

**STUDIES ON EXTRACTION AND CHARACTERIZATION
OF STARCH FROM MOULD - INFECTED BLACK
SORGHUM**

by

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Ujwala Jotiba Patil

(Reg No. 99094)

A Thesis submitted
in partial fulfilment of the requirements for the degree
of

MASTER OF SCIENCE

[AGRICULTURE]

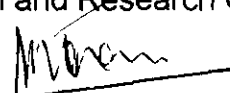
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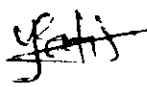
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This is to certify that the thesis entitled “ **Studies on Extraction and Characterization of Starch from Mould-infected Black Sorghum**” submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri in partial fulfilment of the requirements for the degree of **Master of Science (Agriculture) in Biochemistry**, embodies the results of a bonafide research work carried out by **Miss Ujwala Jotiba Patil** under my guidance and supervision and that no part of the thesis has been submitted for any degree or diploma.

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ACKNOWLEDGEMENTS

I avail this opportunity to express my deep sense of gratitude to Dr. J.K. Chavan, Chairman of the Advisory Committee and Professor and Head, Department of Biochemistry, Mahatma Phule Krishi Vidyapeeth, Rahuri for suggesting the research problem, his valuable guidance and sympathetic attitude throughout the entire period of this investigation till final shaping of this manuscript in the present form.

I express my sincere thanks to the members of my Advisory Committee Dr. U.D. Chavan, Assistant Professor of Biochemistry, Dr. K.M. Pol, Associate Professor of Plant Physiology, and Dr. B.H. Khan, Assistant Professor of Statistics, Mahatma Phule Krishi Vidyapeeth, Rahuri for their valuable suggestions and critical reading of the manuscript.

I am extremely thankful to Shri. U.S. Dalvi, Research Associate, Department of Biochemistry for his kind help during the laboratory work. My thanks and appreciation are also due to Prof. A.A. Kale, Asstt. Professor, Prof. D.P. Kachare, Asstt. Professor, Shri. S.V. Damame, Sr. Res Asstt. for their kind co-operation.

I am also grateful to my friends Shailesh, Bipin, Siddharth, Chakkar, Prakash, H.M., Sajid, Sachin and Dipti who always cooperated and inspired me during my post graduate education.

Last but never the least, I hardly find words to express my gratitude to my beloved parents Shri Appa and Sou.Aai, brother Dada, sister Dipu and younger brother Mayu for their intense affection, constant encouragement and inspiration in the life which enabled me to build up a confidence to develop my educational career.

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ABSTRACT

Studies on Extraction and Characterization of Starch from Mould-infected Black Sorghum

by

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2001

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The present investigations were undertaken to standardize a suitable process for the extraction and recovery of starch from moulded and blackened sorghum grains. A market sample of black sorghum was used for the experiments. Initially, the grain soaking time and temperature for maximum water absorption and starch recovery were standardized. Then different grain soaking media such as water, dilute acids and dilute alkali were tested for starch extraction. Two slurry filtration devices viz. musline cloth and 0.045 mm sieve were tested for starch yield. Finally, dilute alkali was used in different changing sequence of grain soaking, blending or residue washing to obtain maximum starch yield. The starch obtained from different methods was analysed for the contents of starch, proteins, sugars, ash and certain functional properties.

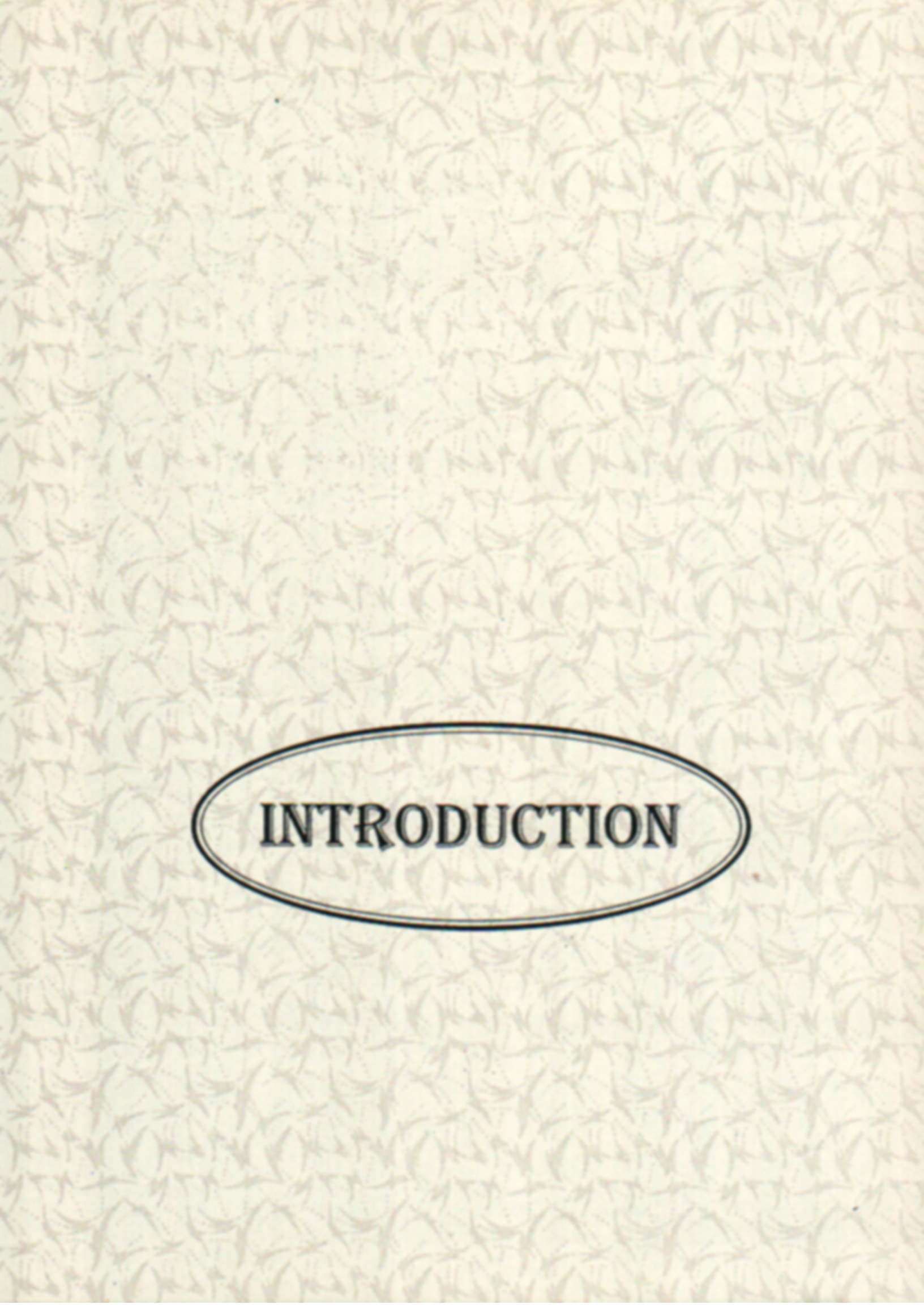
A maximum absorption of water was observed in the grains soaked at 60 °C for 96 hrs. However, the highest starch yield was obtained when the

Abstract (Contd...)

grains were soaked at 60 °C for 144 hrs. The dilute alkali (0.2%) was found to be superior grain soaking and residue washing medium than the dilute acids or water. A significantly higher starch yield was obtained with the use of 0.045 mm sieve (59.3%) as a slurry filtration device as compared to that of musline cloth (33.8%).

The starch obtained with muslin cloth exhibited slightly higher purity but significantly lower recovery. A soaking of grains for 144 hrs at 60 °C in 0.2 per cent NaOH followed by grinding in water and filtration of the slurry through 0.045 mm sieve, centrifugation of the filtrate at 8000 x g for 30 min, washing of the residue in 0.2 per cent NaOH followed by water washing, centrifugation and drying of the residue has been standardized to obtain a maximum of 62.2 per cent bright starch from moulded and blackened sorghum. The product contained 91.6 per cent starch.

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INTRODUCTION

1. INTRODUCTION

Grain sorghum (*Sorghum bicolor* L. Monech) is one of the important cereal crops of India. It is an important food crop particularly for arid and semi-arid tropical areas. In India, the crop is widely grown in the States of Maharashtra, Andhra Pradesh, Madhya Pradesh, Karnataka, Gujarat and Tamil Nadu and often fetches higher market price than wheat and millets. It is a dual purpose crop providing staple food for human consumption and fodder for livestock (Salunkhe *et al.*, 1984).

The sorghum is grown in both seasons i.e. in *kharif* as well as in *rabi*. In India, sorghum was grown on an area of 5714 thousand ha with a production of 4084 thousand tonnes in *rabi* while over an area of 5858 thousand ha with a production of 7004 thousand tonnes in *kharif* during 1996-97. In Maharashtra State, sorghum occupied an area of 3542 thousand ha with a production of 2329 thousand tonnes in *rabi* and an area of 2150 thousand ha with a production of 3911 thousand tonnes in *kharif* of 1992-93 (Anon. 1998-99).

The sorghum is an important source of dietary calorie, proteins, minerals and vitamins to a large segment of population, particularly from the lower income groups and farming community. It is consumed mostly in the form of pan-bread (*roti / bhakari*). Generally, pearly white grain with intermediate corneous endosperm and a thin pericarp without testa produce acceptable pan-bread while waxy, floury grains with coloured spotting, brown types



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and discoloured grains produce unacceptable product (Subramanian and Jambunathan, 1984).

The *rabi* crop always produces pearly white grains. In *kharif*, however, the crop is often caught in late rains during grain development stage. Due to humid climate, it results in the migration of phenolic pigments of the glumes to the pericarp of the grain. Besides, several grain-mould fungi infect the seeds at pericarp layer and secrete coloured pigments at the surface. These pigments get spread all over the grain and make them red, brown to black in colour. The species of *Fusarium*, *Aspergillus*, *Curvularia*, *Alternaria* and *Helminthosporium* have been reported to grow on the developing grain and make them brown to black (Anon. 1980). This is called a black sorghum or superdisco grade in market. The moulds generally grow on the pericarp leaving the endosperm unaffected. However, under severe infestation particularly if infested at early grain developing stage, the entire grain remains underdeveloped or entirely deep blackened. Several coloured pigments are secreted by the grain-mould which make the grains black and almost unacceptable for any edible purpose. Although the reliable estimates are not available, the losses to the tune of 60-65% have been observed. Thousands of tonnes of sorghum is practically wasted every year in the state due to blackening. Estimates placed in the annual economical cost to the National Agricultural GDP due to rain caused quality deterioration of *kharif* grain to the tune of Rs. 200-250 crores (Anon. 2000).

The blackened sorghum can be processed into dehulled white grains by pearling. The partial physical pearling of normal white sorghum using Satake (horizontal), cone (vertical) or McGill Rice polisher resulted into 6-27 per cent broken grains (Chandrashekhar and Desikachar, 1984). The mould affected black grains may suffer greater breaking losses as these grains are relatively more soft and near complete pearling is essential.

Darade *et al.* (1999) have attempted to develop a alkali dehulling process using 4% NaOH for blackened sorghum. This treatment although found to be quite-effective, the cost of processing is high and it requires a large quantity of water for washing the treated grains. A physical pearling treatment of black sorghum has been recently standardized in this laboratory (Chavan *et al.*, 2001). This process is simple, cheaper and easy to practice. However, this technology may be suitable for smaller lots at domestic level because formation of a black sorghum on a large scale every year is not guaranteed to install commercial processing plants. Besides, both black and pearled sorghum exhibit a limited storage life. Hence, it is necessary to attempt to develop technologies for alternative value added product such as starch from such mould-infested black sorghum.

The cereals are widely used for starch extraction by either dry or wet milling processes. The starch is however, obtained usually by a wet milling process. The starch extraction process for corn is well developed on a commercial scale (May, 1987; Anderson and Watson, 1982; Watson, 1967).

Attempts have also been made to isolate starch from barley (Vasanthan *et al.*, 1997). However, the reports on starch preparation from sorghum are limited. The procedure used for starch extraction from sorghum is identical to that used for corn (Buffo *et al.* 1998). However, sorghum differs from corn in grain structure, nature and distribution of starch granules with the grain and starch granule-protein bodies association (Yang and Seib, 1995). The sorghum grains with yellowish pigments are reported to produce starch with dull-coloured starch. Further the tiny starch granules of main horney endosperms are embeded in a thick and heavy cross linked protein matrix in grain sorghum. Hence, the extraction conditions used for corn i.e. use of acid water may not be suitable for sorghum unless high acid concentration is used which might hydrolyse the starch to lower the starch recovery. Hence, there is a need to test the suitability of alkaline conditions to solubilize both proteins and phenolics during steeping and blending of grains to obtain a protein-free starch with higher recovery and bright appearance. The blackened sorghum produce which has practically negligible market can be converted into a starch, eventhough the recovery level is relatively low. Such starch may be utilized in weaning and high-protein foods, medicine, laundry, textile industry etc. Therefore, present investigation on extraction and characterization of starch from black sorghum was undertaken, with the following objectives -

- 1) To standardize the alkaline extraction conditions for starch from black sorghum.
- 2) To characterize the purified starch by physico-chemical properties.

Chapter Opener Page



**REVIEW
OF
LITERATURE**

2. REVIEW OF LITERATURE

2.1 Composition of Sorghum Grain

An exhaustive review on the chemical composition and nutritive value of sorghum and millets are available (Hulse *et al.*, 1980). In many parts of Asia, East Africa and Latin America, nearly two third of the dietary protein requirements come from cereals (Axtell, 1979). The grain protein in a large number of sorghum genotypes varied from 4.4 to 21.1 per cent with a mean value of 11.4 per cent (Hulse *et al.*, 1980). Frey (1977) reported that a negative correlation exists between yield and protein content in several cereals including sorghum. The quality of grain protein depends on the levels of essential amino acids (FAO/WHO, 1973). As in other cereals-lysine is the first limiting amino acid recorded in sorghum (Adrain and Sayerse, 1957). Fractionation studies indicated that the distribution of albumin, globulin and glutelin in sorghum was about 15, 26 and 44 per cent, respectively of the total nitrogen in sorghum grains. In high lysine sorghums, the proportion of prolamine is lower and the albumin-globulin fraction is higher than normal sorghums (Jambhunathan *et al.*, 1975).

The fat content in sorghum ranges from 2.1 to 7.6 per cent, crude fiber from 1.0 to 3.4 per cent and ash from 1.3 to 3.3 per cent. The wide range in minerals and trace element composition indicated that sorghum is a good source of dietary minerals. (Deosthale and Belavady, 1978).

Cereals are the major source of carbohydrates. Cereal grains are composed of upto 85 per cent polysaccharides. In case of sorghum grains, the total soluble sugars range from 0.7 to 4.2 per cent (Subramanian *et al.*, 1980) of which sucrose is the predominant sugar. The flatulence causing oligosaccharides, stachyose and raffinose are also present in varying proportions.

Starch is the major constituent of sorghum accounting for 56 to 75 per cent (Subramanian and Jambunathan, 1982), 62.26 to 73.25 per cent (Suslova and Ishin, 1980; (Subramanian and Jambunathan), 1984)), 60.5 to 64.2 per cent (Torres *et al.*, 1996) of the total dry matter in the grain. Their studies with 100 germplasm accessions indicated that starch content was negatively and significantly correlated with protein content. The physico-chemical characteristics of starch are influenced by the amylose content in sorghum (Miller and Burns, 1970). Waxy sorghums have a low amylose content (Deatherage *et al.*, 1955). In non-waxy sorghum, the amylose content varied from 21.1 to 30.2 per cent while water soluble amylose varied from 3.1 to 12.7 per cent.

2.2 Utilization of Sorghum

The food utilization pattern of sorghum around the world has been recently reviewed (Anon, 1982). In India, it is mainly used in the form of pan-bread (*roti / bhakari*) in addition to a range of traditional products (Salunkhe *et al.*, 1984). Generally, pearly white grains with intermediate corneous endosperm and a thin pericarp without testa produce acceptable

roti, while waxy, floury grains with coloured spotting, brown types and discoloured grains produce unacceptable product (Subramanian and Jambhunathan, 1984). The kharif-grown sorghum crop is often caught in late rains which results into moulding and blackening of grains. Such a produce is unacceptable, fetch negligible market price and results into heavy economical losses to the cultivators and Government agencies. There is a need to find out a suitable solution to this problem either by developing a mould-resistant cultivars, replacing kharif cultivation of sorghum by other suitable crops or process such black sorghum into a value-added products. However, such efforts are quite limited and research in this direction has now started.

2.3 Grain Moulds

The grain moulds appear on the surface of grains soon after the milk-stage and became very severe under high humidity at the grain drying period. Depending upon the types of mould, the colour of seed-coat turns from crimson red to dark black. The moulded grains are lighter in weight and are easily crushed. More (1978) reported that mould occurred on the grains at the physiological maturity stage. In peninsular India, especially in Andhra Pradesh, Karnataka and Maharashtra field mould are very common in kharif crop. The sorghum crop is usually caught in rains of September and October, when grains are being matured. Ear or field moulds are developed under specific condition of high relative humidity (Chavan and Raut, 1987). A large number of fungi consisting of the saprophytic and

parasitic groups are associated with mouldiness. The number and the kind may vary from region to region and also time when they are collected. The most frequently met with are the species of *Fusarium*, *Aspergillus*, *Penicillium*, *Alternaria*, *Colletotrichum*, *Phoma*, *Drechslera* and *Curvularia* (Narayana *et al.*, 1980).

The grain moulds of sorghum have become very serious, particularly so with the increased area under hybrid sorghum in the kharif season. These varieties mature early and get caught in the rainy season. In Maharashtra, it was very seriously affected due to grain moulds.

Bhagwat and Pedgaonkar (1973) reported the incidence of grain moulds on sorghum in Maharashtra State. It was also pointed out that the probability of the severity of the attack due to grain moulds would increase as the area under early maturing varieties during kharif will increase. Williams and Rao (1978) observed that wet conditions make the developing sorghum grains prone to attack by species of *Fusarium* and *Curvularia* besides many saprophytes. Since glume can be the possible source of infection of seed, fungi colonising on glumes and seeds at various stages of grain development need more attention.

2.4 Effect of Moulding and Discolouration on Grain Quality

2.4.1 Effect on colour and appearance

The sorghum grains of different genetic origin are known to possess pearly white, chalky, yellowish, red or light to dark-brown to black colours

(Hulse *et al.*, 1980). The cultivars commonly grown in India or Maharashtra exhibit usually pearly white colour. The consumers have been adapted to such grains that produce a white, soft and sweet *roti*.

When the crop is caught in rains, particularly in kharif season, the pigments of glume and testa, if present, migrate and spread all over the grain surface and impart red to brown colour. Such discolouration is limited only to surface layers and can be eliminated by pearling. However, when the grains are infested by field moulds, a very complex discolouration occurs which leads to blackening of grains.

The cells of the developing kernel produce compounds (pigments) in response to fungal colonization. The pigments may be found in several areas of the kernel. Similarly, *Alternaria* and *Phoma* spp often colonize the pericarp and sporulate on the kernel surface leading to an unacceptable appearance of the grain (Castor and Frederiksen, 1981).

2.4.2 Effect on grain weight

Several investigators have reported thousand grain weight for a large number of sorghum cultivars (Karim and Rooney, 1972; Sullins and Rooney, 1974). The variations in grain weight from 19.0 to 28.5 per 1000 grain weight have been reported (Karim and Rooney, 1972; Ejeta, 1981).

A mould infestation has been reported to decrease the grain weight by 50-70 per cent (Anon., 1978). Sullins and Rooney (1974) had observed 20

per cent loss in grain weight due to moulding. A marked loss in grain weight due to moulding and blackening of sorghum has been observed by Mukuru *et al.*, (1981). Deshmukh (1999) observed a loss of 9.3 to 14.1 per cent in 1000 grain weight due to mould infestation and blackening.

2.4.3 Effects on grain hardness

The grain hardness in sorghum as related to their milling characteristics have been studied (Reichert *et al.*, 1981; Maxon *et al.*, 1972; Chavan and Nagarkar, 1988). Grain moulding and grain weathering fungi can completely degrade the germ and endosperm of sorghum kernels. In these cases, the kernels are extremely light, soft and covered by mould. Nutrients may be available in grains of this kind, but the grains may be unacceptable as a human food and barely acceptable as an animal feed (Castor and Frederiksen, 1981). A decrease in grain hardness by 28.8 to 55.6 per cent due to moulding and blackening has been reported (Deshmukh, 1999).

2.4.4 Effects on nutritional quality

The occurrence of fungal infestation either increase (0.09 - 0.46%) or decrease (0.12 - 26.37%) the grain protein content. The infection by certain species such as *Curvularia lunata*, *C. pallescens*, *Drechslera specifera*, *D. rostrata*, *D. halodes* var. *tritici* increase the grain protein content while by others such as *Fusarium moniliforme*, *F. semitectum*, *Aspergillus niger*, *A. Flavous*, *Cephalosporium acremonium*, *Rhizoctonia species*,

Alternaria tenuis and others resulted in a significant protein reduction (Ingle, 1976). This can be attributed to the special fungal effect rather than the disease effect. Besides, the type of cultivar is important. Some fungi infect the protein rich germ while the few others feed only on pericarp.

Consequent upon mould development, deterioration in nutritive status was expected to be reduced as the developing moulds on the grain derive their energy from carbohydrate and utilize the protein in the grain for synthesis of fungal proteins (Wall and Blessin, 1970). The reduction in protein content in the range of 0.72 to 36.03 per cent in various mouldy grains has been observed (More, 1978). Deshmukh (1999) observed that the moulding and blackening of the grains was found to lower the protein content by 3.0 to 33.3 per cent among the different cultivars studied. The crude fat has been reported to be reduced by 8.95 per cent by *Fusarium semitectum*, followed by *F. monilliforme* (8.44%), *C. lunata* (7.62%), *C. intermedia* (7.41%), *C. pallescens* (6.64%), *Drechslera rostrata* (6.39%), *D. halodes* var. *tritici* (5.88%), *Aspergillus niger* (5.37%), *Phoma insidiosa* (4.85%), *Colletotrichum graminicolum* (4.34%) and *Acremonium strictum* (3.83%) (Ingle, 1976).

The reduction in total sugar content of sorghum grains ranged from 26.14 to 31.37 per cent upon fungal infestation (Ingle, 1976). The total sugar content was reduced by 12.2 per cent. The ash content was reduced by 6.35 to 10.40 per cent due to infection by different fungi. The crude fibre content

showed an increase due to the infection of different fungi. The increase in crude fibre ranged from 3.86 to 15.02 per cent (Ingle, 1976) or 1.5-29.0 per cent (Deshmukh, 1999). An increase in crude fibre is quite obvious as moulds feed on other digestible nutrients and lower their levels in the grains.

2.4.5 Effects on polyphenols

The chemical nature, content and the nutritional significance of polyphenols in grain sorghum have been recently reviewed (Salunkhe *et al.*, 1989), Hahn *et al.*, (1984) and Hahn and Rooney (1985) have recently described the phenols and tannins in different genotypes of sorghum. The phenolic acids are concentrated in the outer layer of kernel. Although phenolic acids do not interfere with the nutritional quality of sorghum, they may form undesirable colour under some food processing conditions.

Variations in the phenolics content of grain sorghum from 3.6 to 10.2 (Harris and Burns, 1970), 4.8 to 8.2 (Harris *et al.*, 1970). Deshmukh (1999) indicated an increase in the content of total phenolics by about 20.64 per cent and 2.69 to 6.88 per cent (Jambhunathan and Mertz, 1973) have been reported for high tannin cultivars. Mc Millan *et al.* (1972) observed a range of tannins from 0.12 to 0.8 per cent in broad spectrum of available sorghum. The other reports on tannin content of sorghum include 0.27 to 0.93 per cent (Maxon *et al.*, 1972), 0.15 to 0.77 per cent (Nelson and Cummings, 1975), 0.13 to 7.22 per cent (Burns, 1971), Radhakrishnan and Sivprasad (1980) reported 0.12 to 2 per cent tannin in some sorghums in

India. However, only 5 per cent of the samples had more than 0.4 per cent tannin. The tannin content of the cultivars commonly grown and consumed in India ranged from 0.43 to 0.64 per cent (Chavan *et al.*, 1979).

The pigments seen at the surface of the discoloured sorghum grain of kharif season may be due to spreading of polyphenols to the pericarp layer and/or due to pigments secreted by the infecting moulds or both. Information on the chemical nature of pigments secreted by the moulds and their interaction with pericarp components is lacking. When the crop is caught in rains, the phenolics in the testa and the glume spread over the surface of grains imparting a red to brown colour. This incidence is often also seen in rabi grown crop. This type of discolouration is dark brown to complete black. Such grains are light, soft and unsuitable for consumption. Further investigations are needed on the nature of pigments secreted by grain moulds.

2.4.6 Mycotoxins in discoloured grains

The occurrence of mycotoxins in food grains, particularly oilseeds, their meals and products is often a very serious problem in their utilization. The mycotoxins may be present in cereals, particularly when these are infested heavily in the field or during storage (Salunkhe *et al.*, 1984). The presence of aflatoxins in stored sorghum grains have been reported (Shotwell *et al.*, 1969, Martin; 1974).

The mould infestation of grains may produce mycotoxins in sorghum. *Fusarium monilliforme* and *Fusarium semitectum* are known to produce zeralenone and T-2 mycotoxins while *Aspergillus* spp produce various aflatoxins in a variety of grains. There are a few reports indicating that these mycotoxins may occur naturally in sorghum grain (Rukmini and Bhat, 1978; Schroeder and Hein, 1975; Stipanovic and Schroeder, 1975). The effects of these mycotoxins on animals is well documented. Many of the storage fungi produce mycotoxins that may be toxic or debilitating to livestock as well as to man (Christensen and Kaufman, 1974). Sorghum grain may have these mycotoxins present in it (Alpert *et al.*, 1971; Shotwell *et al.*, 1969). High concentrations of aflatoxin in grain can be deadly. Grain quality is reduced to such an extent that it cannot be safely used as food or as an animal feed (Castor and Frederiksen, 1981). The grain lots of moulded and blackened sorghum grains have been reported to contain as high as 100 µg/kg of aflatoxins B1 (Chavan *et al.*, 2001).

Unseasonal rains beginning in 1995 damaged the maize and sorghum crops harvested in a few villages of the Deccan Plateau in India. Human consumption of those grains resulted in a foodborne disease outbreak characterised by abdominal pain and diarrhoea. A rapid epidemiological survey was conducted in the affected villages and detailed house to house survey in selected villages. It was found that people in out of 50 villages surveyed were affected and disease was self-limiting. Diarrhoea was reproduced in dayold cockrels fed contaminated grains from affected

households. The grains had *Fusarium* sp. as the dominant mycoflora and contained fumonisin B1 in the range of 0.14-7.8 mg/kg respectively. The higher water activity in the grains left in the field following harvest led to the production of high levels of Fumonisin B1 and consumption of such grains by humans resulted in the disease (Bhat,1997).

2.5 Processing of Moulded and Blackened Sorghum

The sorghum being a coarse grain is often subjected to pearling to lower its bran content and make the product more palatable. The pearling of grains by certain degree is often performed where the bran is only partially or incompletely removed. A similar treatment can also be attempted to moulded and blackened grains since the discolouration is mainly limited to surface layers. Of course, under such situation, near complete removal of bran is necessary to obtain a white dehulled product.

2.5.1 Chemical dehulling

Several investigators have used an approach of chemical treatment of grains, particularly discoloured lots to remove the bran (Blessin *et al.*, 1971; Barta *et al.*, 1966; Chavan *et al.*, 1979). The grains are treated with dilute to strong alkali either at ambient or elevated temperature followed by washing under water and neutralization of residual alkali.

Darade (1997) have standardised a simple process for alkali dehulling of discoloured sorghum. It includes soaking of grains in 4 per cent sodium

hydroxide at 100°C for 5 min, followed by washing under water and neutralization of residual alkali. The process yields over 85 per cent white grains without any breakage. However, it requires a large amount of water, wetting grains which results in loss of sugars and texture of the product. The cost of such treatment was also found to be quite high.

2.5.2 Physical pearling

Reports on polishing sorghum in a rice dehuller are available (Wall, 1967; Raghavendra Rao and Desikachar, 1964; Kapasi Kakama, 1977). Viraktamath *et al.* (1971) used a rice cone polisher and collected polished grains, grits, bran and aspirated husk or glumes as separates. Pearling caused 6.4 to 27.1 per cent brokens and a significant losses in crude proteins, ether extract, fibre, minerals and vitamins (Chandrashekhar and Desikachar, 1984; Varadharajan *et al.*, 1989). Pearling of normal white or coloured sorghum has also been reported from other part of the world (Hahn, 1969; Deobald *et al.*, 1965; Wall, 1967; Kapasi-Kakama, 1977; Shepherd *et al.*, 1970-71; Reichert and Yongs, 1976; Demen *et al.*, 1973; Chibber *et al.*, 1978).

Apart from the polisher used, the breakage during pearling depends upon the variety of sorghum. The hard varieties give less breakage and more whole grain yields than the soft varieties (Reichert *et al.*, 1981). It was observed that the nutritional value was adversely affected by over polishings. However, at a stage of 10-12 per cent degree of polish, maximum grain

recovery with minimum loss of nutritive value can be secured (Raghavendra Rao and Desikachar, 1964). Hahn (1969) showed compositional changes during the pearling of grain sorghum. He observed that the per cent protein, fat, fibre, and ash was reduced after the pearling. The reduction in protein content indicated higher protein content in the outer layers of the grain sorghum (Normand *et al.*, 1965; Hahn, 1969). It is also possible to produce flours with 8 per cent protein or higher after successive removal of layers from sorghum grains (Deobald *et al.*, 1965). The blackened sorghum can be processed into dehulled white grains by pearling. The mould affected black grains may suffer greater breaking losses as these grains are relatively more soft and near complete pearling is essential.

A physical pearling treatment of black sorghum has been recently standardized in this laboratory (Chavan *et al.*, 2001). It involves separation of highly infested grains either by gravity separator or by suspending a black grain lot in 40 per cent NaCl solution and removal of infested light weight floating grains. The sinkers are dried and subjected to physical pearling for 5 min on rice polisher to obtain a clear white produce with about 70-80 per cent clear white grains. The pearling treatment removed completely the surface discolouration and aflatoxins while lowering the contents of fibre by 15.6 per cent and polyphenols by 20 per cent, crude fat and by 24.1 per cent. The pearled product exhibited an excellent *roti* quality but exhibited a shelf life of about 30 days at ambient conditions. The technology is simple and adaptable at domestic level.

Besides, both black and pearled sorghum exhibited a limited storage life. Hence, it may be more convenient to process it either into a cattle feed or into a value-added products such as starch. A processing unit of starch making can be operated with both normal or black-sorghum. This can also solve the issue of a shorter storage life of blackened sorghum. The different alternatives planned for value addition of blackened sorghum under NATP project include preparation of starch and starch based byproducts viz., high quality potable alcohol and utilization as adjunct in breweries (Anon. 2000).

2.6 Sorghum Starch : Extraction and Properties

Wet milling is better method for the extraction of sorghum starch. The potential of grain sorghum for starch production is similar to that of corn since the sorghum wet milling process is almost identical to the one used for corn starch manufacture (Watson, 1967; Subramanian *et al.*, 1994). Industrial wet-milling of sorghum for starch production was developed in the U.S.A. during World War II as an alternative to production of maize starch, (Watson, 1970). In 1948, Corn Products Co. built a modern sorghum wet-milling plant in Corpus Christi, Texas, which ran until the 1970s on an enlarged capacity as the only one in the U.S.A. (Munck, 1995). During those years, valuable information was collected about the process (Watson, 1967; Watson, 1970; Zipf, 1950; Freeman and Watson, 1969). However, little is yet known about the relation of sorghum grain quality factors to end use value (Cagampang, and Kirleis, 1984).

Watson and Hirata (1954) reported a method for determining milliability of steeped grain by visually estimating the amount of starch released when thinly sectioned slices of steeped maize or sorghum kernels were brushed. No correlation was found between the visual milliability score and the yield of starch from wet-milling. Freeman and Watson (1969) reported that gluten-starch separation in graduated cylinders was indicative of milliability. Sorghum of high milliability yielded sharp delineations between the milky white starch layer at the bottom of the cylinder and the less dense, but more pigmented, gluten layer above it (Freeman and Watson, 1969). Watson (1967) reported that best wet-milling results were obtained with plump kernels of high test weight and minimum damage from mould and insects.

Grain sorghum and corn starches, both waxy and normal, are interchangeable, although corn starch granules are bigger in size (Watson, 1970). Wet milling of large tonnages of corn is practiced around the world, yet no wet milling of grain-sorghum is carried. Grain sorghum is a less consistent commodity than corn in case of size, color and defects. The recovery of starch is lost in its germ and fibre fractions because some starch occurs in pericarp and because some of its peripheral cells are not washed during grinding of the steeped grains (Rooney 1973, Watson, 1984). Furthermore, sorghum starch is associated with more thick and cross linked proteins matrix than corn starch (Hamaker *et al.*, 1992). The sorghum starch usually is less bright than corn starch and may be strained with

pericarp or glume pigments in the field or during processing (Watson, 1970; Munck, 1995).

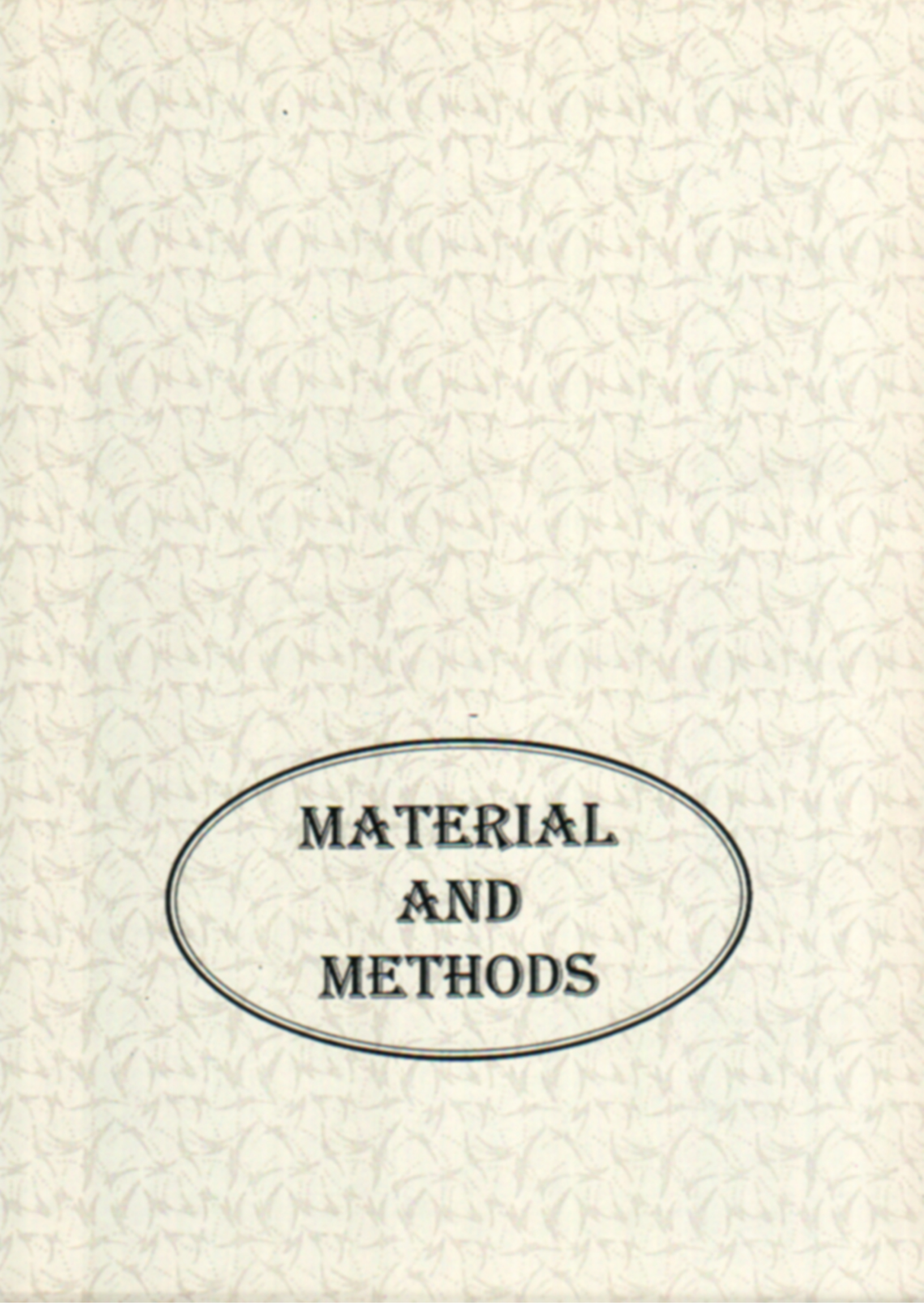
In order to release and recover maximum starch from the protein matrix, relatively strong acidic conditions need to be applied during steeping step for sorghum. This may degrade a significant amount of starch which ultimately lowers the yield. The sulfuric acid is known to hydrolyze starch at higher concentration. Further, sorghum grains often contain yellow, brown, red or black pigments in their pericarp which often contaminate the extracted starch and make it less bright in appearance (Munck, 1995; Yang and Seib, 1996). Such pigments and phenolics can however, be effectively removed during steeping and subsequent blending and filtration, if the grains are steeped or blended or washed with a solution of dilute alkali. The use of dilute alkali will also facilitate a near complete solubilization of a protein matrix. This would eliminate proteins more effectively and improve the yield of bright starch. However, this type of approach for starch making has not been attempted by the investigators.

Starch is the major constituent of sorghum accounting for 56-75 per cent of the total dry matter in the grain. Physico-chemical properties of starch are influenced by amylose content in sorghum (Miller and Burns, 1970). Waxy sorghum starch has a low amylose content (Deatherage *et al.*, 1955). In non-waxy sorghum, amylose content varied from 21.1 to 30.2 per cent, while water soluble amylose varied from 3.1 to 12.7 per cent.

The gelatinization temperature of starch is affected by the proportion of amylose to amylopectin in starch (Hoseney *et al.*, 1981). The swelling capacity and the solubility of the starch at different temperatures have also showed a wide variation for the genotypes studied (Subramanian and Jambunathan, 1982). The polyphenol content and grain characteristics influenced its starch properties (Beta, 2001). Peak gelatinization temperature $T(p)$ occurred over a narrow range from 66-69 °C. $T(p)$ was negatively correlated with the floury endosperm portion of the grain.

Sorghum starches had higher peak viscosity in pasting than commercial maize starch. The time taken to reach viscosity from initial viscosity rise was less for sorghum starches than maize starch. However, sorghum starches had a higher rate of shear thinning than maize starch. There was a significant positive correlation between grain polyphenol content and starch. Starch gel hardness was negatively correlated to pasting properties and paste breakdown (Beta, 2001). Specific dietary limitations in the utilization of cooked and baked sorghum products for human are caused by factors such as the high gelatinization temperature of the starch and high viscosity of the cooked products causing acceptability and digestion problems (Axtell *et al.*, 1982).

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**MATERIAL
AND
METHODS**

3. MATERIAL AND METHODS

3.1 Material

3.1.1 Black sorghum grains

The mould infected and blackened sorghum grains to the extent of 80 per cent by number and proportion were procured from the local market in November 2000. The grains were cleaned, dried to about 10 per cent moisture in hot air oven and stored at 4 °C in capped plastic jars until used for experiment.

3.1.2 Chemicals

All the chemicals used in this investigations were of analytical grade (BDH, E. Merck Qualigen etc.)

3.2 Methods

3.2.1 Water Absorption Studies

The initial experiment was conducted to find out the time and temperature of soaking the grains for maximum water absorption. Ten gram of black sorghum grains were soaked in 30 ml of distilled water in duplicate for 24 to 120 hrs at 30 °C, 40 °C, 50 °C and 60 °C in beakers. The soaking water was changed after every 24 hrs. These soaked grains were drained, blotted on a filter paper to remove the surface water and weighed. The gain in weight by the grains was determined and expressed in percentage.

3.2.2 Standardization of Starch Extraction Process

3.2.2.1 Effect of soaking period and temperature on extraction of starch

The black sorghum grains (50 g) were soaked in 150 ml of distilled water for 24 to 168 hrs separately at ambient temperature (27 ± 2 °C) and 60 °C. The soaking water was changed after every 24 hr. The soaked grains were blended with water (1:2 w/v) in a laboratory blender for 2 min. The blending was repeated for two more minutes after a pause of 1 min. The slurry was screened through a double layered musline cloth. The residue on the musline cloth was washed with additional water to solubilize and filterate out the remaining starch. The filterate was centrifuged at 8000 x g for 30 min. The supernatants were discarded and the residue was suspended in 0.2 per cent sodium hydroxide to solubilize the proteins. The contents were allowed to stand for 3-4 hrs to settle the starch. The yellowish upper sodium hydroxide layer was decanted and the settled starch was resuspended in sodium hydroxide until the sodium hydroxide layer became colourless. The settled starch was then washed with water and the contents were screened through the 0.045 mm sieve. The filterate was centrifuged and the starch obtained was recorded as residue in centrifuge tubes and dried at ambient temperature.

3.2.2.2 Effect of soaking medium on extraction of starch

Fifty gram of black sorghum grains were separately soaked in 0.1 per cent sodium hydroxide, sulfuric acid or lactic acid at 60 °C for

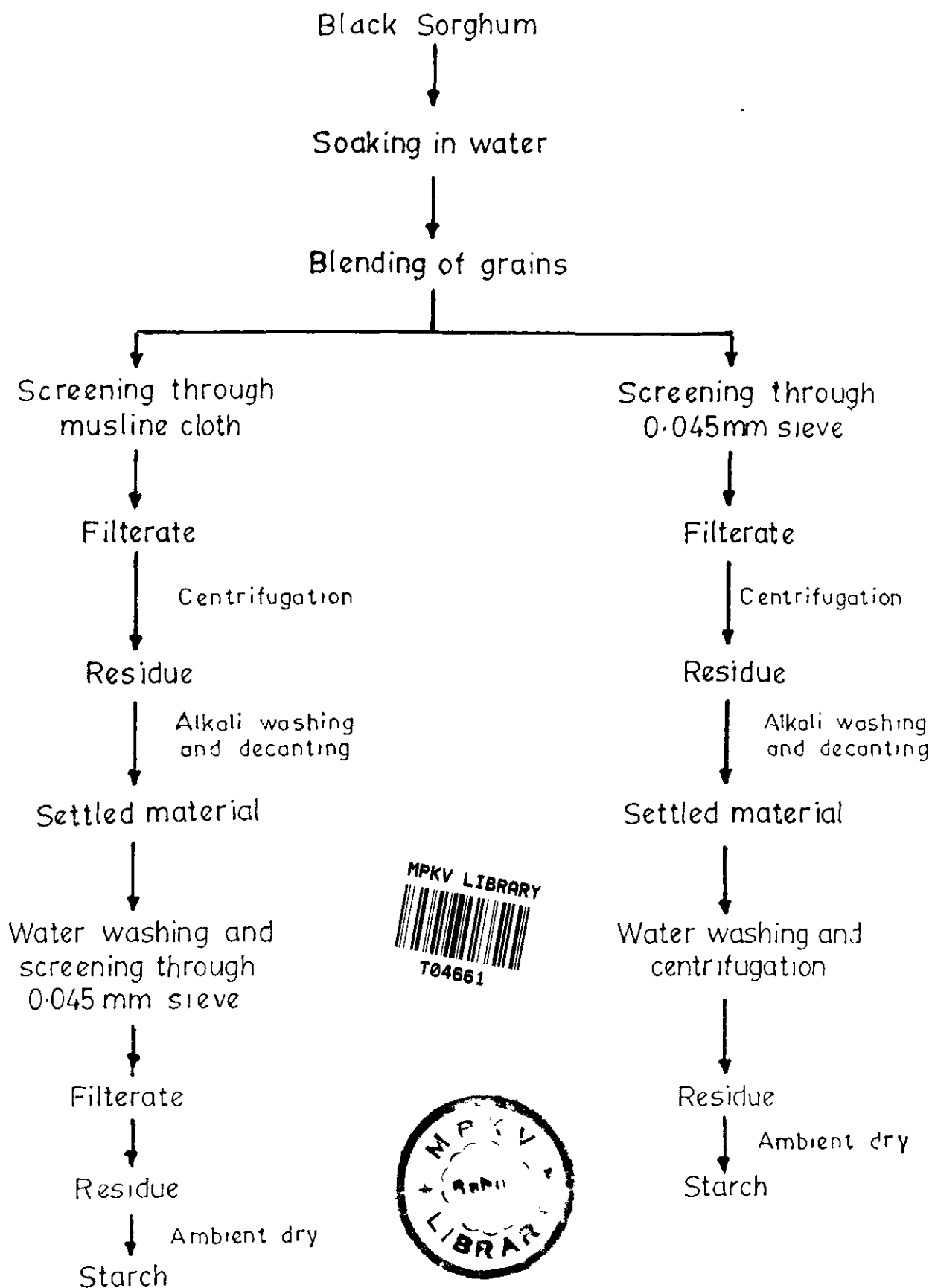
144 hrs. The starch was extracted from the soaked grains and dried as described above 3.2.2.1. The blending of soaked grains was carried out in water while the suspension to solubilize and remove proteins was performed in respective soaking medium.

3.2.2.3 Effect of NaOH concentration on extraction of starch

Fifty gram of grains were soaked separately in 0.1, 0.2 and 0.5 per cent NaOH at 60 °C upto 144 hrs. The starch was extracted and dried as described in section 3.2.2.1. The blending of soaked grains was carried out in water while the suspension treatment was performed with respective soaking medium. Finally, the starch was washed with water, centrifuged and dried at ambient temperature.

3.2.2.4 Effects of screening method on starch extraction

Fifty gram of black sorghum grains were soaked in water (1: 3 w/v) at 60 °C upto 144 hrs in duplicate and blended in water for 2 min. The slurry was screened through either double layered musline cloth or 0.045 mm screen. The filtrate obtained with the use of musline cloth was processed for recovery of starch as described in section 3.2.2.1. The starch residue obtained from the filtrate by direct use of sieve was suspended in 0.2 per cent NaOH to remove the proteins. The NaOH was decanted out and the settled starch was washed with water and centrifuged. The starch recovered was dried at ambient condition. The process details are outlined in Fig.1.



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Fig. 1 Starch extraction processes

In order to obtain a maximum recovery of good quality starch, the soaking medium, blending medium, suspension medium and method of screening the blended slurry are important process components. In order to work out a best combination of soaking, blending and suspension medium, different combinations were tested. Each combination was considered as a separate method. In all six combinations were tried. The details are presented alongwith results in Section 4.7.

3.2.3 Chemical Analysis

3.2.3.1 Moisture, crude protein and total ash

The moisture, crude protein (% N x 6.25) and total ash content in the samples were estimated by the standard procedures of AOAC (1990).

3.2.3.2 Starch

The total starch content in grains and isolated starch was determined by the method of McCready *et al.* (1950).

Reagents :

- 1) Standard glucose** - Hundred milligram of unhydrous glucose was dissolved in 100 ml of distilled water. Then, 10 ml of the stock solution was diluted to 1000 ml and from this 0, 1, 2, 4 10 ml was used for the calibration of standard curve of glucose.

- 2) **Anthrone sulphuric acid solution** - Two gram of anthrone was dissolved in 1 L of ice-cold 95 per cent sulphuric acid.
- 3) **Ethyl alcohol (80%)** - One litre of 80 per cent ethanol was prepared by diluting 840 ml of 95 per cent ethyl alcohol with water.
- 4) **Perchloric acid (52 %)** - It was prepared by diluting 722.2 ml of reagent grade perchloric acid to 1000 ml using distilled water.

Procedure :

The whole grain meal (60 mesh) or isolated starch product (0.2g) was taken into a 50 ml centrifuge tube. To this, a few drops of 80 per cent ethanol were added to wet the sample. Then, 25 ml of hot 80 per cent ethanol was added, stirred thoroughly and after 10 min centrifuged at 5000 x g for 10 min. Alcoholic solution was decanted and discarded. Then 30 ml of fresh hot 80 per cent ethanol was added stirred and centrifuged as before. This washing treatment was repeated twice more until a test with anthrone was negative.

To the residue, 5 ml of water was added, cooled in ice-water and while stirring 6.5 ml of diluted perchloric acid (52%) was added. The content was stirred again for 5 min with a glass rod and occasionally thereafter for 15 min keeping the mixture cold. To this 20 ml of water was added and centrifuged at 1000 x g for 10 min. Aqueous starch solution was poured into a 100 ml volumetric flask. Again 5 ml of water was added to the

residue, cooled in ice-water and stirred while adding 6.5 ml of perchloric acid reagent. The content was solubilized as before for 30 min with occasional stirring centrifuged at 10,000 x g for 10min and the supernatant of the tube was transferred into the 100 ml flask containing the first extract. Combined solutions were diluted to 100 ml and filtered.

Colour development

Five millilitre of the filtered starch solution was pipetted out into a 25 x 250 mm borosilicate glass tubes, ice-cooled and 10 ml of fresh anthrone reagent was added. The contents in each tube was mixed thoroughly and all the tubes were then heated together for 7.5 min at 100 °C. The tubes were cooled rapidly to 25 °C in a waterbath and colour intensity was recorded at 630 nm. Standard curve was then prepared by using 0 to 100 µg of glucose containing the same amount of perchloric acid as that in the starch aliquots and this calibration curve was used to obtain the yield of glucose from starch. Glucose concentration found was then multiplied by 0.90 to obtain the value of starch in the sample.

3.2.3.3 Total sugars

Defatted sample was finely ground in pestle and mortar and 0.5 g was extracted with 80 per cent boiling ethanol. The supernatant obtained after centrifugation at 5000 x g for 15 min was collected and extraction was repeated twice. The combined ethanolic extract was then evaporated to

about 8 to 10 ml and diluted to 25 ml with distilled water. This extract was used for the determination of sugars.

To 5 ml ethanolic extract, 15 ml diluted hydrochloric acid (1:1) was added and kept for 24 hrs for inversion. It was neutralized with 5 N sodium hydroxide. The extract was cleaned with saturated lead acetate and delead with sodium phosphate. The hydrolysed sugars were estimated as reducing sugars (Nelson, 1944).

Reagents

Reagent A

Twenty five gram of sodium carbonate (anhydrous), 25 g sodium potassium tartarate, 20 g sodium bicarbonate and 200 g sodium sulphate (anhydrous) were dissolved in about 800 ml distilled water and volume made to 1000 ml.

Reagent B

Fifteen gram of copper sulphate was dissolved in 100 ml distilled water and 1 to 2 drops of conc. Sulphuric acid were added.

Copper reagent

It was prepared by mixing 25 parts of reagent A and 1 part of reagent B and was prepared fresh every week.

Arsenomolybdate reagent

Twenty five gram of ammonium molybdate was dissolved in 450 ml of distilled water. To this, 2 ml of conc. H_2SO_4 was added. Three gram of sodium arsenate was dissolved separately in 25 ml of distilled water and added to above solution. The contents were mixed and incubated at $37^{\circ}C$ for 48 h.

Glucose stock solution

Glucose solution was prepared by dissolving 100 mg of D-Glucose in 100 ml of distilled water. Ten ml of stock solution was diluted to 100 ml with distilled water. This solution contained 100 μg glucose per ml.

Colour development

To 1 ml diluted extract 1 ml of alkaline copper tartarate reagent was added. The contents were mixed and heated for 10 min in a boiling water bath. The tubes were then cooled, 1 ml of arsenomolybdic acid reagent was added and mixed well. This mixture was diluted to 8 ml with distilled water and mixed on vortex mixer. The absorbance was read at 520 nM on spectronic-20.

Preparation of standard curve

Different concentrations of glucose viz., 0, 10, 20, 40, 60, 80 and 100 μg were taken in a series of test tubes and volume made to 1 ml with distilled water. The rest of the procedure of colour development was the

same as described as above. A standard curve was plotted as concentration of glucose against the absorbance and used to calculate total sugar in the extract.

3.2.4 Functional properties of Starch

The oil and water absorption capacities of the starch obtained were estimated by the procedure of Beuchat (1977).

3.2.4.1 Oil absorption capacity

The empty dry centrifuge tubes (50 ml) were weighed accurately. One g starch was added in triplicate and then reweighed accurately. To each tube, exactly 10 ml of edible oil was added, followed by stirring and keeping the tubes, standing for about 1 hr. Each tube was then centrifuged at 5000 x g for 10 min. The supernatant was drained off from each tube and tubes were weighed exactly, alongwith the residue. The fat absorption capacity of starch granules was calculated as follows -

$$\text{Oil absorption capacity of starch/g} = \frac{\text{Wt. of tube + Oil} - \text{Wt. of tube and untreated}}{\text{Wt. of sample (g)}} \frac{\text{treated residue (g)}}{\text{sample (g)}}$$

3.2.4.2 Water absorption capacity

For determining the water absorption capacity the same procedure as above (oil absorption capacity) was used. The only difference was that instead of edible oil water was added to starch.

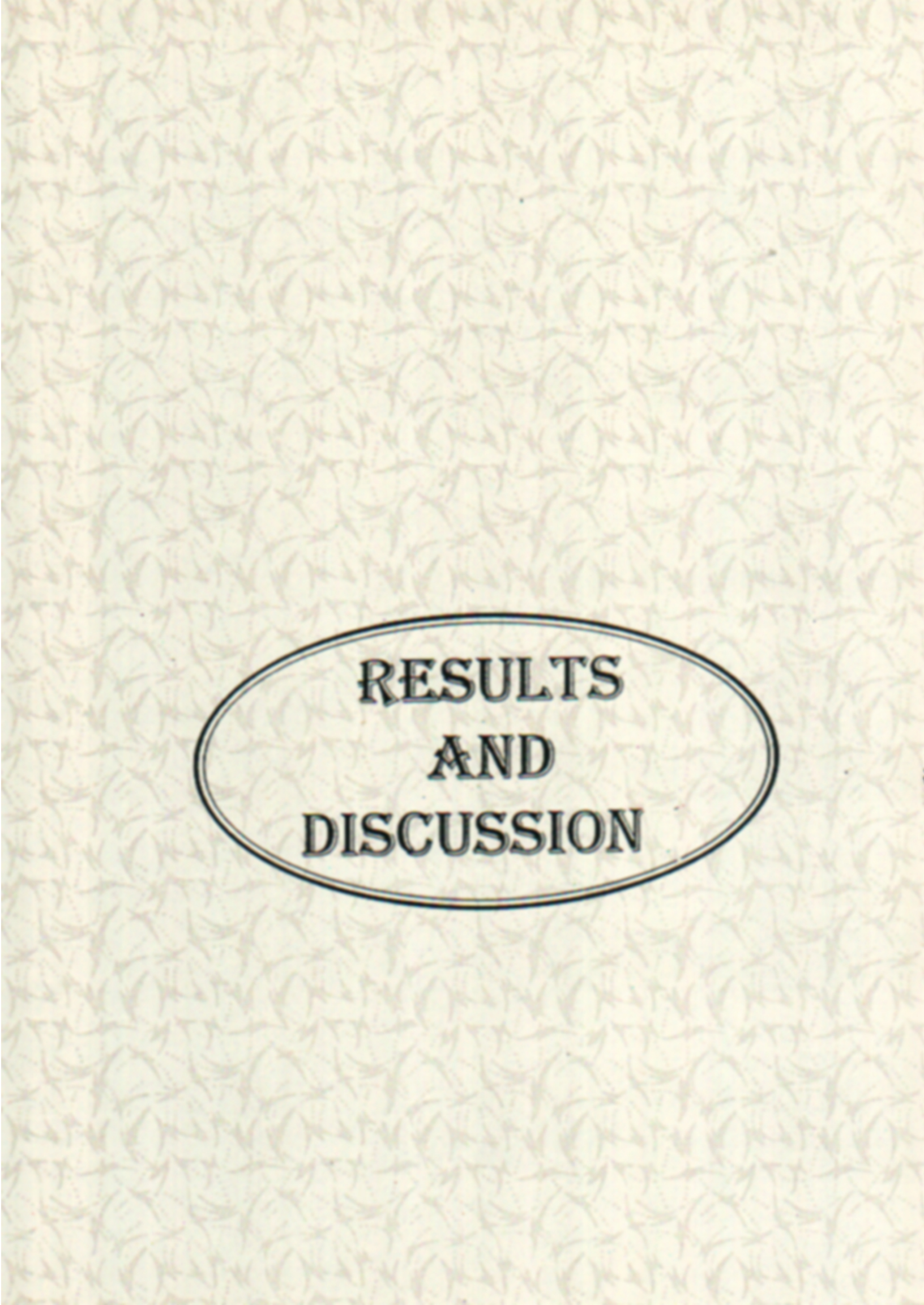
3.2.4.3 Gelatinization temperature

The gelatinization temperature (GT) was recorded as temperature in selcius at which the starch granules are completely converted into a uniform gel when heated in excess quantity of water. One gram starch sample was weighed accurately in triplicate and transferred to 20 ml screw capped tubes. Ten ml water was added to each sample tube. The samples were slowly heated in water bath, until they formed uniform gels. At complete gel formation, the temperature was recorded as gelatinization temperature.

3.2.5 Statistical analysis

The data obtained were analysed for standard error by the procedure described (Panse and Sukhatme, 1957).

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**RESULTS
AND
DISCUSSION**

4. RESULTS AND DISCUSSION

Attempts have been made to standardize the alkali extraction process for making starch from black sorghum. Various combinations of soaking, blending and suspension medium were tested to obtain a bright coloured starch with maximum recovery and purity. The product isolated was analysed for chemical composition and certain physical properties. The results obtained are briefly presented and discussed in this section.

4.1 Water Uptake by Grains

The grains of black sorghum were soaked for 24 to 120 hrs at 30 to 60 °C and the per cent weight increase in the grains were recorded (Table 1). The moisture uptake by the grain was found to increase with increase in both soaking period and soaking temperature. At 30 °C, the grain weight was found to increase by 38.7 per cent after 24 hrs soaking. A continued soaking of grains upto 120 hrs, further increased the grain weight by uptake upto 44.2 per cent. A similar trend was also observed for soaking of grains of 40, 50 or 60 °C. The soaking temperature exhibited a marked influence on the water uptake by the grains. A maximum weight gain of 44.1 per cent after 96 hrs at 30 °C was found to increase to 46.8 per cent when the soaking was carried out at 60 °C for the same period. These results indicated that the soaking of grains for 96 hrs at 60 °C is necessary and optimum for maximum swelling of the grain.

The cereal grains are required to be steeped or soaked in water to facilitate water imbibition by the grains. The imbibed water is taken up by

Table 1. Water uptake by black sorghum grains at different soaking times and temperatures

Soaking Period (hrs)	Weight gain of grains after soaking at °C			
	30	40	50	60
24	38.7 ± 1.2	40.1 ± 1.06	42.6 ± 0.75	43.0 ± 0.65
48	41.1 ± 0.9	43.5 ± 0.93	54.2 ± 0.80	46.2 ± 0.72
72	43.6 ± 1.6	45.2 ± 1.02	45.6 ± 0.77	46.0 ± 0.74
96	44.1 ± 1.8	46.0 ± 0.90	46.5 ± 0.85	46.8 ± 0.75
120	44.2 ± 1.6	46.9 ± 0.98	47.0 ± 0.80	47.0 ± 0.60

the starch and proteins in the grains. This causes the swelling of starch granules and protein bodies. The imbibed water also makes the grain soft and milliable during subsequent blending. A fully swollen grains are easy to disintegrate completely without a marked breakage of starch granules in the blending medium. Hence, a maximum swelling of grains during steeping is useful for maximum starch recovery.

Yang and Seib (1995) soaked the grains for 10 hrs at 58 °C and found a maximum water-uptake of about 35.5 per cent after 5 hrs of soaking for normal white sorghum. Buffo *et al.* (1998), however, found 48 hrs soaking at 51 °C was necessary to obtain a maximum starch recovery for white sorghum. These reports indicated the necessity of soaking the grains before disintegration. Our results indicated that for black sorghum soaking at 60 °C for 96 hrs is optimum for maximum swelling of the grains.

4.2 Effects of Time and Temperature of Soaking on Starch Extraction

The grains were soaked for 24 to 168 hrs at ambient (27 ± 2 °C) and 60 °C before blending and subsequent starch extraction. The data obtained on yield of starch are presented in Table 2. The starch yield was found to increase from 17.9 per cent for 24 hrs soaking to 32.4 per cent for 168 hr soaking of grains at 27 °C. Soaking of grains at higher temperature (60 °C) increased the starch recovery from 18.4 to (24 hrs soaking) 33.8 per cent (144 hrs soaking). These results indicated that a maximum starch recovery

Table 2. Effect of period and temperature of soaking of grains on the extraction and recovery of starch from black grains

Sr. No.	Soaking Period (hrs)	Starch recovery (%) at	
		$27 \pm 2^{\circ}\text{C}$	60°C
1.	24	17.9 ± 0.50	18.4 ± 0.90
2.	48	20.2 ± 1.30	20.8 ± 1.10
3.	72	23.7 ± 0.80	24.7 ± 0.80
4.	96	27.2 ± 1.60	27.6 ± 1.20
5.	120	28.5 ± 0.80	32.1 ± 1.60
6.	144	32.0 ± 0.95	33.8 ± 0.85
7.	168	32.4 ± 0.80	34.0 ± 0.66

can be obtained by soaking the grains at 60 °C for 144 hr. However, the starch recovery even with this treatment was only 33.8 per cent, which is quite low. Hence, it is concluded that only water steeping of grain is not efficient treatment for starch extraction from black sorghum.

The starch recovery from the grains is related to steeping period of the grains. Direct blending of unsoaked grains with water could extract only accessible starch from grain-sorghum (Yang and Seib, 1995). The recovery of the starch was only 14 to 18 per cent from dry grains. Soaking of the grains at 58 °C in water containing 0.2 per cent SO₂ for 4 hr produced about 50 per cent starch recovery. Further increase in soaking period upto 10 hr did not increase starch recovery markedly. Decortication of grain sorghum gave a somewhat reduced recovery of starch. These results indicated that a short steeping of grains with SO₂ is not efficient in releasing the starch from protein matrix.

The use of sorghum for making starch is discouraged because of the incomplete recovery of starch and its off-colour (Freeman and Watson, 1969, ; Watson, 1984) and high wax content in grain sorghum (0.3 % dry basis) (Watson and Hirata, 1954; Miller and Burns, 1970). Purification of starch from grain-sorghum is very difficult than purification of starch from corn. The grain sorghum contains 65 per cent horny or corneous endosperm. Starch granules in the horny endosperm are small, and the protein matrix is thick and heavily cross-linked (Watson and Hirata, 1954).

Softening the thick protein matrix requires the increased level of sulphuric acid during steeping which may cause starch breakdown during blending. Small starch granules sediment more slowly than large ones, so they are relatively difficult to recover. The grain sorghum kernels contain a layer of small densely proteinaceous endosperm cells (peripheral endosperm) that lie just under the aleuron layer. During wet processing these cells tend to be intact when released and many are small enough to pass through the screens used to remove fibrous particles from the starch milk (Watson and Hirata, 1954). These high protein cells contain starch. About 3-4 per cent starch in grain sorghum is located in the middle layer (mesocarp) of the pericarp. The starch in the mesocarp layer tend to remain with the bran on the screen (Watson, 1984). Hahn *et al.* (1969) therefore, suggested a low cost process to isolate only readily accessible starch from grain sorghum. Yang and Seib (1995) reported an abbreviated wet-milling process to isolate readily accessible starch from grain sorghum. The process does not produce waste water, however, recovery of starch was only 14 to 18 per cent and rest of the dry matter was proposed to be used as animal feed.

Keeping in view, the problems encountered in efficient extraction of starch from grain-sorghum by using water with a reasonably lower levels of SO₂, or by using abbreviated wet-milling process (low recovery), there is a need to develop alternative soaking medium for starch extraction from grain sorghum. It is, therefore, thought to employ other acids and dilute alkali at different stages of starch extraction (soaking, blending, suspension).

The use of alkaline solution in starch extraction particularly from black sorghum will be more efficient to solubilize the protein matrix and fungal pigments to avoid the starch degradation during blending and to obtain a maximum recovery of bright starch.

4.3. Effect of Soaking Medium on Extraction of Starch

The starch was extracted from black sorghum with four different soaking media viz. water, dilute NaOH, dilute H₂SO₄ or dilute lactic acid (Table 3). Among the different soaking media employed, the maximum recovery of starch was obtained with 0.1 per cent NaOH (52%) followed by lactic acid (34.4%) or water (33.8%) while the lowest yield of starch was obtained with H₂SO₄ (23.4 %) as soaking medium. These results indicated that dilute alkali is more efficient in solubilizing and eliminating the protein matrix from starch as compared to the acidic conditions.

The physical appearance of starch obtained from four different soaking and washing media is shown in Fig.2. It is seen that the starch obtained by using dilute alkali as soaking and washing media is more bright in appearance as compared to other samples. These results indicated that dilute alkali is a superior soaking and washing medium, than the acids to obtain higher recovery of starch with bright appearance.

Fig. 2. The physical appearance of starch obtained from four different soaking and residue washing media -

1. NaOH (0.1%)
2. H₂SO₄ (0.1%)
3. Lactic acid (0.1%)
4. Water

Table 3. Effect of soaking medium on extraction and recovery of starch from black sorghum

Sr. No.	Grain soaking medium (%)	Extracted starch (%)
1.	NaOH, 0.1	52.00 ± 2.4
2.	H ₂ SO ₄ , 0.1	23.38 ± 1.8
3.	Lactic acid, 0.1	34.4 ± 1.9
4.	Water	33.8 ± 2.1

The grains were soaked in respective soaking medium at 60 °C for 144 hr, blended in water, slurry is screened through muslin cloth and the filtrate residue washed with the same medium followed by water, centrifuged and dried as starch.

4.4 Effect of NaOH Concentration as Soaking Medium on Extraction of Starch

Three levels of NaOH concentrations (0.1, 0.2, 0.5%) were tested as soaking media to extract the starch from black sorghum (Table 4). Among the various concentrations used a maximum starch extraction was observed at 0.5 per cent level (54.8%) followed by at 0.2 per cent NaOH (54.4%). The differences in the yield of starch between 0.2 and 0.5 per cent NaOH were negligible. Hence, it is concluded that 0.2 per cent NaOH is optimum concentration of soaking media to obtain maximum recovery of starch.

The appearance of starch isolated with these three levels of dil.alkali is shown in Fig.3. The starch obtained by using either 0.1 or 0.2 per cent NaOH as soaking and washing media exhibited a bright appearance while that produced with 0.5 per cent NaOH showed a dull creamy look. These results further supported the conclusion that 0.2 per cent NaOH concentration is optimum for extraction of starch from black sorghum.

4.5 Effect of Screening Method on Extraction of Starch

The grains of black sorghum were soaked in water and blended in water. The slurry was screened either through muslin cloth or through 0.045 mm sieve to remove the non-starchy insoluble material. The filtrate was allowed to stand, decanted and the residue washed with 0.2 per cent NaOH to remove the proteins. The starch recovery was 33.8 per cent when musline cloth was used to screen the slurry while it was 59.3 per cent when

Fig. 3. The physical appearance of starch obtained by using three levels of dilute alkali -

1. 0.1 per cent
2. 0.2 per cent
3. 0.5 per cent

Table 4. Effect of increasing concentration of alkali as soaking medium on starch extraction and recovery

Sr. No.	NaOH concentration (%)	Starch recovery (%)
1.	0.1	52.0 ± 2.8
2.	0.2	54.4 ± 2.6
3.	0.5	54.8 ± 2.8

The grains were separately soaked in dilute NaOH of respective concentration for 144 hr at 60°C, blended in water and the slurry is screened through muslin cloth. The filtrate residue washed with respective medium followed by water, centrifuged and dried as starch.

0.045 mm sieve was used to filter the slurry (Table 5). It is thus clear that the direct use of 0.045 mm sieve is more effective in obtaining higher recovery of starch than the use of musline cloth. These results indicated that a significant amount of starch is held up in the overs in the musline cloth. This may be due to incomplete or inefficient washing of the slurry residue with the musline cloth. It was experienced that a significant material remained as overs in case of musline cloth while a markedly less residue was left when the sieve was used to filter the starch slurry. The increase in the starch recovery by using sieve (0.045 mm) can be attributed to a complete filtration of starch granules during screening of the slurry.

4.6 Effect of Combination of Soaking, Blending and Washing Media on Starch Extraction

The water and 0.2 per cent NaOH were used in different combinations as soaking, blending or residue washing medium. The starch slurry was filtered through 0.045 mm sieve. The data on the starch recovery are presented in Table 6. A maximum recovery of starch (62.2%) was obtained when dilute alkali was used as both soaking and residue washing medium. When the dilute alkali was used as both soaking and blending media, the recovery of starch was found to be slightly reduced (61.8 %). The lowest recovery was observed when water was used as both soaking and blending media (59.3%). These results indicate that a maximum starch recovery from black sorghum can be obtained by soaking the grains directly in 0.2 per cent NaOH solution followed by blending the grains in water and washing the

Table 5. Effect of screening method on and recovery of starch

Sr. No.	Screening method	Starch recovery (%)
1.	Musline cloth	33.8 ± 1.6
2.	Sieve (0.045 mm)	59.3 ± 2.7

The grains were soaked in water and blended in water. The slurry of one lot was screened through musline cloth while that of second lot through 0.045 mm sieve. Rest of the procedure is as outlined in Fig.1.

Table 6. Effect of combinations of soaking, blending and washing media on extraction and recovery of starch

Sr. No.	Soaking medium	Blending medium	Residual washing	Starch recovery (%)
1.	Water	Water	NaOH	59.3 ± 2.6
2.	NaOH	Water	NaOH	62.2 ± 2.8
3.	NaOH	NaOH	Water	61.8 ± 2.4

filtrate residue with dilute alkali. The details of the standardized process are outlined in Fig. 4.

The starch was extracted from black and normal white sorghum by using a standardized method. The physical appearance of the starches from both the type of grains was equally bright (Fig.5) indicating the effectiveness of the method in eliminating the pigmentation, particularly from black sorghum. The per cent recovery of the starch from black (62.2%) and normal white sorghum (68.4%) cannot be compared as these samples are of different origin.

4.7 Yield and Recovery of Starch by Different Methods

The amount of starch product recovered from black sorghum by using different methods of extraction, their starch contents and actual recovery of starch on the basis of initial starch present in the grain are summarized in Table 7. The black sorghum grains contained 64.7 per cent starch. The yield of starch product by different methods of extraction ranged from 33.8 to 62.2 per cent. The yield of starch product was found to be lowest when water was used as soaking and blending medium and when the musline cloth as screening device. The various starch products were found was used to contain 91.6 to 96 per cent pure starch. A maximum starch content was observed in the samples soaked in dilute NaOH and residue washed with dilute NaOH.

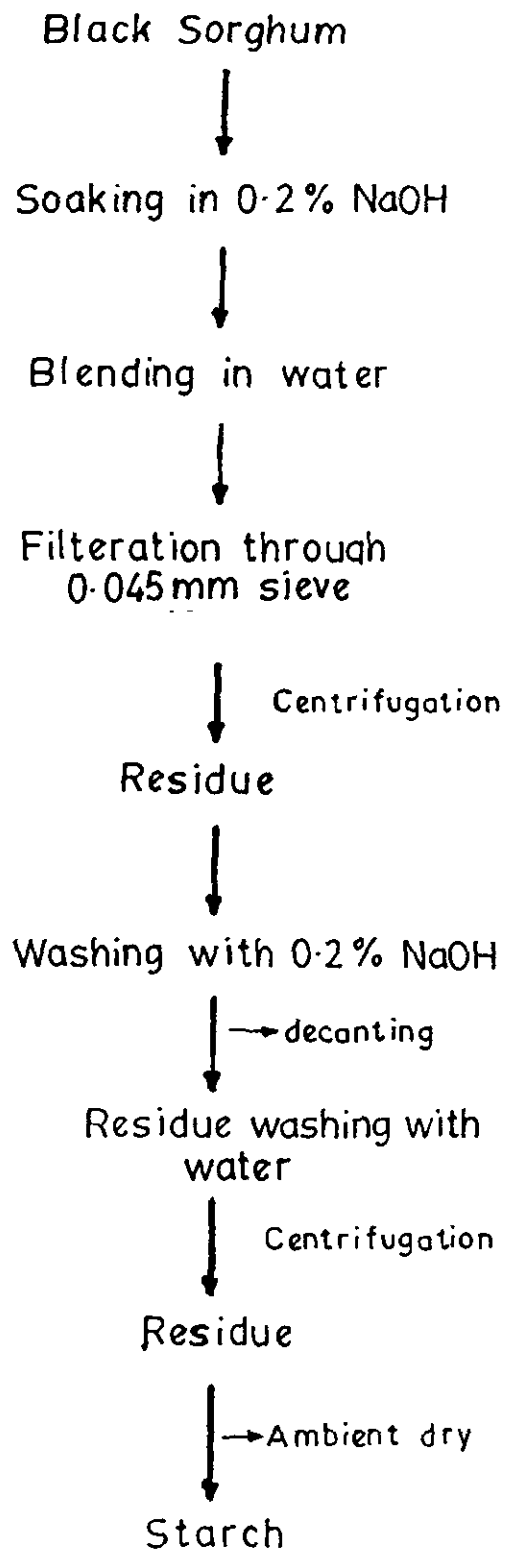


Fig. 4 Standardized process for extraction of starch from black sorghum

Fig. 5. The physical appearance of starch obtained by standardized process from -

1. Black sorghum grains
2. White sorghum grains
3. Starch obtained from black sorghum grains
4. Starch obtained from white sorghum grains

Table 7. The yield, content and recovery of starch by different methods of extraction

Method/ Sr.No.	Discription of Method			Per cent starch in native black grain	Starch product from grain (%)	Actual starch content in extracted (%)	Starch recovery on initial grain basis (%)
	SM	BM	RW				
1.	W	W	N	64.7	33.8 ± 1.20	96.0 ± 1.59	50.2 ± 1.60
2.	N	W	N	64.7	52.0 ± 1.80	93.8 ± 1.65	75.4 ± 1.75
3.	N	N	W	64.7	48.3 ± 1.90	94.7 ± 1.57	70.7 ± 1.70
4.	W	W	N	64.7	59.3 ± 1.80	92.0 ± 1.83	84.3 ± 1.81
5.	N	W	N	64.7	62.2 ± 1.95	91.6 ± 1.92	88.0 ± 1.79
6.	N	N	W	64.7	61.8 ± 1.10	92.2 ± 1.50	88.1 ± 1.67

Method 1 to 3 - With musline cloth
Method 4 to 6 - With 0.045 mm sieve
SM - Soaking medium,
BM - Blending medium
RW - Residue washing medium
W - Water
N - NAOH (0.2%)

The purity of starch obtained was generally higher when musline cloth was used as a filtration device (93.8 to 96%) than the 0.045 mm screen (91.6 to 92.4%). The recovery of starch on the basis of initial starch content in the grain was found to range from 50.2 to 88.1 per cent. A lowest recovery was observed with water (50.2%) as soaking and blending medium. The recovery of starch was significantly higher when the sieve was used as a filtration device as compared to musline cloth. A highest recovery of 88 per cent starch was observed when the grains were soaked in diluted NaOH solution, blended in water, the slurry filtered through 0.045 mm sieve and the residue was washed with diluted NaOH.

4.8 Chemical Composition of Different Starch Product

The data obtained on composition of extracted and dried starch products from different methods are presented in Table 8. The starch content was found to range from 91.6 to 96.0 per cent, the moisture from 4.0 to 5.0 per cent, protein from 0.47 to 1.66 per cent, total sugars from 0.41 to 0.47 per cent and total ash from 0.1 to 0.5 per cent in the starch product prepared by different methods. The purity of starch was found to be highest when water was used as soaking and blending medium (96%) than when dilute alkali was employed. The purity of starch was also found to be higher when musline cloth was used as filtration device as compared to the sieve, although the total product yield was significantly lower with musline cloth. The starch product extracted by using water as soaking and blending

Table 8. Chemical composition of starch extracted by different methods

Method of starch extraction	Starch (%)	Moisture (%)	Protein (%)	Total sugars (%)	Total Ash (%)
Method-1	96.0 ± 1.59	4.5 ± 0.33	0.47 ± 0.10	0.47 ± 0.02	0.50 ± 0.03
Method-2	93.8 ± 1.65	4.0 ± 0.35	1.03 ± 0.09	0.45 ± 0.01	0.25 ± 0.02
Method-3	94.7 ± 1.60	5.0 ± 0.29	1.24 ± 0.08	0.43 ± 0.03	0.10 ± 0.04
Method-4	92.0 ± 1.57	4.5 ± 0.25	1.06 ± 0.06	0.41 ± 0.05	0.39 ± 0.02
Method-5	91.6 ± 1.92	4.8 ± 0.30	1.23 ± 0.07	0.45 ± 0.04	0.40 ± 0.03
Method-6	92.2 ± 1.50	4.8 ± 0.25	1.66 ± 0.06	0.43 ± 0.03	0.42 ± 0.01

medium were found to contain significantly lower levels of contaminating proteins as compared to starch product obtained from other methods. The differences in the contents of total sugar or total ash among the different starch products were not of any significant order. The results therefore indicated that the purity of starch was higher with musline cloth while the yield of starch product was higher with the use of sieve as a filtration device.

4.9 Functional Properties of Extracted Starch

The functional properties viz. water and oil absorption capacities gelatinization temperature (GT) of the starch product obtained from different methods are presented in Table 9. The water absorption was found to range from 0.90 to 1.20 g/g while the oil uptake varied from 0.99 to 1.32 g/g starch. The starch obtained by soaking and blending the grains in dilute alkali (Method-3) exhibited highest water uptake while the starch product obtained by soaking and blending the grains in water (Method 6) showed highest oil absorption. The differences for either oil or water absorption by starch products from other methods were not of much significant order. The GT was found to range 76.8 to 80.2°C for different starches. In general, the practical properties of different starches were found to be more or less similar.

It has been reported that sorghum can be wet-milled for starch production by an identical process used for corn (Watson, 1967). However, it

Table 9. Functional properties of extracted starch

Method No.	Water absorption capacity/g	Oil absorption capacity/g	Gelatinization temperature, °C
1.	0.90 ± 0.06	0.99 ± 0.05	78.6 ± 1.2
2.	1.12 ± 0.07	1.18 ± 0.06	77.2 ± 1.1
3.	1.20 ± 0.06	1.11 ± 0.07	78.0 ± 1.01
4.	0.92 ± 0.07	1.32 ± 0.04	80.2 ± 1.10
5.	0.93 ± 0.05	0.99 ± 0.06	76.8 ± 1.30
6.	0.95 ± 0.06	1.01 ± 0.05	78.3 ± 1.15

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is now known that sorghum grain differs markedly from that of corn with respect to proportion of horny endosperm, size and distribution of starch granules, nature of protein matrix in which the starch granules are embedded, the range of phenolic pigments, the oil content in the germ and difficulty of releasing starch granules from the peripheral cells of the pericarp during blending of the steeped grains (Watson, 1970; Watson, 1984; Hamakar, *et al.*, 1992; Munck, 1995; Yang and Seib, 1996). Hence, the soaking of grains in water containing SO₂ and grinding of the soaked grains in water as practised in corn wet-milling may not be applicable *mutatis mutandis* for sorghum. The modifications with respect to soaking, blending and residue washing medium and other conditions of steeping and grinding are essential to obtain a maximum recovery of bright starch.

Attempts have been made to isolate starch from sorghum with bright appearance by reducing the soaking period (Yang and Seib, 1996), or the direct grinding of dry grains with water to obtain readily accessible starch (Yang and Seib, 1995). However, the recoveries of starch were quite low in these studies. Buffo *et al.* 1998 have reported that the factors such as proximate composition and physical properties of the grains influence the extraction of starch. The initial water absorption by the grains was reported to be important for higher starch recovery. It was indicated to develop steps to break the protein matrix for obtaining optimum separation of the starch components. It is clear from the reports that steeping of grains either in water or sulfuric acid may not be suitable for sorghum. Besides, sorghum

grain, particularly moulded and blackened types contain variety of phenolic pigments which need to be removed during soaking and subsequent steps. The dilute alkali may be more efficient as a soaking and blending medium than the sulfurous acid for sorghum. This will break down the thick protein matrix, release tiny starch granules and also solubilize the phenolic pigments more efficiently. The dilute alkali has been employed for soaking, grinding or residue washing of grains to obtain a starch from food legumes (Beta, 2001). Hence, we have tested the use of dilute alkali in different combinations to produce starch from black sorghum. The recovery of starch product was found to be significantly higher with the use of alkali in place of either water or diluted acids without a marked loss in quality.

The yield of starch product, purity of starch and its brightness are important characters in extraction and isolation of starch from any source for commercial purposes. The purified starch with good quality have many applications in processed foods, medicines etc as functional and energy ingredient. The purity of starch may not be of utmost importance for certain food applications. The moulded and blackened sorghum which otherwise with negligible food or market value can be processed into a value added product such as starch by a process standardized in this investigation. The starch product with relatively lower purity may have several acceptable applications in variety of processed foods.

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**SUMMARY
AND
CONCLUSIONS**

5. SUMMARY AND CONCLUSIONS

Grain sorghum is one of the important cereals grown in India for both food and feed purposes. It is a staple source of dietary proteins, energy, minerals and certain vitamins to a large segment of people particularly from low socio-economic groups and farming community from several states including Maharashtra. It is cultivated both in *kharif* and *rabi* season and the *kharif* crop is often caught in late rains during grain-development stage. The moist and humid conditions result into the severe infestation of grains by field moulds such as species of *Fusarium*, *Curvalaria*, and *Aspergillus*. The moulds grow on the surface of the grains and secrete variety of pigments, the grain becomes moulded and blackened at maturity. The degree of moulding and blackening vary widely depending upon the type of cultivar grain filling stage, severity of rainfall and humid conditions.

The moulding and blackening of *kharif* sorghum crop to varying levels results into heavy economic losses to both the growers and Government because such grains are unacceptable for human consumption or feed purposes due to the presence of black pigmentation and sometimes toxins. The economic losses can be significantly reduced by processing of moulded and blackened sorghum into value added products such as pearled grains, starch etc. The technologies for chemical dehulling and direct physical pearling for moulded and blackened sorghum grains have been standardized in this laboratory. However, a large volume of water is required for chemical dehulling and the pearled product was found to exhibit a short self life of about only 30 days at ambient conditions,

Several investigators have attempted to prepare starch from sorghum by using the process commonly employed for corn starch. The process involves steeping of grains in water containing SO_2 followed by grinding of soaked grains in water and subsequent separation of starch and other fractions. However, sorghum grain differs from that of corn with respect to structure of grain, nature of endosperm, size and distribution of starch granules and nature of protein matrix. Hence, steeping and grinding of grains in water ^{or} in mild acidic conditions is not effective in solubilizing the protein matrix and obtaining maximum starch recovery from sorghum.

Keeping in view these facts, experiments were planned to extract and isolate the starch from black sorghum (market sample) by using various combinations of water, dilute acid or alkali at various stages of starch extraction process. The extracted and purified starch was analysed for proximate composition and some functional properties. The results obtained are briefly summarised in this section :

1) The rate of water absorption was found to increase with increase in the soaking period or soaking temperature. A maximum water absorption was found in the grains soaked at 60°C for 96 hrs.

2) The starch recovery from the grains was found to increase with increase in soaking period and temperature. A maximum starch recovery was found when the grains were soaked at 60°C for 144 hrs.

3) Among the different grain soaking media employed, the highest starch recovery was observed for dilute alkali (52%) followed by lactic acid (34.4%), water (33.8%) and H_2SO_4 (23.38%). Thus, dilute alkali was found to be the most efficient solvent for soaking the grains and washing the starch residue. A concentration of 0.2 per cent NaOH was found to be optimum for soaking the grains and washing the starch residue to obtain maximum starch recovery.


4) Among the two devices used for filtration of starch slurry, the use of 0.045 mm sieve was found to produce maximum starch yield as compared to the use of musline cloth.

5) The starch product obtained by using musline cloth for filtration purpose exhibited lower yield (33.8 to 52.0 %) and recovery (50.2 to 75.4 %) but higher purity (93.8 to 96.0 %). The use of 0.045 mm sieve for filtration, however, produced the higher starch yield (59.3 to 62.2 %), starch recovery (84.3 to 88.1 %) but slightly lower starch purity (91.6 to 92.0 %).

6) The starch products obtained from different methods exhibited a gelatinization temperature. of 77.2 to 80.2°C, a water absorption of 0.90 to 1.20 g/g and an oil absorption of 0.99 to 1.32 g/g. The differences in the contents of moisture, protein, sugars and total ash were not of much significant order.

It is therefore concluded that the black sorghum grains can be conveniently processed into a good quality bright starch with yield of about 62.2 per cent by the process standardized in this investigation. Further studies are however, essential to minimize a soaking time and volume of soaking medium to avoid the effluent problem on commercial scale.

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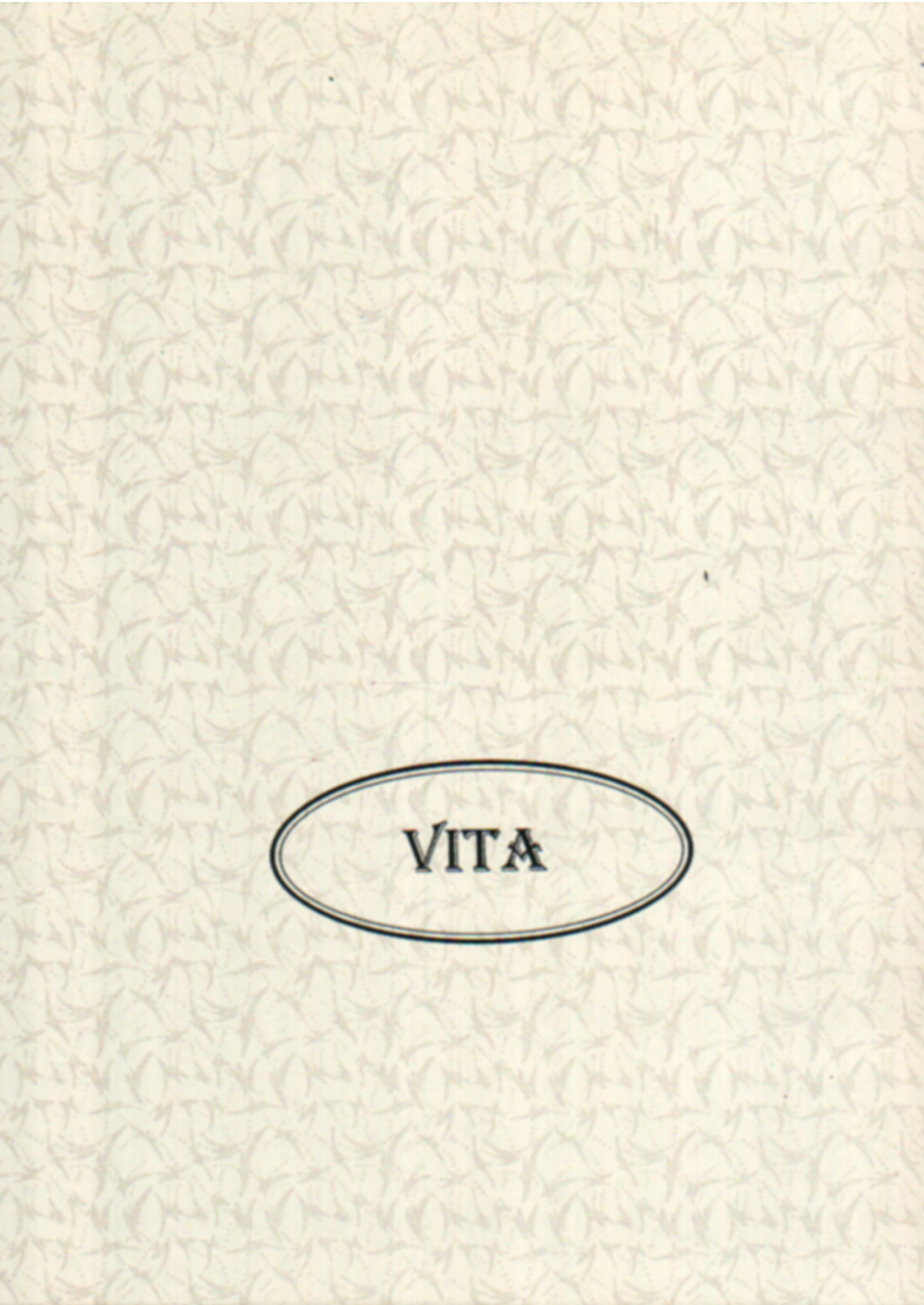
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Chapter Opener Page



VITA

7. VITA

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