similar during storage under ambient conditions.

- Reheating of gruels stored for 3 hours and beyond may lower the bacterial load to safe levels however it may not destroy the toxins liberated by these microorganisms. Therefore, further studies are required to establish the safety of stored gruels on reheating.

VI. **To study the effect of addition of jaggery and salt on viscosity and viscosity reduction power of various ARFs on gruels prepared from (1) cereal & pulse mixes, (2) donated foods, and (3) commercial weaning foods.**

VI.1 The gruels prepared traditionally contain jaggery or sugar as a sweetening agent. If neither is affordable salt is added. Fat is utilised in varying amounts depending on the availability and cost (Deshpande 1987). Jaggery and fat besides lending palatability to the gruel also improve the energy density. It has been proposed that fat and sugar be included in a young child's diet alongwith special processing like toasting, sprouting, malting etc. in order to increase the energy density and reduce the bulk (Hofvander and Underwood 1985, Dearden et al 1980). Therefore, the effect of addition of fat (groundnut oil), jaggery (unrefined sugar) and salt on viscosity in cereal and cereal + pulse gruels was studied. Oil was incorporated at 10% level and jaggery at 75% or salt at 2.5% of total solid contents.

The above was based on observation of actual method of preparation of sweet gruels (n=162) and salted gruels (n=15) by urban slum mothers of Baroda (John 1989).

The cereals selected were wheat, sorghum, pearlmillet and corn. ARFs from above cereals were utilized. All the above cereals were mixed with redgram and bengalgram dhal in the ratio of 4:1. Cereal, cereal + pulse, cereal + pulse + oil + jaggery and cereal pulse + oil + salt gruels of 15%, 20%, 25% and 30%. Solid content were prepared and the viscosity of each recorded in order to establish the highest solid content for each gruel. Table 33 presents the data.

Table 33 **Effect of total solid contents on the viscosity of gruels prepared from cereal+pulse mix**

Gruel	Viscosity (cp units)[1] at solid content of				Acceptable gruel
	15%	20%	25%	30%	
W	6800	18000	37600	80000	25%
S	16700	44800	78400	-	25%
PM	6400	16700	37200	78400	30%
C	10400	24400	68400	80000	25%
W + RG	6800	18200	38000	-	25%
W + BG	7040	18400	40400	-	25%
S + RG	18400	50200	80000	-	20%
S + BG	19200	52000	-	-	20%
PM + RG	7200	17000	38000	80000	25%
PM + BG	8000	17800	39200	-	25%
C + RG	12000	32000	76000	-	25%
C + BG	12400	33200	80000	-	25%
W+RG+O+J	1200	2800	5400	7200	30%
W+BG+O+J	1600	11920	6200	8420	30%
W+RG+O+S	32000	62800	-	-	20%
W+BG+O+S	34200	63600	-	-	20%
S+RG+O+J	760	5120	6900	52000	30%
S+BG+O+J	1200	6200	8120	60000	30%
S+RG+O+S	18200	50800	-	-	20%
S+BG+O+S	19000	60000	-	-	20%
PM+RG+O+J	870	1940	2320	4760	30%

Gruel	Viscosity (cp units)[1] at solid content of				Acceptable gruel
	15%	20%	25%	30%	
PM+BG+O+J	920	2200	2420	5200	30%
PM+RG+O+S	7840	19700	56000	-	25%
PM+BG+O+S	8200	20200	62000	-	25%
C+RG+O+J	696	1120	2800	6440	30%
C+BG+O+J	720	1280	3200	7200	30%
C+RG+O+S	13560	26000	74800	-	20%
C+BG+O+S	14280	30200	-	-	20%

1 – Mean of three observations

– Viscosity beyond the range of the instrument.

W - Wheat; PM - Pearlmillet; S - Sorghum;

C - Corn; RG - Redgram; BG - Bengalgram;

O - Oil; J - Jaggery; S - Salt.

As can be seen from the data presented in table, viscosity of each cereal gruel varied. Pearlmillet gruel had lowest viscosity followed by wheat, corn and sorghum at all solid levels. The difference in paste viscosity of gruels from various cereals arises due to compositional differences. Besides starch, proteins also contribute substantially to the viscosity of a product namely proteins like gluten. Besides the total starch content the amylose and amylopectin content and ratio affects the paste viscosity (Radley 1968). The paste characteristics of a flour are also attributed to the α-amylase content of the grain (Harinder and Bains 1987). In absence of data on the composition and characterisation of each component of various cereals used in the study it is difficult to explain the

variations observed. However, the study established the highest solid content for each cereal gruel as given in table 33.

Addition of pulse flour increased the viscosity of each cereal gruel. Incorporation of fat, jaggery and salt affected the viscosity of the gruel. Table 34 presents the percent change in viscosity on incorporating pulse flour, oil & jaggery and oil & salt in the various cereal gruels.

Addition of pulse flour was found to increase the viscosity by 0.6% - 36% in various gruels. Cereal gruels prepared using bengalgram flour showed content of pulses is reported to be approximately 40% and it forms the major carbohydrate fraction. The cold paste viscosity of black gram dal is non-starchy polysaccharide. Addition of sodium chloride to black gram flour is reported to reduce the consistency of the same as studied using various rheological

Table 34 : Percent change in viscosity of cereal gruels on addition of pulse flour, oil, salt and jaggery

Gruel	Percent change in viscosity at solid content levels			various [1]
	15%	20%	25%	30%
W + RG	+ 0.6	+ 1.1	+ 1.1	-
W + BG	+ 3.5	+ 2.2	+ 7.4	-
S + RG	+10.2	+12.0	+ 2.0	-
S + BG	+14.9	+12.0	-	-
PM + RG	+12.5	+ 1.7	+ 2.1	+ 2.0
PM + BG	+25.0	+ 6 5	+ 5 3	-
C + RG	+15.3	+31.1	+11.1	-

Gruel	Viscosity (cp units) at solid content of			Remarks
	15%	20%	25%	30%
C + BG	+19.2	+36.1	+16.9	-
W+RG+O+J	-82.3	-84.4	-85.6	-91.0
W+BG+O+J	-76.5	-89.3	-83.5	-89.5
W+RG+O+S	+78.7	+248.8	-	-
W+BG+O+S	+80.1	+253.3	-	-
S+RG+O+J	-95.4	-71.5	-81.6	-35.0
S+BG+O+J	-92.8	-86 1	-89.6	-
S+RG+O+S	+ 8.9	-13.3	-	-
S+BG+O+S	+13.7	+33.9	-	-
PM+RG+O+J	-86.4	-88.3	-93.7	-93.9
PM+BG+O+J	-85.6	-86.8	-93.4	-
PM+RG+O+S	+22.5	+17.9	+55.9	-
PM+BG+O+S	+25.0	+ 6.5	+ 5.3	-
C+RG+O+J	-93.3	-95.4	-95.9	-91.9
C+BG+O+J	-93.0	-94.7	-95.9	-95.3
C+RG+O+S	+30.3	+ 6.5	+ 9.3	-
C+BG+O+S	+37.3	+23.7	-	-

1 – percentage change in viscosity as compared with the cereal gruel.

– could not be calculated in absence of either or both values.

W - Wheat; PM - Pearlmillet; S - Sorghum;

C - Corn; RG - Redgram; BG - Bengalgram;

O - Oil; J - Jaggery; S - Salt.

constants i.e. yield stress, consistency index and flow behaviour. This reduction in is explained as follows. Sodium chloride holds a considerable amount of water and reduces the swelling of starch. Therefore, the resultant product has a low cold as well as hot paste viscosity (Reddy et al 1989). The above observation is in total contradiction to that of Olkku and Rha (1978) and the present study. Data presented in table show an increase in viscosity (5% - 250%) of all cereal+pulse gruels on addition of salt. The change varied with the type and concentration of substrate used and no obvious trends were seen. Olkku and Rha (1978) observed a 100% - 200% increase in the viscosity of pure starch dispersions on addition of salt. The authors suggested the possible role of sodium chloride in increased amylose - amylopectin and amylopectin - amylopectin interaction. The above observation has been further substantiated with scanning electron micrographs of salt added starch dispersions.

Addition of oil and jaggery resulted in reduction in viscosity of all the gruels. The percentage redution in viscosity on addition of oil and jaggery was similar in gruels from corn and sorghum (92 - 95%), follwed by pearlmillet gruel (85 - 87%) and wheat gruel (77 - 82%). Several investigators have reported similar findings. Olkku and Rha (1978) reported that deffating of grains yielded high viscosity pastes and addition of lipids lowered the viscosity. They further report on viscosity reducing properties of sugar which traps water and prevents gelatinisation of starch. Fat molecules coat the starch and prevent galatinisation. As a pure starch slurry cools down it solidifies while, addition of fat or oil produces a liquid slurry on cooling (Dearden et al 1973).

The above experiment clearly demonstrates the beneficial effect of addition of fat and jaggery in terms of reduction in viscosity. The same is however not achieved on incorporation of salt alongwith fat. Our previous studies report a 45% - 60% reduction in viscosity of cereal gruels on addition of

fat and salt and around 80 - 84% on addition of jaggery and fat (Deshpande 1987). However, the cereal + pulse gruels did not show such remarkable change in viscosity on addition of salt + oil and in certain instances the resultant slurry had a viscosity higher than the slurry without fat and salt.It is evident from the above data that addition of fat besides lowering the viscosity also contributes significantly to the energy density of the gruels and so does jaggery. This increase in energy density does not lend to an increase in the volume and viscosity and is therefore of paramount significance.

Table 35 presents data on the energy density of selected gruels with fat and jaggery or fat and salt along with their control counterparts. The gruels selected are those with viscosity suitable for further studies on action of various ARFs.

Table 35 : Energy density of selected gruels from cereal + pulse with added oil and jaggery and oil and salt

Gruel	Solid content (%)	Energy density (Kcal/g)
W+RG	25%	0.86
W+BG	25%	0.87
W+RG+O+J	30%	2.16
W+BG+O+J	30%	2.18
W+RG+O+S	20%	0.86
W+BG+O+S	20%	0.87
S+RG	20%	0.69
S+BG	20%	0.70

Gruel	Solid content (%)	Energy density (Kcal/g)
S+RG+O+J	30%	2.17
S+BG+O+J	30%	2.19
S+RG+O+S	25%	1.09
S+BG+O+S	25%	1.10
C+RG	30%	1.02
C+BG	30%	1.04
C+BG+O+J	30%	2.17
C+RG+O+S	25%	1.07
C+BG+O+S	20%	0.86
PM+RG	25%	1.07
PM+BG	25%	1.09
PM+RG+O+J	30%	2.20
PM+BG+O+J	30%	2.22
PM+RG+O+S	30%	1.34
PM+BG+O+S	30%	1.36

– Could not be calculated in absence of either or both values.

W - Wheat; PM - Pearlmillet; S - Sorghum;
C - Corn; RG - Redgram; BG - Bengalgram;
O - Oil; J - Jaggery; S - Salt.

Data from table 35 suggest a definite increase in energy density of cereal+pulse gruels on addition of oil+jaggery which ranged between 2.25 - 2.50 times. Incorporation of oil+salt contributed marginally (0.95 - 1.2 times) towards enhancement of energy density of the gruels. In certain instances namely wheat+pulse and corn+bengalgram in combination with oil+salt yielded a product with extremely high viscosity and an acceptable gruel could be obtained only if the solid content was decreased. Thus, the improvement in energy density achieved by incorporation of oil was set off by reduction in the solid contents and the gruels with oil+salt had a similar or even lower energy density as compared against their control counterparts prepared without the addition of oil+salt. However, all the above gruels were studied for viscosity reduction on addition of ARF and the effect of oil+salt and oil+jaggery if any on the amylolytic power of ARF.

The selected cereal+pulse, cereal+pulse+oil+jaggery and cereal+pulse+oil+salt gruels were studied for viscosity reduction on addition of ARFs developed from wheat, corn, pearlmillet and sorghum at graded levels namely 1-7% of solid contents. ARF from pearlmillet at 4% solid contents was found to yield maximum reduction in viscosity followed by sorghum, corn and wheat ARF under similar experimental conditions. Therefore, pearlmillet ARF at 4% of total solids was utitlised for further experiments and the results are presented in table 36.

Table 36 : Viscosity reduction in gruels on addition of pearlmillet ARF at 4% solid contents

Gruel	Total solid contents(%)	Viscisity (cp units)	% reducton in viscosity
W+RG	25	38000	-
W+RG+A	25	4480	87
W+RG+O+J+A	30	2740	93
W+BG	25	40400	-
W+BG+A	25	6940	88
W+BG+O+J+A	30	2980	93
W+BG+O+S+A	20	5960	85
S+RG	20	50200	-
S+RG+A	20	4140	92
S+RG+O+J+A	20	2800	94
S+RG+O+S+A	20	3200	94
S+BG	20	52000	-
S+BG+A	20	4320	92
S+BG+O+J+A	20	3120	94
S+BG+O+S+A	20	3640	93
PM+RG	30	38000	-
PM+RG+A	30	3800	90

Gruel	Total solid contents(%)	Viscisity (cp units)	% reducton in viscosity
PM+RG+O+J+A	30	2800	93
PM+RG+O+S+A	30	3200	92
PM+BG	25	39200	-
PM+BG+A	25	3980	91
PM+BG+O+J+A	25	2960	92
C+RG	30	76000	-
C+RG+A	30	3180	96
C+RG+O+J+A	30	2220	97
C+RG+O+S+A	30	3000	96
C+BG	30	80000	-
C+BG+A	30	3600	95
C+BG+O+J+A	30	2920	96
C+BG+O+S+A	30	3120	96

1 – Mean of three observations

W - Wheat; PM - Pearlmillet; S - Sorghum;
C - Corn; RG - Redgram; BG - Bengalgram;
O - Oil; J - Jaggery; S - Salt.

It is evident from the data presented in table 36 that pearlmillet ARF incorporated at 4% of total solids brought about 87%-97% reduction in viscosity. The final viscosity of all the gruels was similar irrespective of the type, concentration and initial viscosity of the gruels. Thus incorporating ARF at 4% solid levels was adequate to convert the thick gruels into thin gruels with viscosity desirable for young child feeding (2000-4000 cp units) and acceptable energy density (1.1 - 2.2 Kcal/g).

VI. 2 **Viscosity reduction in donated foods**

The donated foods namely corn soya milk (CSM), cornsoya blend (CSB) and energy food are utilised in the National Nutrition Programs for the mass feeding of the under threes. Therefore, it was thought worthwhile to study the effect of adding ARF at the time of cooking and to the dry mix on the viscosity reduction in the cooked product. Addition of ARF to the mix would simplify the procedure of handling and storing the mix and preparing the gruel. Gruels of 15 - 30% solid content were prepared from CSM, CSB and energy food. Energy food contains added jaggery and fat. Hence, jaggery and oil were added at 75% and 10% solid contents level respectively in gruels from CSM and CSB. Our previous experiments indicated an optimum requirement of ARF at 4% of total solid contents for desirable viscosity reduction. Therefore, pearlmillet ARF which was found to give the best results, was incorporated at 4%, 5%, 6% and 7% of total solid contents in the dry mix. Th emix was seived thoroughly for uniform distribution of the ARF throughout the mix and stored in an airtight container. This mix was utilised for the preparation of gruels. Similarly gruels were prepared from CSM, CSB and energy food by incorporating ARF at 4%-7% of total solids at the time of cooking and the comparative viscosity reduction was

studied. Table 37 presents the data on viscosity reduction in gruels with 25% solid content with pearlmillet ARF added at 4% - 7% of total solids, either to the dry mix or to the gruel during cooking.

Table 37 : Viscosity reduction in gruels from CSM, CSB and Energy Food on addition of ARF before and during cooking

Gruel	ARF (% solid contents)	Stage of ARF addition	Viscosity1 (cp units)	% Viscosity reduction
CSM	0	-	23800	
	4	BC	4173	82
	4	DC	4200	82
	5	BC	3600	85
	5	DC	3480	85
	6	BC	2400	90
	6	DC	2420	90
	7	BC	1800	92
	7	DC	1860	92
CSB	-	-	20800	-
	4	BC	4666	77
	4	DC	5026	76
	5	BC	3800	82
	5	DC	3920	81
	6	BC	2720	87
	6	DC	2800	86
	7	BC	2170	89

Gruel	ARF (% solid contents)	Stage of ARF addition	Viscosity1 (cp units)	% Viscosity reduction
Energy Food	7	DC	2120	90
	-	-	38000	-
	4	BC	2780	93
	4	DC	2800	93
	5	BC	2220	94
	5	DC	2246	94
	6	BC	2120	94
	6	DC	2200	94
	7	BC	1600	96
	7	DC	1680	96

1 – Mean of three observations

BC – Before cooking

DC – During cooking

The data presented in table 37 clearly indicate that irrespective of the substrate, initial viscosity and the stage of addition of ARF to the gruels, the final viscosity of each gruel was comparable under similar experimental conditions. The desirable viscosity reduction was achieved on incorporating pearlmillet ARF at 4% level and further increase in the amount of ARF was not required. The above results are highly encouraging. They clearly establish the feasibility of obtaining low viscosity gruels by incorporating pearlmillet ARF at 4% level to a dry mix namely CSM, CSB and Energy Food. The amount of ARF incorporated can be

increased to 5% of solid contents to take care of any loss occuring during handling and storage of mixes in bulk. These aspects need to be studied indepth further

VI.3 Viscosity reduction in commercial weaning foods

Four commonly available cereal weaning foods were purchased in bulk from the local market. Weaning food A, B and D were wheat based and C was a rice based product. They were roller dried products fortified with sugar and selected minerals and vitamins.

For initial viscosity trials each product was reconstituted with boiling water as per the manufacturer's instructions which have been described in detail in chapter III. The percent solid content, energy density and viscosity of the reconstituted gruels is given in table 38.

As can be seen from the table the reconstituted gruels had a desirable viscosity but low energy density. Since all the commercial mixes are reconstituted

Table 38 : Total solid content, energy density and viscosity of commercial weaning foods on reconstitution

Mix	Solid content (%)	Viscosity[1] (cp units)	Energy Density (Kcal/g)
A Farex	27.7	4720	1.10
B Farex-Veg.	20.8	2560	0.88
C Nestum rice	10.1	2260	0.42
D Cerelac Wheat	25.0	5400	1.05

1 - Mean of three observations.

with boiling water or milk, it was thought appropriate to incorporate ARF in the the mix and study the viscosity reduction on reconstitution. Pearlmillet ARF at 4% of total solid contents was incorporated in all the mixes. Gruels with higher solid contents, than prescribed by the manufacturer, were prepared from ARF added mixes. The solid contents were increased gradually and the viscosity of reconstituted product recorded. This was repeated to obtain maximum possible solid contents level for each mix while keeping the viscosity within the desirable range and table 39 presents the data obtained.

Table 39 : Maximum solid content attainable for commercial weaning gruels on incorporation of ARF

Mix	Solid content (%)	Viscosity (cp units)	Energy density (Kcal/g)
A	38	4680	1.50
B	31	2300	1.30
C	20	2200	0.83
D	35	3400	1.50

The above data indicate the possibility of **obtaining a low viscosity product from an ARF added mix on reconstitution with boiling water.** Incorporation of pearlmillet ARF at as little as 4% solid contents level in the commercial weaning mixes improved their energy density by 1.5 - 2 times. This is an important finding which can be exploited at a commercial level to give high energy density gruels. This aspect needs further detailed investigation to work out the mode and cost of incorporation of ARF.

VII. **To compare the in vitro starch digestibility of gruels with ARF versus gruels without ARF**

It has long been known that the physico-chemical form of starch affects both the rate and extent of its hydrolysis by amylolytic enzymes and that there are corresponding differences in the digestibility of starch in foods. Further, the physical form of the starch polysaccharides may undergo several transformations during processing and storage of a starch- based food. Raw starch granules are semi-crystalline and birefringent material. During processing namely by heating in presence of excess water at a characteristic temperature, which is around 70°C for most starches, the granular order is lost. The granules swell to many times their original size and the amylose is preferentially solubilized. The degree of solubilisation and loss of crystallinity varies with the heat-moisture treatment given. Upon cooling, the dispersed starch polysaccharides reassociate or retrograde. It is predominantly the amylose fraction which forms a network and crystallizes. Thus a starch polysaccharide may occur in quiet diverse forms. These forms may show varying degree of resistance to enzymolysis (Ring et al 1988). Starch which escapes digestion in the small intestine is acted upon by the colonic bacteria producing bloat and flatus (Bond and Levitt 1972). This is of paramount significance in the feeding of young children. Therefore, it was thought worthwhile to study the digestibility of various starch based gruels in raw, cooked and ARF added form.

The in vitro technique was utilised for studying the starch digestibility of the following :

(1) Cereal gruels viz. wheat, corn, pearlmillet and sorghum gruel.

(2) Cereal gruels in combination with greengram and bengalgram dal.

(3) Corn Soya Milk and Corn Soya Blend.

(4) Energy food.

Table 40 summarises the results.

As is evident from the data presented in Table 40 the raw gruels prepared from cereals or cereal & pulse combination had a low starch digestibility. Corn had the lowest starch digestibility followed by sorghum, wheat and pearlmillet. Inclusion of greengram and Bengalgram did not have any obvious adverse effect on the starch digestibility of raw gruels. Raw gruels prepared from CSM, CSB and Energy Food had higher digestibility compared to the cereal or cereal & pulse gruels. The higher starch digestibility recorded in raw gruels from CSM, CSB and Energy Food could be due to the fact that these are processed, ready to reconstitute mixes. The starch digestibility improved by 21-300% on cooking the gruels. Though raw corn gruel had poor digestibility the cooked gruel from corn had digestibility comparable with that of other gruels.

The percentage improvement in starch digestibility of cereal and pulse gruels was proportionately lower on cooking as compared to the gruels prepared from cereals alone. Therefore, the cooked gruels from cereal & pulses had a lower overall starch digestibility than gruels prepared from cereals alone.

Table 40 : In vitro starch digestibility of raw versus cooked gruels

Sample	% starch digestibility of[1]					
	Raw gruel			Cooked gruel		
	30 min	60 min	90 min	30 min	60 min	90 min
Wheat	50	62	67	70	90	92
Corn	20	53	58	80	92	93
Pearlmillet	63	67	72	82	94	96
Sorghum	42	50	57	60	80	82
Wheat+Green-gram dal	48	59	63	58	80	82
Wheat+Bengal-gram dal	47	59	64	55	81	82
Corn+Green-gram dal	19	50	53	62	72	82
Corn+Bengal-gram dal	18	51	54	60	72	80
Pearlmillet+ Greengram dal	62	67	72	73	75	79
Pearlmillet+ Bengalgram dal	60	65	71	72	76	80
Sorghum+ Greengram dal	40	52	57	58	72	79
Sorghum+ Bengalgram dal	38	50	56	56	71	76
CSM	70	72	74	80	84	94
CSB	68	70	72	72	78	90
Energy Food	72	78	79	82	86	9

(1) Mean of three observations in duplicate

Lower starch digestibility of raw corn has been reported by several investigators (Mouliswara et al 1993, Ring et al 1988). The poor digestibility of starch from raw corn has been attributed to the crystalline form, degree of crystallinity and the granule size. However, the exact mechanism by which these factors affect the starch digestibility is not clear (Ring et al 1988). On cooking the starch granules lose crystallinity, gelatinise and swell. This results in improved accessibility of enzyme to C- C bonds in the starch and an increase in the rate of hydrolysis of starch (Whistler et al 1967). Chandrasekhar and Desikachar (1988) have reported low starch digestibility of raw sorghum and corn. The same has been attributed to the presence of a higher percentage of amylose which is hydrolysed at a slower rate than amylopectin.

The lowered starch digestibility of gruels from cereal and pulse could be either due to the type of starch contributed by the pulses or any other factor contributed by the pulses which inhibits amylolysis. Ring et al (1988) have reported a lower hydrolysis of pea starch as compared to the cereal starches. The authors attribute this to the high amylose content and crystalline form of the pea starch. Thomson and Yoon (1984) have suggested a mechanism by which polyphenols namely tannic acid, phytic acid and catechin present in pulses can affect starch digestibility. The authors suggest possible direct interaction between polyphenols and amylase or through binding of calcium ions required for catalising amylase activity.

In the subsequent experiment ARF from pearlmillet (which was found to be the most active of all) was incorporated at 4% of total solid contents to all the gruels and the in vitro starch digestibility was studied. The results are presented in Table 41.

As is evident from the data in Table 41 the starch digestibility improved markedly on addition of ARF in all the gruels. This could primarily be due to the conversion of starch into lower molecular weight compounds by the action of ARF. When such a substrate is acted upon by digestive enzymes the rate and degree of hydrolysis is rapid. Thus, all the gruels with ARF showed a higher percentage starch digested at any point of time as compared to their control counterparts. Further, the gruels with ARF also showed a more rapid rate of hydrolysis as depicted in Figure 15.

Table 41 : Effect of addition of ARF on in vitro starch digestibility of gruels

Sample	% starch digested[1]					
	Control[2]			Experimental[3]		
	30 min	60 min	90 min	30 min	60 min	90 min
Wheat	70	90	92	90	92	100
Corn	80	92	93	92	93	99
Pearlmillet	82	94	96	94	96	99
Sorghum	60	80	82	80	88	90
Wheat+Greengram dal	58	80	82	80	82	97
Wheat+Bengalgram dal	55	81	82	83	86	98
Corn+Greengram dal	62	79	82	79	89	99
Corn+Bengalgram dal	60	72	83	78	89	96
Pearlmillet+ Greengram dal	73	75	79	78	95	97

Sample	% starch digested[1]					
	Control[2]			Experimental[3]		
	30 min	60 min	90 min	30 min	60 min	90 min
Pearlmillet+ Bengalgram dal	72	76	80	77	94	99
Sorghum+ Greengram dal	58	72	79	75	93	96
Sorghum+ Bengalgram dal	56	71	76	72	95	98
CSM	80	84	94	83	97	100
CSB	72	89	90	88	96	98
Energy Food	82	83	92	84	98	99

(1) Mean of three observations in duplicate

(2) Gruel without ARF

(3) Gruel with ARF

As can be seen from Figure 15, maximum starch digestion occurred within first 15 minutes in all the gruels with added ARF while, similar degree of digestibility was achieved at the end of 30 minutes in gruels without ARF. In absence of data from parallel studies it is difficult to corroborate the findings of present study.

It may however be concluded here that, the starch digestibility improves on cooking and, the extent and rate of hydrolysis improves further on addition of ARF. The higher rate of hydrolysis may imply rapid emptying of gastric contents which is important in determining the feeding frequency in young children. However, further indepth studies are required to establish the same. However, easy digestibility of ARF added gruels may prove to be the single most beneficial factor in feeding of young malnourished children.