

**This research manuscript, a first flower in
My life is affectionately dedicated to my
beloved Mother Late *Ena (Kaku)*,
Father *Appa* and elder Brother
Anna who are the continuous
source of inspiration for
me in every walk
of life.**

..... *Ram*

SCREENING OF PROMISING SAFFLOWER (Carthamus tinctorius L.)
CULTIVARS FOR OIL CONTENT AND QUALITY

by

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A Thesis submitted to the
MAHATMA PHULE KRISHI VIDYAPEETH, RAHURI
DIST: AHMEDNAGAR, MAHARASHTRA STATE (INDIA)

in partial fulfilment of the requirements for the degree
of
MASTER OF SCIENCE (AGRICULTURE)
in
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DEPARTMENT OF BIOCHEMISTRY,
POST GRADUATE INSTITUTE,
MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI, DIST. AHMEDNAGAR, M.S. (INDIA)

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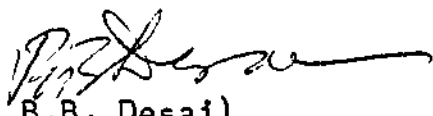
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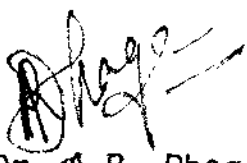
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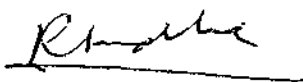
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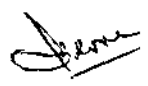
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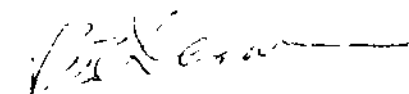
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The assistance and help received during the course of this investigation have been duly acknowledged.

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Place : Rahuri

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(N. K. Umrani)

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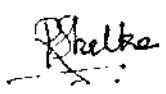
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Date : 23 / 12 / 1994


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ABSTRACT

SCREENING OF PROMISING SAFFLOWER CULTIVARS FOR OIL
CONTENT AND QUALITY

by

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The present investigation was undertaken to study the oil content, oil quality and quality of meal protein of safflower seeds. Fifteen samples were analysed for oil content and eight samples analysed for protein quality parameters.

There were significant differences among the cultivars in respect of oil content ranging from 29.16 to 32.67% and crude protein ranging from 10.5 to 18.4%. The safflower seed proteins were deficient in methionine content but the tryptophan content was up to standard. Among the cultivars studied, CO-1 had higher levels of protein and oil contents. The safflower seed oil varied in respect of its acid value, iodine value, saponification value and peroxide value from

0.391 to 0.626%, 134.13 to 145.93, 136.25 to 196.32 and 2.4 to 3.66 respectively. The methionine and tryptophan content of the defatted safflower meal ranged from 1.02 to 1.76% and 1.11 to 1.50 (g/16g N), respectively, whereas the tannin content of safflower meal varied from 0.528 to 0.724%.

The overall results of investigation indicated that both the oil and protein quality of the seed of safflower cultivars studied were nutritionally under safe limits.

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Introduction

1. INTRODUCTION

The safflower (Carthamus tinctorius L.), also called Kardi, Kusumb, false or bastard saffron, cartamo and suff is one of the important and oldest crops in the world of oilseeds trade. It is being grown for centuries in India and other parts of Asia, Africa and Europe as a source of an oilseed and dye, though present expression and use as source of an edible oil came much later. In ancient India, the plant was cultivated by small farmers both for dye and oil. The seeds were used to extract oil, while florets were added to rice, bread and pickles to give them attractive orange colour. With the introduction and availability of cheaper synthetic food dyes, the use of safflower as source of dye has diminished slowly during recent time. Currently it is cultivated as a commercial oilseed crop in different parts of the world.

Safflower is widely grown in the region of arid and semi-arid tropics of India, Mexico, U.S.A., Ethiopia and Australia. Although safflower contributes about 0.5% of the total oil production of the world, India ranks first followed by Mexico, U.S.A., Ethiopia and Australia in safflower production (FAO, 1990). Out of the total world production of 922 (000 MT) safflower seeds, India contributed to 491 (000 MT) i.e. about 53 per cent. In India, Maharashtra is the largest producer of safflower followed by Karnataka and Andhra Pradesh (Salunkhe et al., 1992).

Safflower is mainly grown for edible oil. The green tender leaves which are discarded during thinning of the crop are used as vegetable in India and Burma. The cake remaining after extraction of decorticated seeds for oil, being rich in protein is used as cattle feed, while cake from undecorticated seeds is generally used for manuring purposes. The hulls, removed from decortication of seeds are used to provide bulk in high grade ration for beef cattle.

The most common safflower has a white normal hull, with high linoleic acid and contains 40% total carbohydrates, 13% protein, 6% moisture, 38% oil and 3% minerals. A wide variation of 27 - 50% in the oil content of safflower seeds has been reported (Patil, 1987). The protein content of the whole seed ranges from 11 to 24%. However, after the extraction of oil from seeds, the cake or meal obtained as byproduct in the oil industry carries most of the seed protein. The cake contains 46.06 to 55.5% proteins (Betschart, 1975). The safflower protein is of a high biological value, only lysine being a critically limiting amino acid. The biological quality of protein changes only slightly by the meal processing (Weiss, 1971). The deoiled cake is not used for human consumption due to its high content of fibre and bitter tasting phenolic glucosides (Lyon et al., 1979).

The safflower oil is considered as premium cooking oil due to its high content of linoleic acid, high iodine value,

light yellow colour and a characteristic pleasant flavour. The safflower is widely used in the form of salad oil, hydrogenated fat, margarine, myonnaise and in several type of processed foods (Salunkhe and Desai, 1986).

The safflower oil contains low percentage of saturated fatty acids (5 - 10%) and high percentage of polyunsaturated fatty acids (90 - 95%). Due to its high content of polyunsaturated fatty acids, mainly linoleic, the keep ing quality of oil is low owing to oxidative changes taking place in hot climates. Though safflower oil and protein have better nutritional quality, the information on storage property of safflower seed oil and protein quality is scanty. It was, therefore, felt necessary to study the quality of safflower seed oil and protein with the following objectives :

1. To evaluate fifteen promising safflower cultivars for their oil content and proximate composition
2. To study the oil and protein quality of fifteen safflower cultivars

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Review of Literature

2. REVIEW OF LITERATURE

The literature pertaining to the chemical composition of safflower seed and oil quality is reviewed in this chapter.

2.1. Chemical composition of safflower seed

The chemical composition of safflower seed can be influenced by many factors such as cultivar, geographic location, agronomical practices, soil fertility and management factors like fertilizers and use of growth regulators. The seed composition of safflower has been reported by several workers (Apple white, 1966; Betschart, 1975; Haby et al., 1982; Singh and Yusuf, 1981; Sangale et al., 1982; Bratuleanu and Gracu, 1986). The range of average composition of safflower seeds from different geographical origin as compiled by Weiss (1983) indicated significant variations with the moisture, 4 to 8%, oil, 12 - 47%, protein, 11 - 24%, ash 1.5 to 3.0%. For Indian commercial type the content of moisture was 7 %, oil, 32%, protein, 15%; ash, 1.7% and total carbohydrates, 44.3%. Safflower seed has been reported to contain 14.9 % protein, 27.5% oil, 2.0% ash, 3.2% total sugars and 40.6% crude fibre (Latha and Prakash, 1984).

2.1.1. Protein

2.1.1.1. Protein content

The protein content of the whole safflower seeds ranges from 11 to 24% (Van Etten et al., 1963; Guggolz et al.,

1968; Betschart, 1975; Salazar-zazueta and Price, 1989). Van Etten et al. (1963) reported that the kernel (55.5% of seed weight) contained 55.9% protein while the hulls (44.5% of seed weight) contained only 3.1% protein. The cultivars with thin hull had slightly higher proportion of protein than normal thick types, due to increased proportion of kernel in the seeds. The protein content is often calculated by multiplying N content with a factor of 6.25 or 5.45. There is a need to use uniform factor for protein calculation in safflower. Betschart (1975) reported that the protein content (using 6.25 factor) of whole seeds, kernel (full fat) and hulls was 16.44, 22.38 and 6.75%, respectively. Since the safflower seeds are exclusively extracted for oil, the cake or meal manufactured as byproduct of oil industry contains most of the proteins. The expeller press cake contains 55.5% protein, while commercial desolventized contains 45.06 % (Betschart, 1975). Using a factor of 5.45 for calculating protein content, Salazar-zazueta and Price (1989) reported a protein content of 24.87% in defatted whole meal and 66.93% in dehulled, defatted safflower meal. The commercial safflower seed cake contains varying amounts of hull and residual oil which cause variation in its protein content. Most of the storage proteins of safflower are concentrated in the kernel. Based on the solubility and electrophoretic properties, Salazar-zazueta and Price (1989) observed that the major storage proteins in safflower seeds were alkali-

soluble glutelins (69%). The other fractions of water- and salt-extractable proteins accounted for 17 to 5%, respectively. The prolamin fraction was negligible. Singh and Sinha (1978) found that the protein content decreased with an increase in storage time.

2.1.1.2. Amino acid composition

The amino acid composition of safflower kernel, hull, defatted meal with or without hulls have been reported by several workers (Van Etten et al., 1963; Evans and Bandemer, 1977; Guggolz et al., 1968; Palter et al., 1969; Betschart and Saunders, 1979).

The safflower proteins are rich in glutamic acid (18 - 25 g/10g N), aspartic acid (8 - 12 g/16g N) and their amines. Hence the factor of 5.45 can be used for protein calculation instead of common factor of 6.25 used for several proteins. Since the proportion of total protein in the hull is very small, the amino acid composition of whole meal and kernel are quite similar (Van Etten et al., 1963). In case of essential amino acid composition of safflower, the meal proteins are different in lysine, methionine, threonine and isoleucine when compared with FAO/WHO (1985) pattern. Lyman et al., (1956) reported the essential amino acid content of safflower seed meal to be lysine, 2.71; methionine, 1.54; and tryptophan, 1.13 g/16g N. A significant variation in the contents of

methionine, cystine, threonine and tyrosine between cultivars of safflower has been reported (Evans and Bandemer, 1967; Guggolz et al., 1968). The environmental factors significantly influenced the levels of lysine, although the thickness of hull did not affect the amino acid composition markedly (Guggolz et al., 1968). The lysine content ranged from 2.51 to 3.22 g/16g N in the world collection of safflower (Palter et al., 1969).

2.1.2. Lipid

2.1.2.1. Lipid content

The most common safflower cultivated on commercial scale has a white normal hull with high linoleic acid, although the plant types differing in hull and major fatty acid contents are available. In India, almost entire safflower crop is of normal type while it is over 90% in U.S.A. A large number of safflower varieties analysed for oil content showed a range of 27 to 50% oil (Patil, 1967). An average range of oil content of 39.00 to 49.00 % (Knowler, 1969), 21.3 to 40.1% (Kumar et al., 1982), 33.5 to 46.9% (Bratcher et al., 1969), 30.0 to 40.0% (Hadjichristodoulou, 1985), 32.7 to 36.0% (Haby et al., 1982), 22.6 to 47.7% (Guggolz et al., 1968), and 29.8 to 48.0% (Sangale et al., 1982) have been reported. These reports clearly indicate the genetic variability for oil content in different genotype of safflower available.

The common types with normal hull usually contain 25.0 to 40.0% oil. Oil content from six Indian plant species belonging to different and less familiar botanical families were studied for their fatty acid composition (Gupta et al., 1983). According to Singh and Sinha (1978), there was an increase in the contents of oil and invert sugars during storage of safflower seeds.

2.1.2.2. Oil characteristics

Safflower oil is a drying oil, intermediate between soybean and linseed in total unsaturation. The oil has a pale or golden yellow colour and bland or nutty flavour depending upon the method of extraction. It has the highest refractive index, specific gravity, density, iodine value and linoleic acid content than most other common edible oils (Salunkhe et al., 1992).

The linoleic acid is a major fatty acid (55 - 81%) found in safflower oil followed by oleic acid (7 - 42%), stearic acid (1 - 10%) and palmitic acid (2 - 10%). Other fatty acids also have been reported to be present in safflower oil but they are in trace amounts (Durate et al., 1978). The unsaturated fatty acids make up for about 90%, while the saturated fatty acids form remaining 10%.

The oil constitutes mainly of triglycerides and other natural lipid constituents like phospholipids (0.5%)

and unsaponifiable lipids (0.3 - 1.3%) are in minor quantities (Burkhardt, 1971; Weiss, 1983). The unsaponifiable lipids in safflower oil mostly constitute sterols and terpenes (Fedeli et al., 1972). Composition of the fractions of sterols was related to fatty acid composition and significant correlations were found between campesterol, D⁷ - Stigmasterol, oleic and linoleic acids (Conte et al., 1983). The crude safflower oil contains about 0.63% total sterols, while the refined oil contains about 0.56% (Bernardini, 1985).

2.1.3. Carbohydrates and crude fibre

The whole seeds of safflower contain 18.5% carbohydrates and 21.7% crude fibre (Salazar-zazueta and Price, 1989). Dehulling and / or defatting of the seed meal markedly influence the level of carbohydrates and crude fibre. The defatted whole meal contains 37.73% total carbohydrates, while dehulled and defatted meal contains 19.93%. Similarly, the crude fibre content of 21.7% in the whole seed increased to 35.0% in defatted whole meal and 4.12% in dehulled and defatted meal. Defatting increases the level of carbohydrates and fibre in the defatted whole meal while dehulling significantly decreases the level of both carbohydrates and fibre in the meal. The hulls are higher in the total carbohydrates due to greater contents of fibre pentosans, lignin and unhydrouronic acid.

The defatted kernel contained 24.6% carbohydrates out of which 7.6% was sugars (Guggolz et al., 1968, Saunders, 1970). Among the sugars, sucrose (47%) and raffinose (35%)

contributed major proportion. Tamhane (1923) did not find any starch, glucoside or tannin in resting seeds of safflower. Safflower seed has been reported to contain 3.2% total sugars and 40.6% crude fibre (Latha and Prakash, 1984).

2.1.4. Minerals

The total mineral content in the whole safflower seed ranges from 2.1 to 3.5% (Guggolz et al., 1968; Deosthale, 1981). The hulls contain about 4.5% mineral matter while the kernel had about 3.2%. The removal of hulls or defatting of kernel increases the mineral matter content of meal upto 8%. Among the different mineral elements, the mean contents of phosphorus, magnesium, calcium and iron were 367, 241, 214 and 4.6 mg/100 g meal, respectively (Deosthale, 1981). The defatted meal can be a rich source of calcium, phosphorus and iron. Significant varietal differences are noticed for the calcium content of safflower seed.

2.2. Protein quality of safflower seeds

The biological value of the protein changed only slightly by the processing of meal (Weiss, 1971). Evans and Bandemer (1967) in an experiment with rats observed that supplementation of meal with 0.4% methionine alone did not improve the protein nutritive value (PNV). However, addition of 0.4% lysine and 0.4% isoleucine along with 0.4% methionine increased the PNV from 31 to 61. In a similar

experiment with Tetrahymena pyriformis, the PNV increased from 12 to 56, when the meal was supplemented with methionine, isoleucine and lysine. These observations indicate that lysine and isoleucine are the most limiting amino acids in safflower protein.

According to Betschart and Saunders (1978), the protein efficiency ratio (PER) of unheated meal improved from 1.14 to 1.31 when the safflower meal was supplemented with 0.75% lysine. The PER of expeller press cakes increased from 1.10 to 1.56 by supplementing with soyaflower (1 : 1). The digestibility of protein from heated or unheated meal was lower. The supplementation of meal proteins with lysine improved the true digestibility (T.D.), while supplementation with soyaflower decreased it. The heating caused during expeller pressing did not influence the PER or T. D. of safflower proteins markedly indicating that protein quality of safflower is inferior mainly due to deficiency of lysine. According to Lyman et al. (1956) the essential amino acid content of safflower seed meal as methionine (1.54) and tryptophan (1.19) g/16g N.

2.3. Oil quality of seeds

The quality of any edible oil is evaluated based on attributes like nutritional and cooking quality, physical and sensory quality and storage stability. Among the commercial edible oils, safflower oil is the richest in

linoleic acid and highest in iodine value. Hence the nutritional quality of safflower oil is considered as the highest amongst the edible oils. (However, higher content of polyunsaturated fatty acids may not be desirable for the keeping quality.) The Committee on Dietary Allowances (1980) has described criteria for evaluating the dietary value of edible oils based on the ratio of the content of linoleic acid to the sum of palmitic and stearic acid $L / (P + S)$. On this basis safflower oil exhibits a much higher ratio of 9 : 1 (Carpenter et al., 1974), supplying more than recommended polyunsaturates. However, it can be regarded to exhibit low dietary value and keeping quality. A low $L / (P + S)$ ratio of about 2 - 4 is considered nutritionally desirable. The ecological factors during seed formation may influence the quality of seeds to a great extent (Weiss, 1971). The following physico-chemical characteristics of safflower oil have been reported : Iodine value, 136 to 146; saponification value 186 to 196; and free fatty acid, 0.15 to 1.09 (Vibhakar et al., 1981). While characteristics of solvent extracted Egyptian safflower oil were : saponification value, 183.6, iodine value, 145.5, and acid value (0.68%) (Abu-Nasr, A.M., 1963). [The higher iodine value (greater than 70) and lower saponification value (lower than 200), acid value (lower than 2.0) and peroxide number (lower than 10.0 meq/kg) are desirable for any edible vegetable oil (Salunkhe et al., 1991).]

2.4. Phenolic compounds

The use of defatted safflower meal in human consumption is limited, primarily due to the presence of fibrous hull and second content of tannins, which give a bitter taste to safflower meal. Safflower meal was intermediate in total phenolics, the principal acids obtained on hydrolysis of esters being trans-sinapic acid (Dabrowski et al., 1984; Kohler, 1966; and Raj et al., 1968).

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Material and Methods

3. MATERIAL AND METHODS

3.1. Material

3.1.1. Seeds

The seeds of fifteen safflower varieties were obtained from the Associate Director of Research (NARP), Scarcity Zone, Solapur of Mahatma Phule Krishi Vidyapeeth.

3.1.2. Chemicals

Most of the chemicals used in the present study were of analytical grade from B.D.H., Sarabhai M. Chemicals and E. Merck, Bombay. The DL-Methionine, L-tryptophan were obtained from Sisco Research Laboratory, Bombay.

3.2. Methods

A design of the experiment planned was completely randomized design (CRD).

3.2.1. Extraction of oil

The samples were crushed and crude oil was extracted using petroleum ether and used for determination of different oil constants viz. acid value, peroxide value, iodine value and saponification value.

3.2.2. Chemical analysis

The moisture, minerals, crude protein and crude fibre were estimated from the fat meal, while the methionine,

tryptophan and phenolic compounds were estimated from the defatted meal. All analytical work reported was carried out in quadruplicates.

The moisture, minerals, crude fibre, crude protein ($N \times 6.25$), acid value, peroxide value, iodine value and saponification value were estimated by following standard methods of A.O.A.C. (1980).

3.2.2.1. Protein quality parameters

3.2.2.1.1. Methionine

This was estimated by the method of Mc Carthy and Paille (1959) :

Reagents :

1. Hydrochloric acid, 2 N
2. Sodium hydroxide, 10%
3. Sodium nitroprusside, 10%
4. Sodium hydroxide, 10 N
5. Glycine, 3%
6. Concentrated orthophosphoric acid
7. Standard methionine solution : The DL-methionine (100 mg) was dissolved in 2 ml of 20% hydrochloric acid and further diluted to 100 ml with water. This solution contained 1 mg DL-methionine/ml.

Methionine extraction

Methionine was extracted according to the following method of Gupta and Das (1955) :

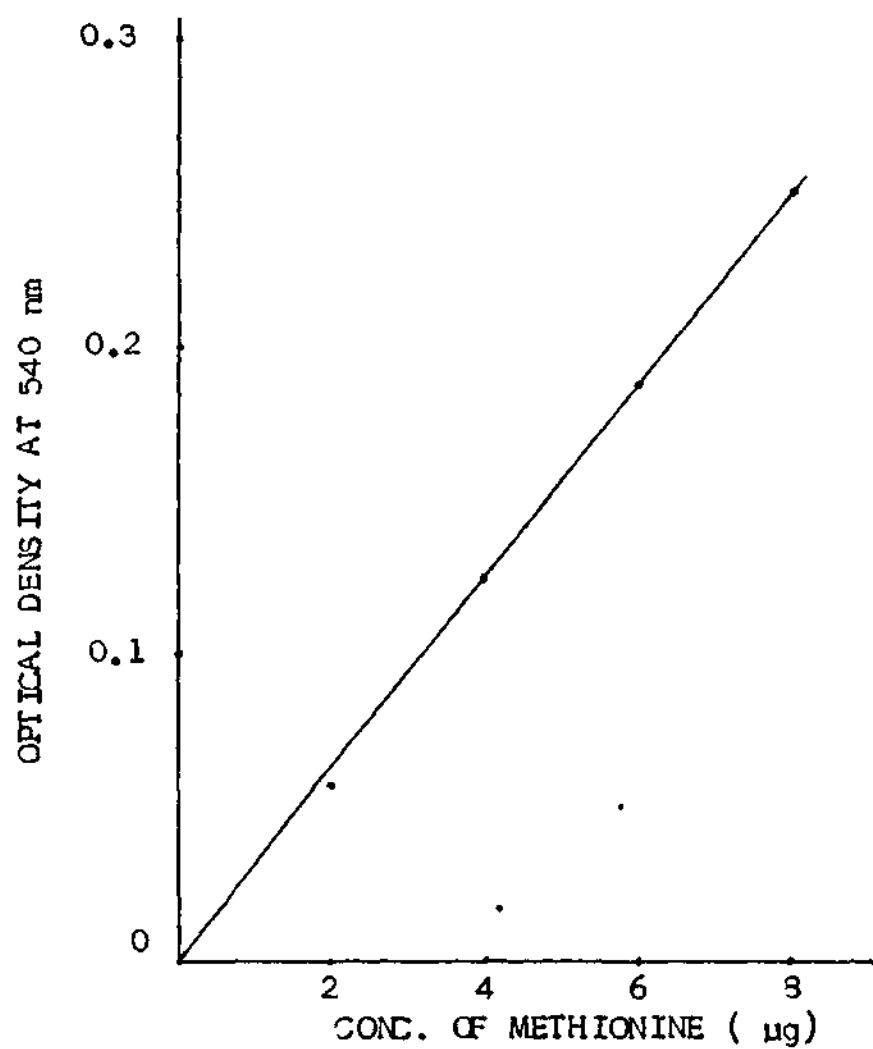


FIG. 1. STANDARD CURVE FOR METHIONINE

Two grams of defatted sample was autoclaved with 25 ml of 2N HCl at 15 lb pressure for one hour. The hydrolysate was treated with a pinch of activated charcoal to get rid of the colour, heated to boiling for few minutes and filtered by giving 3-4 quick washings with hot water. The filtrate and washings were pooled. The colour free extract was neutralised with 10 N NaOH to bring the pH to about 6.5 and volume was made to 100 ml.

The colour was developed according to the method described by Mc Carthy and Paille (1959). A 50 ml extract was taken in 250 ml conical flask and 6 ml of 10% NaOH were added. This was followed by addition of 0.3 ml of sodium nitroprusside and contents were kept for 10 minutes with occasional shaking. Then 2 ml of glycine solution was added, shaken well and allowed to stand for 10 minutes. Afterwards, 4 ml of concentrated orthophosphoric acid was added, shaken vigorously and allowed to stand. The colour intensities were measured after 10 minutes of Spectronic 20 spectrophotometer at 540 nm.

Standard curve and calculations

For a standard curve, different concentration of methionine (0, 2, 4, 6, 8, 10 mg) were taken in quadruplicate and 25 ml of 2 N HCl were added to each. The remaining procedure was similar as used for samples. The methionine content of samples was calculated from standard curve (Fig. 1)

and reported as per cent on moisture free basis in meal and g per 16g N.

3.2.2.1.2. Tryptophan

Tryptophan in the samples was determined by the colorimetric method of Spice and Chambers (1949) :

Reagents :

1. Sulphuric acid, 19 N and 20.4 N
2. Sodium nitrate, 0.045%
3. P-dimethyl aminobenzaldehyde
4. Standard tryptophan : The tryptophan (20 mg) was dissolved in 100 ml water and 10 ml of this solution was diluted to 100 ml which contained 20 mg/ml of tryptophan.

Procedure : The ground defatted samples (40 mg) were taken in quadruplicate and 30 mg of p-dimethyl aminobenzaldehyde were added to each flask. The fourth flask in each experiment was a blank (without sample). Then, 10 ml of 19 N sulphuric acid was added to all the flasks. After keeping the flasks in dark for 20 hours at 30° C, 0.1 ml of 0.045% sodium nitrate was added to each flask except in blanks, shaken gently and kept for 30 minutes for colour development. The contents were filtered through glass wool and the colour intensities were read at 600 nm on Spectronic 20.

Standard curve

The standard tryptophan solution of 20 to 120 ug concentration was taken in conical flasks in quadruplicate.

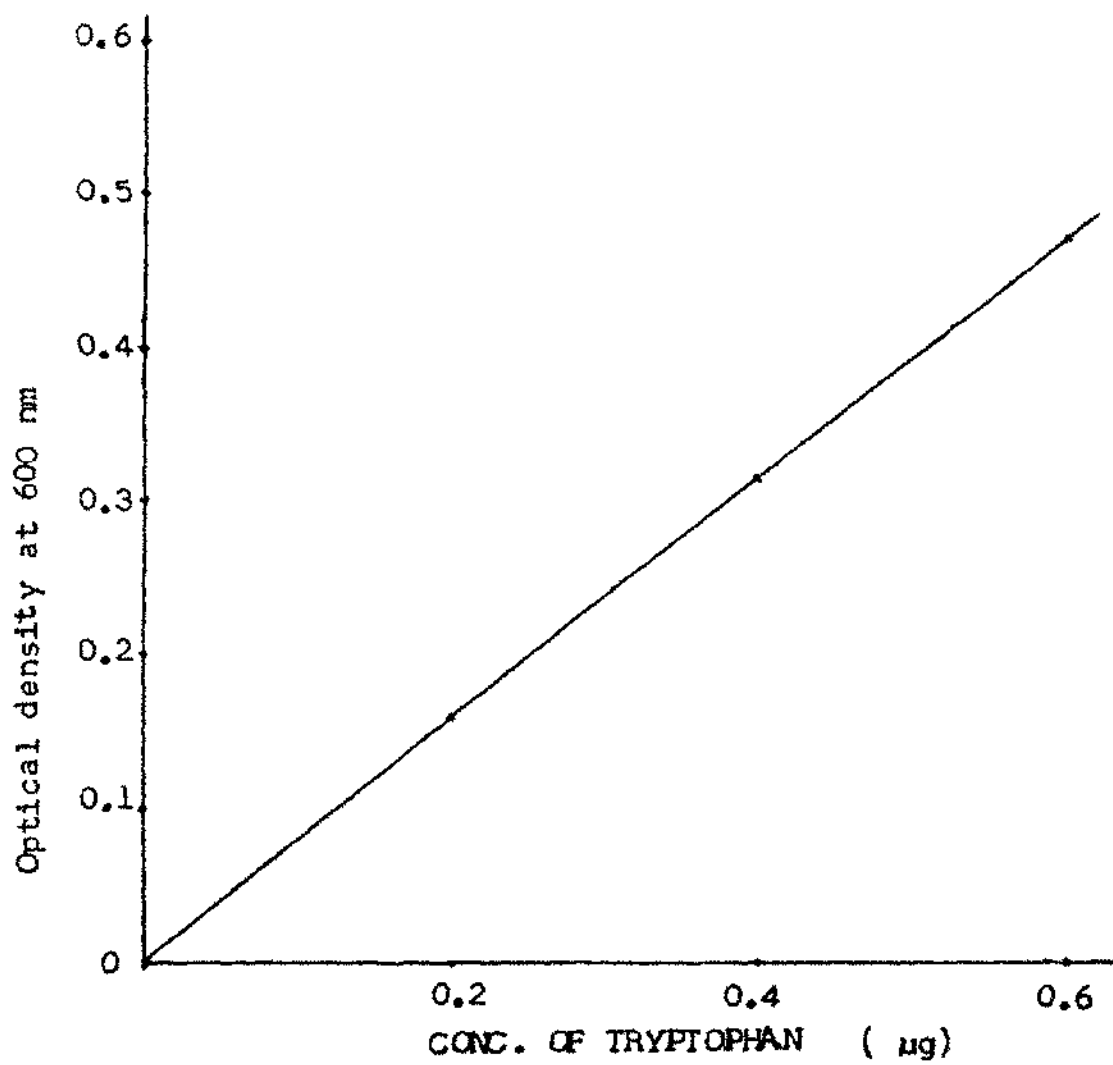


FIG. 2. STANDARD CURVE FOR TRYPTOPHAN

The water was added to make up volume to 0.6 ml in each flask. Then 9.4 ml of 20.4 N sulphuric acid was added to each flask in order to make 19 N as final strength of the acid. Then 30 mg of p-dimethylaminobenzaldehyde was added to each flask. The flasks were kept in dark for 20 hrs at 30° C. Rest of the procedure was same as described for samples. The tryptophan content of each sample was determined from the standard curve (Fig. 2) after making the necessary corrections for blank. The tryptophan was expressed as per cent and g/16g N on moisture free basis in meal.

3.2.2.2. Oil quality parameters

3.2.2.2.1. Acid value

Reagents :

1. Fat solvent, alcohol
2. Sodium hydroxide, 0.1 N
3. Phenolphthalein indicator

Procedure : An oil sample (1 gm) was accurately weighed and dissolved in 25 ml of fat solvent in conical flask. Phenolphthalein indicator (3 to 4 drops) was added to flask and mixed thoroughly. The contents of flask were titrated with 0.1 N sodium hydroxide until faint pink colour persisted for 20 to 30 seconds. The amounts of alkali required for neutralization was recorded and used for calculation. A blank determination was also run simultaneously.

3.2.2.2.2. Iodine value

Reagents :

1. Fat solvent : Chloroform
2. Hanus reagent : 13.2 g pure iodine or 13 g of sublimated iodine in acetic acid containing 3 ml of bromine solution was made to 1 litre with acetic acid and kept in dark
3. Potassium iodine, 15 %
4. Sodium thiosulphate, 0.1 N
5. Starch indicator, 1 %

Procedure : An oil sample (0.20 g) was weighed accurately in 250 ml of glass stoppered flask and dissolved in 10 ml of chloroform. Then 25 ml of Hanus reagent was added to it, occasionally. Subsequently 10 ml of potassium iodide solution was added and mixed thoroughly using 100 ml freshly boiled and cooled water for washing down any free iodine on stopper. The contents of the flask were titrated with 0.1 N sodium thiosulphate untill yellow colour turned almost colourless. Then, few drops of starch indicator were added and the titration was continued until blue colour entirely disappeared. The quantity of sodium thiosulphate required for titration was recorded and used for calculation of iodine value. A blank was also run simultaneously.

3.2.2.2.3. Peroxide value

Reagents :

1. Acetic acid-chloroform reagent was prepared by mixing three volumes of acetic acid with two volumes of cloroform.

2. Saturated potassium iodide solution was prepared by dissolving excess potassium iodide in freshly boiled water.

3. Standard sodium thiosulphate solution : A 0.1 N sodium thiosulphate solution was prepared by dissolving 24.8 g sodium thiosulphate in one litre water, diluted to 0.01 N with freshly boiled and cooled water. The reagent was standardized by using potassium dichromate.

4. Starch solution 1%.

Procedure : An oil sample (1 g) was accurately weighed in erlenmeyer flask. To this 30 ml of acetic acid-chloroform reagent was added followed by addition of 0.5 ml of saturated potassium iodide solution. The contents were swirled to dissolved potassium iodide solution and kept for 2 minutes with occasional shaking. After 2 minutes shaking, 30 ml of water was added. The contents were slowly titrated with 0.01 N sodium thiosulphate with vigorous shaking until yellow colour was almost disappeared. Then starch indicator was added and titration was continued with vigorous shaking until blue colour just disappeared. A blank determination (without oil sample) was also conducted simultaneously.

3.2.2.2.4. Saponification value

1. Hydrochloric acid, 0.5 N : prepare accurately 0.5 N HCl by dissolving approx. 44.5 ml of conc. HCl and make vol. 1000 ml.

2. Alcoholic KOH : Dissolve 28 g of KOH in small quantity of distilled water make one litre with alcohol

keeping temperature below 15.5°C , while alkali being dissolved. This solution should be clear.

3. Phenolphthalein indicator : 1 % in 95% alcohol

4. Oil or fat sample

Procedure :

1. The sample must be completely moisture free. Weigh accurately 5 gm of oil and add 50 ml of alcoholic KOH from burette by allowing it to drain for a definite period of time.
2. Prepare a blank by taking 50 ml alcoholic KOH allowing it to drain at the same duration of time.
3. Connect the air condenser to the conical flask and boil them gently on hot water bath for about one hour.
4. After the flask and condenser get cooled rinse down the inside of the condenser with little of water and then remove the condenser.
5. Add about one ml of phenolphthalein indicator to solution and titrate it with standard (0.5 N HCl) until pink colour just appears.

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3.2.2.3. Total sugar

Total sugar from the samples were estimated by the procedure of Nelson (1944) as follows,

Reagent A :

Twenty five grams of sodium carbonate (anhydrous)
25 g sodium potassium tartarate, 20 g sodium bicarbonate

and 200 g sodium sulphate (anhydrous) were dissolved in distilled water and volume made to one litre.

2. Reagent B :

Fifteen grams of copper sulphate were dissolved in 100 ml distilled water and 1 to 2 drops of concentrated sulphuric acid were added.

3. Copper reagent :

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

4. Arsenomolybdate reagent :

Twenty five grams of ammonium molybdate were dissolved in 450 ml of distilled water. To this 21 ml concentrated sulphuric acid was added. Three grams of sodium arsenate were dissolved separately in 25 ml distilled water and added to above solution. The contents were mixed and incubated at 37° C for 48 hrs. fresh reagent was prepared every week.

5. Standard glucose solution :

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

Extraction of sugars :

One gram of finely powdered fat free material was extracted with 80 % (V/V) boiling ethanol on a shaker for 1 hr. followed by centrifugation at 1000 rpm for 15 minutes. The extraction was repeated four times and combined. The combined ethanolic extract was then evaporated to about 5 ml and the contents were diluted to 100 ml with distilled water.

Procedure : To 5 ml of ethanolic extract, 5 ml diluted hydrochloric acid (1 : 1) was added and kept for 24 hrs. for inversion. It was neutralised with 5 N sodium hydroxide. The extract was cleared with saturated lead acetate and delead with sodium phosphate. One ml of the hydrolysed sugar solution was mixed with one ml of copper reagent. The contents were heated for exactly 20 min. in boiling water bath. After cooling one ml of arsenomolybdate reagent was added and mixed well. This mixture was diluted to 8 ml and absorbance was read at 520 nm against blank. Total sugar were calculated from a standard curve (Fig. 3) for D-glucose prepared by using different concentration of standard glucose solution and expressed as per cent.

3.2.2.4. Phenolic compounds

Phenolic compounds content was determined by using folin-Dennis reagent as described by Chavan et al. (1979).

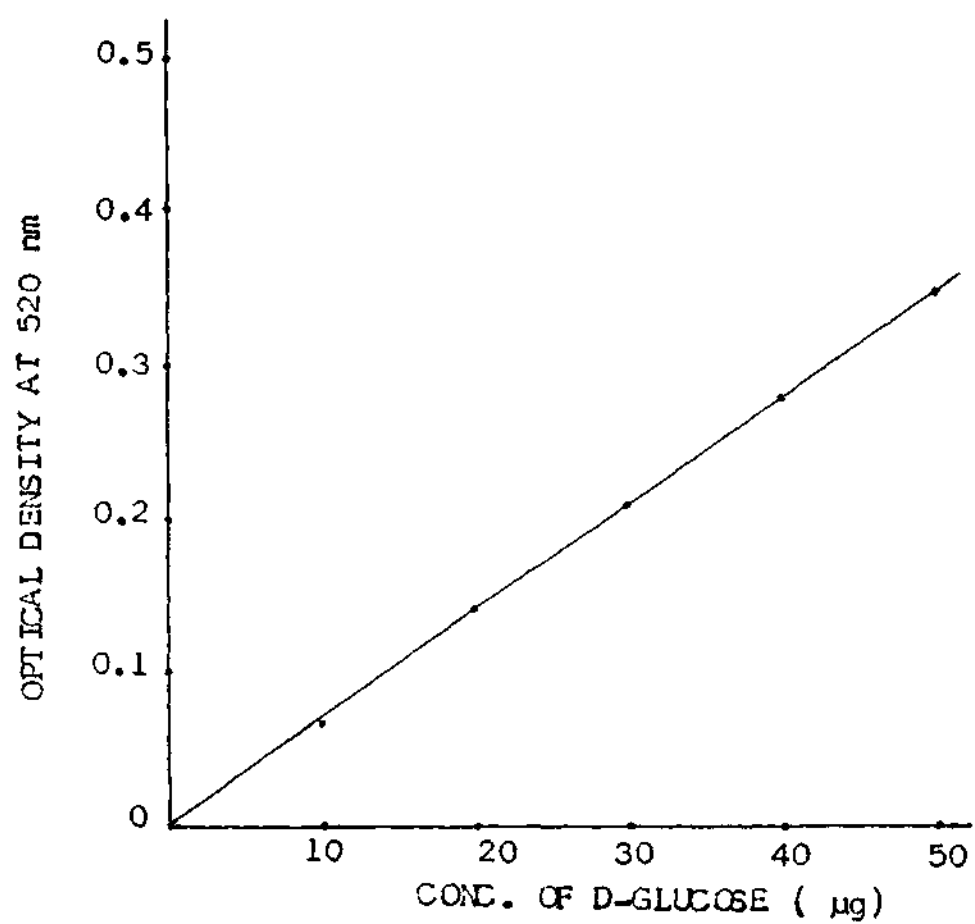


FIG. 3. STANDARD CURVE FOR TOTAL SUGARS

Reagents :

1) Folin-Dennis reagent : One hundred grams of sodium tungstate 20 grams phosphomolybdic acid and 50 ml of concentrated phosphoric acid were added to 750 ml of distilled water. The mixture was allowed to reflux for two hours on hot water bath and made to 1000 ml.

2) Alkaline reagent : Sodium carbonate 350 grams was dissolved in 1000 ml at 80° C, allowed to stand for overnight at room temperature and then filtered through glass wool.

3) Standard tannic acid solution (0.1 %) : One hundred milligrams of tannic acid was dissolved in 100 ml of distilled water.

Procedure : One gram of ground sample was accurately weighed and transferred to 500 ml conical flasks, four flasks, each containing one gram sample were prepared and 150 ml of distilled water was added to each flask. Internal standards were prepared by adding 10, 15, 20 ml of standard tannic acid solution in the first three flask and fourth flask was left as control. The contents of conical flasks were gently heated, boiled for 30 min and centrifuged at 5000g for 20 min. The supernatant was collected in 250 ml volumetric flasks and volume made with distilled water.

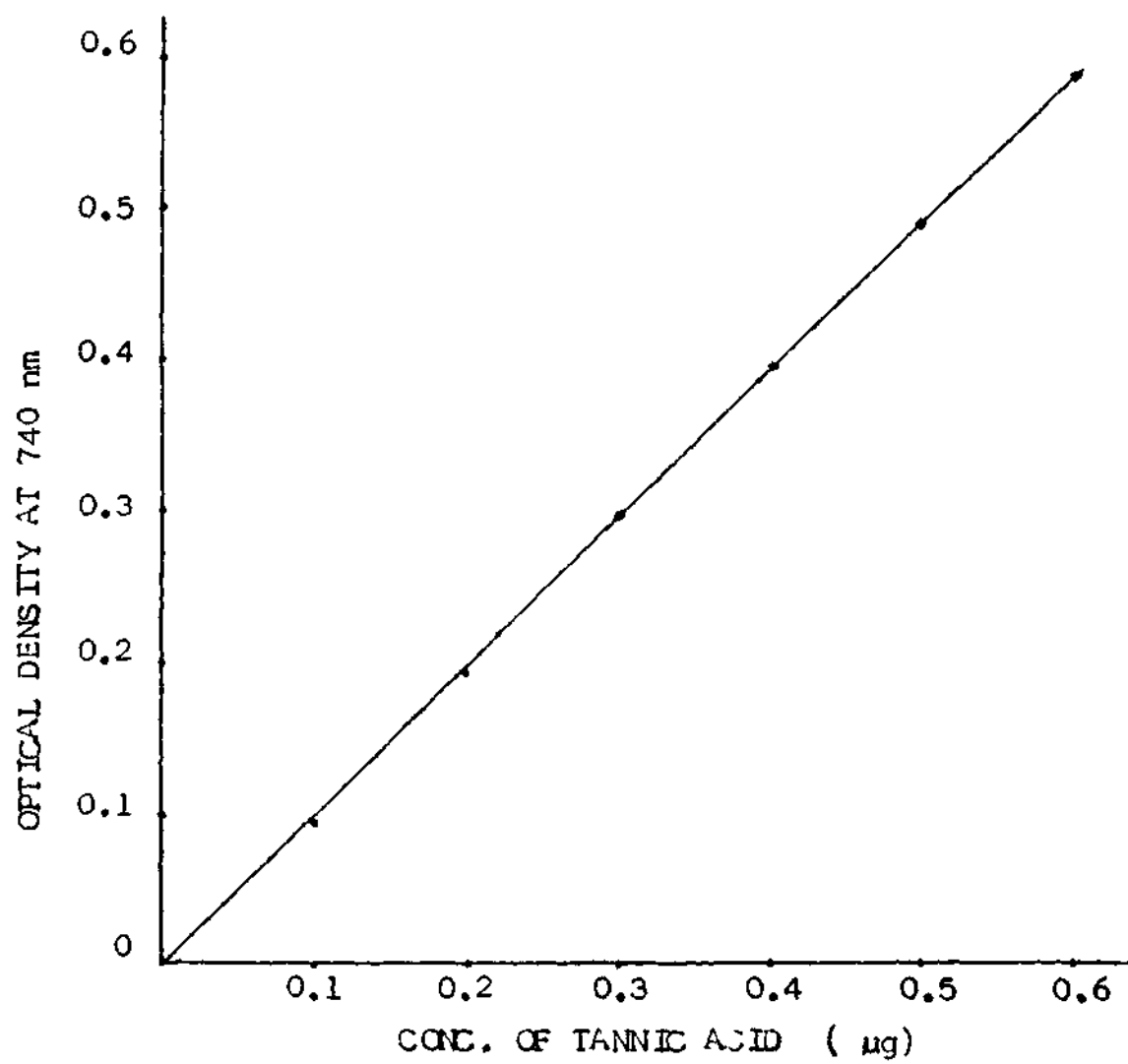


FIG. 4. STANDARD CRUVE FOR TANNIN

Colour development :

Ten ml of supernatant was taken in 100 ml volumetric flask to which 75 ml distilled water added and the volume was made. The flasks were allowed to stand for 30 minute and absorbance was read at 740 nm on Spectronic 20. A standard graph was prepared by plotting absorbance Vs tannic acid concentration. Absorbance of the control wherever it cuts 'Y' axis was taken as a new origin. The difference in absorbance in first and second origin was taken or measure of tannin content in the sample.

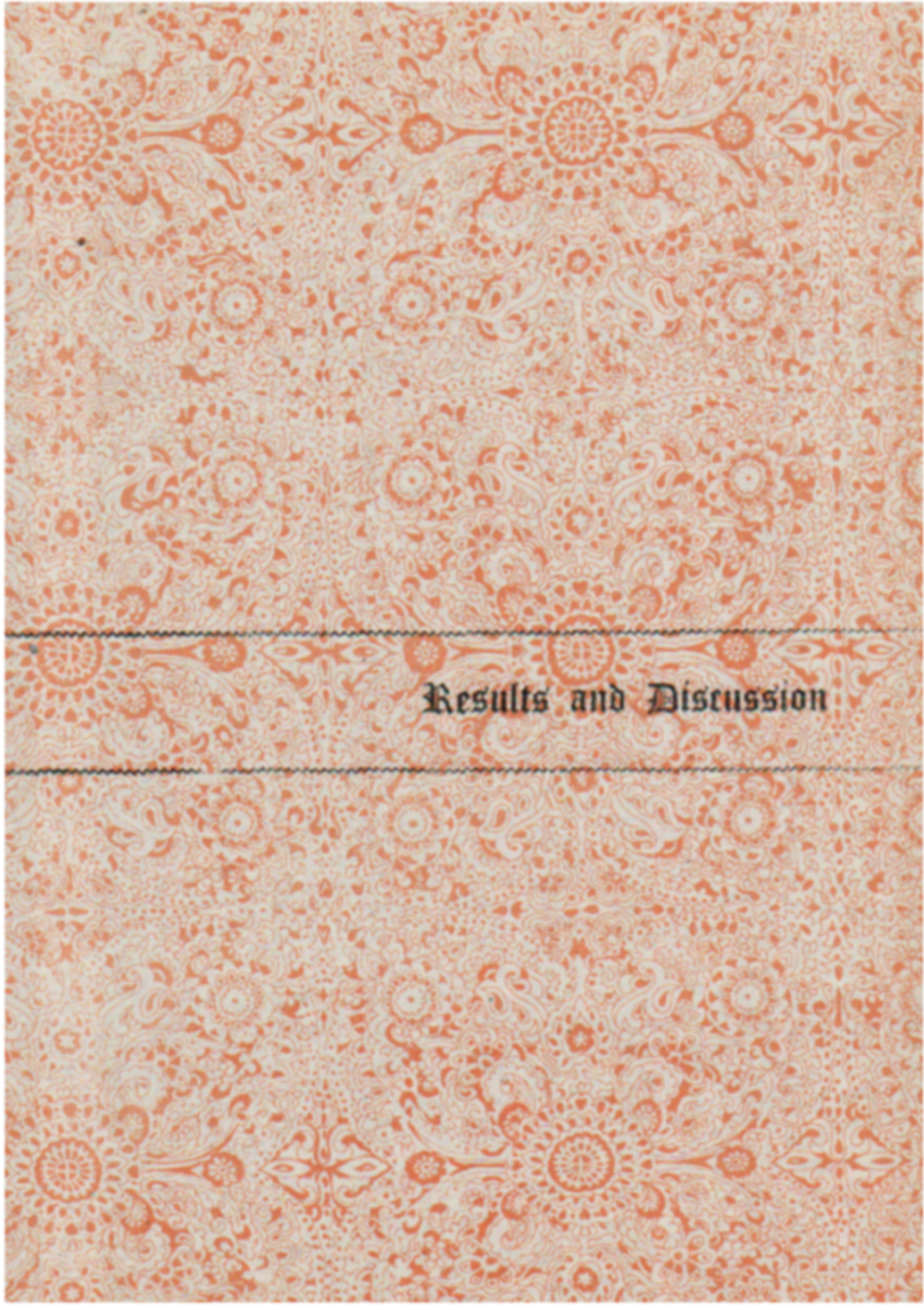
All determinations were performed in quadruplicate and the standard error and critical differences are calculated.

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Results and Discussion

4. RESULTS AND DISCUSSION

Fifteen promising cultivars of safflower differing in their oil content potential were analysed for the proximate composition, protein quality, oil quality and phenolic compounds. The results of these studies are presented and discussed in their section under the following headings :

4.1 Oil content of safflower cultivars

4.2 Proximate composition

4.3 Protein quality of seeds

4.4 Oil quality of seeds

4.5 Phenolic compounds

4.1 Oil content of safflower cultivars

Safflower oil had a pale or golden yellow colour and was bland or had a nutty flavour depending on the method of processing used. Safflower oil exhibits a higher refractive index, specific gravity, density, iodine value and linoleic acid than most of the other common edible oils.

The whole seeds of safflower cultivars were crushed and the oil was extracted. The data on proximate composition is presented in Table 1. The oil content ranged from 29.16 to 32.67 %. The cultivar Tara had the highest oil content (32.67 %) and the mean oil content of safflower cultivar was 30.5%. These results are in agreement with the range of oil content of safflower seeds from 21.3 to 40.1% (Kumar et al., 1982; Patil, 1987 and Sangale et al., 1982).

Table 1. Proximate composition of safflower seeds

Sr. No.	Cultivars	Moisture (%)	Total mineral (%)	Crude fat (%)	Crude protein (N x 6.25) (%)	Crude fibre (%)	Total sugars (%)
1.	Bhima	7.0	2.62	31.55	10.505	44.945	3.38
2.	A-1	7.9	2.16	29.75	14.006	42.964	3.22
3.	HUS-305	9.0	2.43	32.33	12.250	40.000	3.99
4.	JSF-1	8.1	2.17	29.9	11.380	45.000	3.45
5.	A-300	9.1	2.12	30.10	11.38	43.460	3.84
6.	K-1	7.13	2.38	29.91	14.88	42.63	3.07
7.	Manjira	9.1	2.33	30.63	13.131	42.199	2.61
8.	JLSF-38	7.2	2.26	29.69	15.757	41.713	3.38
9.	BLY-152	8.3	2.46	30.18	12.256	42.274	4.53
10.	Sharda	7.5	2.24	30.14	14.007	43.353	2.76
11.	N-7	7.2	2.40	29.16	15.757	41.723	3.76
12.	Tara	8.6	2.43	32.67	14.882	38.658	2.76
13.	S-144	8.1	2.83	30.41	16.633	39.117	2.91
14.	CO-1	8.0	2.21	31.46	15.757	37.653	4.92
15.	NRS-209	8.0	2.11	29.78	18.384	38.126	3.3
Mean		8.035	2.343	30.510	14.064	41.582	3.458
S.E. $\pm$		0.78	0.09	0.33	0.84	0.52	0.246
C.D. at 5%		N.S.	0.28	0.99	2.41	1.56	0.74

N.S. = Non significant

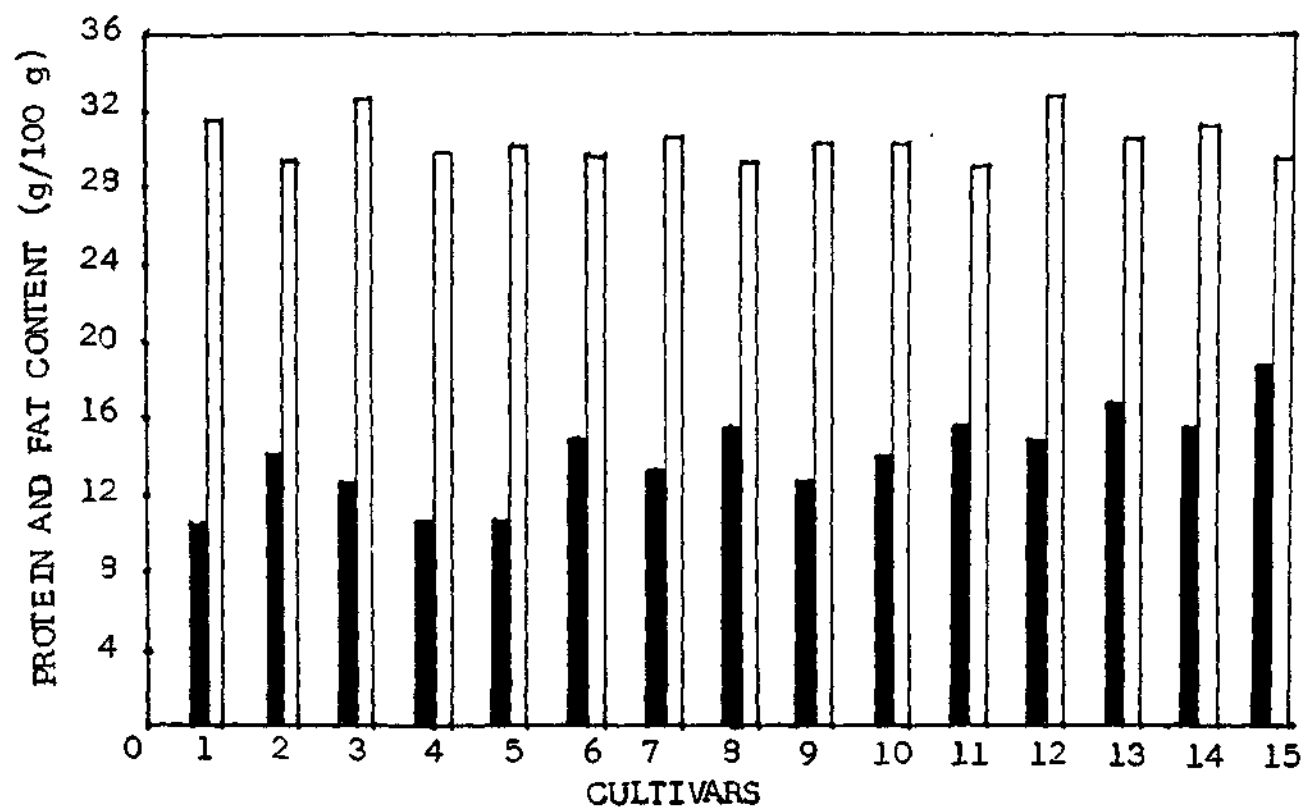


FIG. 5. PROTEIN AND FAT CONTENTS OF SAFFLOWER CULTIVARS

No. Cultivars

1. Bhima
2. A-1
3. HUS-305
4. JSF-1
5. A-300
6. K-1
7. Manjira
8. JLSF-99
9. BLY-152
10. Sharda
11. N-7
12. Tara
13. S-144
14. CO-1
15. NRS-209

PROTEIN : 

FAT : 

4.2. Proximate composition

The whole seeds of safflower cultivars were analysed for the content of moisture, crude proteins, crude fat, total minerals, crude fibre and total sugars. The results were presented in Table 1. The moisture content in the safflower seed, did not significantly differ and it averaged from 7.0 to 9.1%. The crude protein ($N \times 6.25$), crude fat, total minerals, crude fibre were from 10.50 to 18.38 %, 29.16 to 32.67 %, 2.11 to 2.93 %, and 37.65 to 45 %, respectively. The range of total sugar content was from 2.61 to 4.92%. The proximate composition of the safflower seeds indicated that cultivar CO-1 was promising; having better performance in oil content and protein. The results obtained are in conformity with the earlier reports. Safflower cultivars from different origin as compiled by Weiss (1983) indicated significant variations with the moisture content from 4 to 8%; oil, 12 to 47%, protein, 11. to 24% and ash 1.5 to 3.0 %. Indian commercial safflower cultivars had moisture, 7%; oil 32%; protein, 15%; ash 1.7%; and crude fibre 44.3%. Latha and Prakash (1984) reported that total sugars in safflower meal was 3.2% .

4.3. Protein quality of seeds

4.3.1. Methionine content

The essential amino acids composition of safflower meal are lysine, leucine, isolucine, valine and methionine.

The proteins are deficient in lysine, methionine, threonine and isoleucine in comparison with the FAO (1935). The quality of protein is influenced by its essential amino acid contents. Methionine is limiting amino acid in oil seed proteins.

The methionine content of safflower seed expressed on meal and protein basis is presented in Table 2. The methionine content of the cultivars studied ranged from 1.02 to 1.76 g/16g N, the highest being in Manjira, closely followed by A-1 and A-300 (1.56 g/16g N). These values are in close agreement with early reports. The methionine content of safflower seed meal reported was 0.5 to 1.59 g/16g N (Lyman et al., 1956). While the standard for methionine is 3.5 g/16g N (FAO, 1935) indicating that safflower meal is deficient in this essential amino acid. Evans and Bandemer (1967) observed that supplementation of safflower meal with 0.4% methionine alone did not improve protein nutritive value (PNV). The PNV increased when the meal was supplemented with lysine and isoleucine in addition to methionine.

4.3.2. Tryptophan

Tryptophan is the another essential amino acid present in safflower seed meal. Tryptophan of safflower seed expressed on meal and protein basis is presented in Table 3. Tryptophan has been found to be a limiting amino acid in oil seed proteins. The tryptophan content of safflower seed meals ranged from

Table 2. Methionine content of defatted safflower seed meal of various cultivars

Sr. No.	Cultivars	g/100 g meal	g/16g N
1.	Bhima	0.153	1.45
2.	A-1	0.162	1.56
3.	HUS-305	0.126	1.02
4.	A-300	0.178	1.56
5.	K-1	0.224	1.50
6.	Manjira	0.232	1.76
7.	JLSF-88	0.202	1.28
8.	CO-1	0.230	1.45
Mean		0.198	1.44
S.E. $\pm$		0.0037	
C.D. at 5%		0.011	

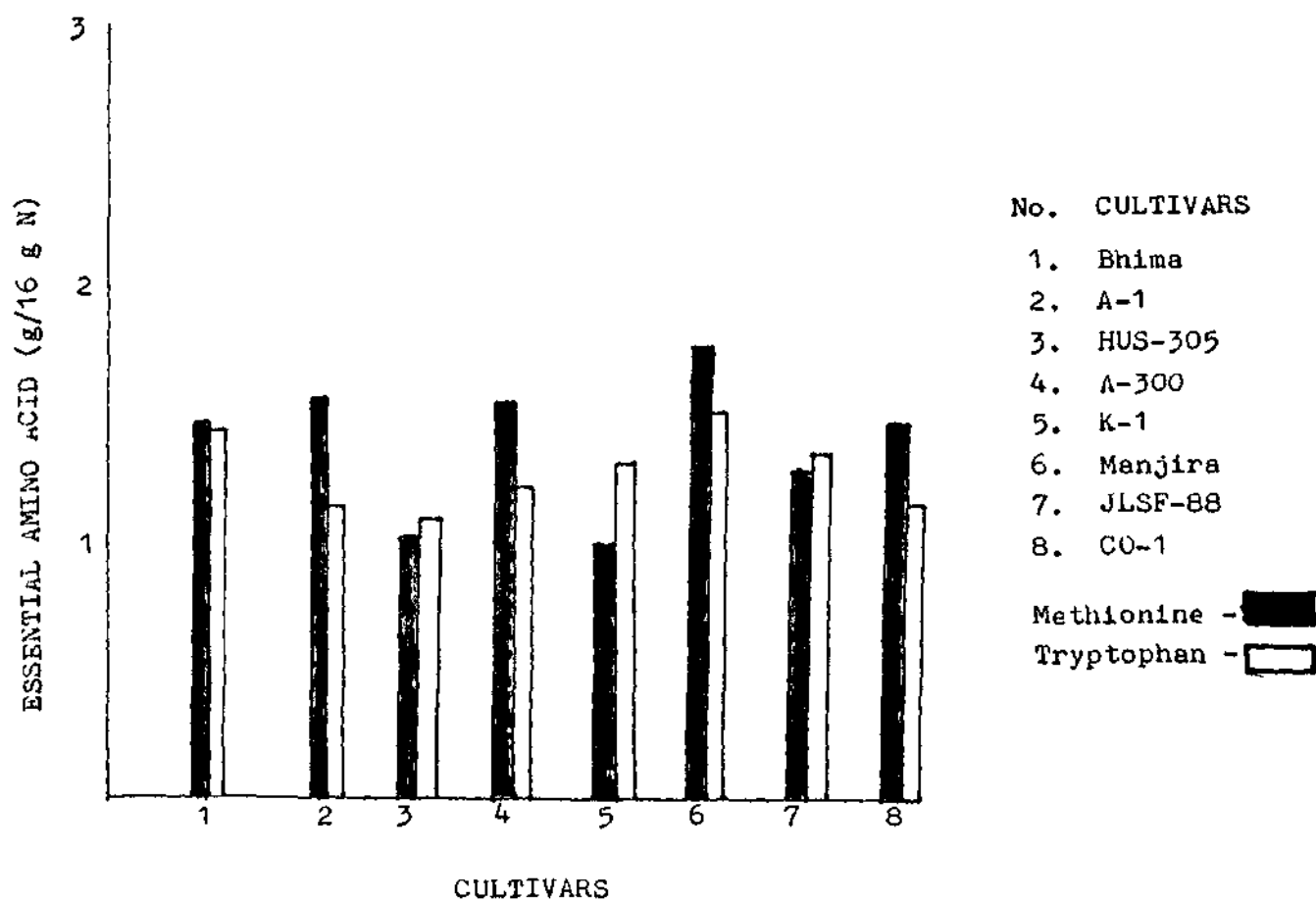


FIG. 6 : CONTENT OF AMINO ACID (g/16 g N) IN SAFFLOWER SEED

Table 3. Tryptophan content of defatted safflower seed meal of various cultivars

Sr. No.	Cultivars	g/100 g meal	g/16g N
1.	Bhima	0.152	1.44 ✓
2.	A-1	0.173	1.15
3.	HUS-305	0.136	1.11
4.	A-303	0.135	1.19
5.	K-1	0.196	1.31
6.	Manjira	0.197	1.50 ✓
7.	JLSF-33	0.212	1.34
8.	CO-1	0.183	1.16
Mean		0.171	1.27
S.E. $\pm$		0.005	
C.D. at 5%		0.014	

1.11 to 1.50 g/16g N, the highest being in Manjira closely followed by Bhima (1.44). These values are in agreement with the earlier reports (Lyman et al., 1956).

4.4. Oil quality of seeds

4.4.1. Acid value

(The acid value of the safflower oil indicates its level of free fatty acids,) which increase during storage. (Keeping quality of oil, therefore, relies upon the acid value)(Damame, 1988). The data obtained for acid value are presented in Table 4. There was a significant difference in the acid value of oil among the 15 cultivars. The acid value of oil ranged from 0.39 to 0.62% , which was highest (0.626%), in the cultivar CO-1, closely followed by Bhima (0.623%). It was lowest (0.391%) in cultivar A-300, The mean acid value of safflower cultivar was 0.455%. These values are in agreement with the results reported earlier (Abu-Nasr, 1969). The desirable acid value for any vegetable oil is lower than 2.0 (Salunkhe et al., 1992).

4.4.2. Iodine value

(The iodine value indicates the degree of unsaturation of the oil. It is a useful parameter in studying oxidative rancidity of oils, since the higher the unsaturation the greater the tendency of oils to go rancid (Vibhakar et al., 1981).) The iodine value of safflower oil is presented in

Table 4. Acid values of different safflower cultivars
in seed oil

Sr. No.	Cultivars	Acid value (%)
1.	Bhima	0.623
2.	A-1	0.504
3.	HUS-305	0.439
4.	JSF-1	0.487
5.	A-300	0.391
6.	K-1	0.392
7.	Manjira	0.436
8.	JLSF-88	0.398
9.	BLY-152	0.408
10.	Sharda	0.408
11.	N-7	0.443
12.	Tara	0.443
13.	S-144	0.426
14.	CO-1	0.626
15.	NRS-209	0.411
Mean		0.455
S.E. $\pm$		0.004
C.D. at 5%		0.01

Table 5. Significant difference in the iodine value of safflower oil was noticed among the 15 safflower cultivars. The iodine value was highest (145.93) in the cultivar CO-1, closely followed by HUS-305 (145.49); while it was lowest in Bhima (134.19). The mean iodine value was 139.35. These values are in agreement with the earlier reports available (Abu-Nasr, 1963). The desirable iodine value for any edible vegetable oil should be greater than 70 (Salunkhe et al, 1992).

4.4.3. Peroxide value

The peroxide value indicates the extent of peroxidation of oil during storage. Rancidity is brought about by the action of oxygen (oxidative rancidity) or micro-organisms (ketonic rancidity) in oil. In oxidative rancidity oxygen is taken up by the oil with the formation of low molecular weight carbonyl compounds viz. peroxides, ketones, epoxides and aldehydes. These increases peroxide value during storage (Damame, 1998).

The data presented in Table 6 show significant differences among the safflower cultivars. The peroxide value was highest (3.66 meq/kg oil) in cultivars, NRS-209, closely followed by JLSF-88 (3.49 meq/kg oil) and it was lowest in S-144 (2.40 meq/kg oil), with the mean peroxide value of 3.04 meq/kg oil. The peroxide value for any edible vegetable oil should be lower than 10 meq/kg (Salunkhe et al., 1992).

Table 5. Iodine value of various safflower cultivars
in seed oil

Sr. No.	Cultivars	Iodine value
1.	Bhima	134.18
2.	A-1	140.07
3.	HUS-305	145.49
4.	JSF-1	143.58
5.	A-300	134.70
6.	K-1	137.64
7.	Manjira	134.22
8.	JLSF-88	136.41
9.	BLY-152	137.30
10.	Sharda	140.10
11.	N-7	139.60
12.	Tara	139.59
13.	S-144	144.15
14.	CO-1	145.93
15.	NRS-209	139.82
Mean		139.35
S.E. $\pm$		1.13
C.D. at 5%		3.39

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Table 6. Peroxide values of different various
safflower cultivars in oil

Sr. No.	Cultivars	Peroxide value (meq/kg)
1.	Bhima	3.28
2.	A-1	3.07
3.	HUS-305	3.28
4.	JSF-1	2.57
5.	A-300	2.98
6.	K-1	3.48
7.	Manjira	3.19
8.	JLSF-88	3.49
9.	BLY-152	2.69
10.	Sharda	2.77
11.	N-7	2.75
12.	Tara	3.28
13.	S-144	2.40
14.	CO-1	2.86
15.	NRS-209	3.66
Mean		3.04
S.E. $\pm$		0.07
C.D. at 5%		0.22

4.4.4. Saponification value

The saponification value is a measure of molecular weight of constituent fatty acids present in the oil. The data presented in Table 7 show significant difference in the saponification value among the safflower cultivars. The observed range of saponification value was 186.25 to 196.32 with the highest (196.32) in the cultivar Sharda, which was closely followed by Bhima (194.25). The values recorded in this study are in agreement with the earlier reports (Vibhakar et al., 1981). The desirable saponification value for any edible vegetable oil is expected to be lower than 200 (Salunkhe et al., 1992).

4.5. Phenolic compounds

Safflower meal is known to contain two phenolic glucosides viz. 2-hydroxy arctiin and metairesinol monoglucoside which give carthamic activity and bitter taste, respectively (Kohler, 1966).

The phenolic compounds i.e. tannins of seeds of eight safflower cultivars express on meal basis are presented in Table 8. The data show significant difference among the eight cultivars. The use of safflower meal for human consumption is limited, primarily due to the presence of fibrous hull and the tannin content which give bitter taste.

Table 7. Saponification values of various safflower cultivars in seed oil

Sr. No.	Cultivars	Saponification value
1.	Bhima	194.25
2.	A-1	191.28
3.	HUS-305	187.51
4.	JSF-1	194.94
5.	A-300	187.25
6.	K-1	187.85
7.	Manjira	188.45
8.	JLSF-88	185.25
9.	BLY-152	192.21
10.	Sharda	196.32
11.	N-7	189.98
12.	Tara	188.09
13.	S-144	187.83
14.	CO-1	191.37
15.	NRS-209	189.70
Mean		189.76
S.E. $\pm$		0.48
C.D. at 5%		1.40

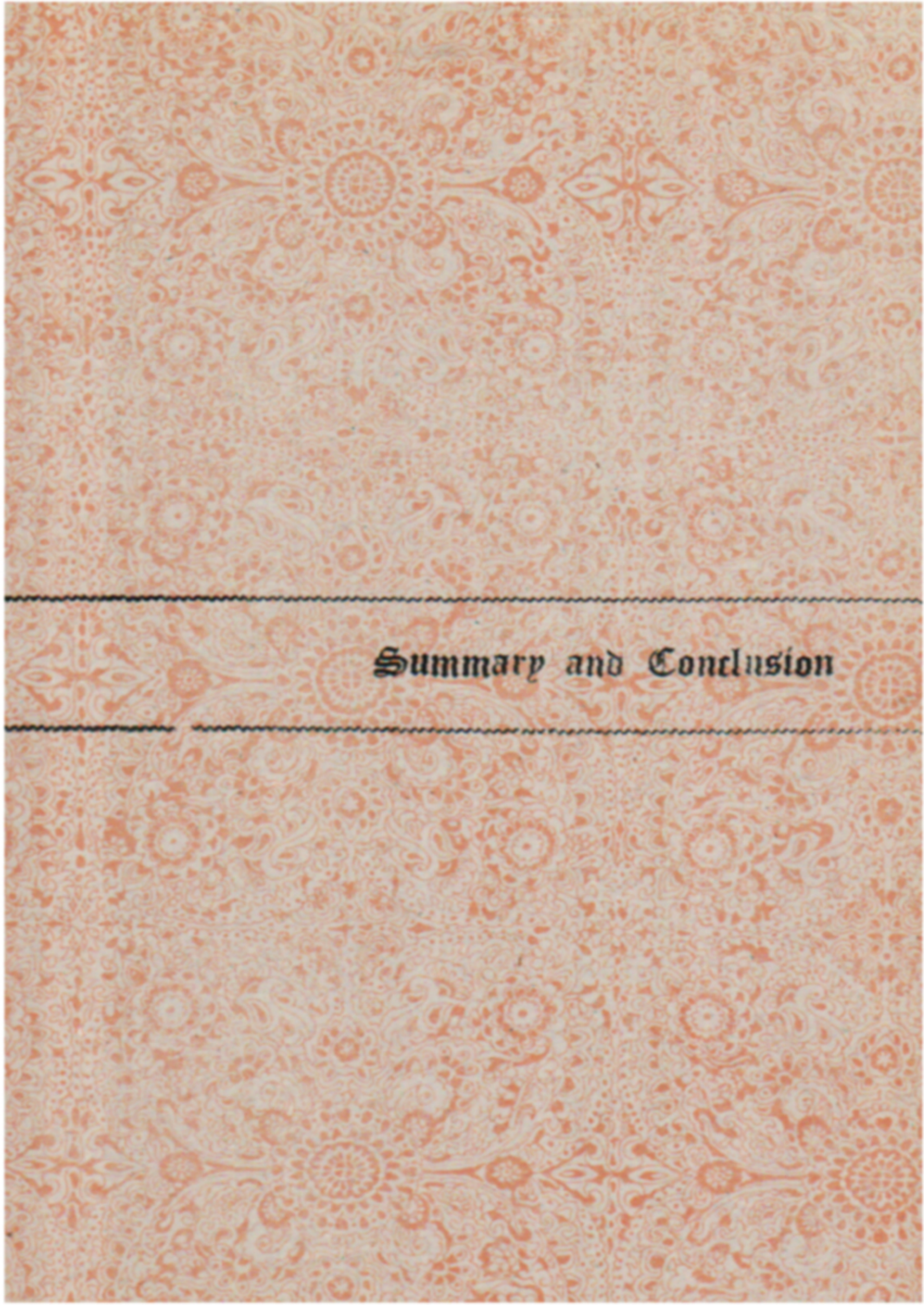
Table 3. Phenolic compounds of various cultivars
in safflower seed meal in tannins form

Sr. No.	Cultivars	Tannins (%)
1.	Bhima	0.676
2.	A-1	0.632
3.	HUS-305	0.636
4.	A-300	0.609
5.	K-1	0.528
6.	Manjira	0.554
7.	JLSF-88	0.724
8.	CO-1	0.584
Mean		0.617
S.E. $\pm$		0.0061
C.D. at 5%		0.019

The tannin content is concentrated in the seed meal (Raj et al. 1983). The tannin content of the safflower cultivars ranged from 0.528 to 0.724%. The highest was in JLSF-88 (0.724%), closely followed by Bhima (0.676%). The values recorded here are in agreement with the earlier reports (Raj et al., 1983).

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Summary and Conclusion

5. SUMMARY AND CONCLUSIONS

Safflower is one of the most important oilseed crops of India grown in arid and semi-arid zones. India produces more than 53% of world's safflower. The normal hull type safflower contains higher amount of hull. New cultivars are being developed to reduce hull and increase kernel contribution. However, the information of these cultivars is meagre. Almost all safflower is crushed for oil and the cake remaining after oil extraction is fed to cattle. Though the cake is a rich source of protein, it can not be used for human consumption as it contains high amount of fibre and bitter compounds. The oil is the richest source of linoleic acid, an essential fatty acid. Because of higher degree of unsaturation, safflower oil undergoes degradation faster than any other edible oil during storage. It is, therefore, though necessary to know the proximate composition, oil content, protein quality and oil quality of safflower seeds of some promising cultivars. The results obtained are briefly summarised in this chapter.

1. Significant differences in crude fat and crude protein contents of seeds among different safflower cultivars were noticed. The crude fat and crude protein ranged from 29.16 to 32.67 and 10.50 to 18.39 %, respectively.

2. Significant differences in crude fibre and total sugars were observed with a range of 37.65 to 45.00 and 2.61 to 4.92 %, respectively.

3. The safflower meal contained phenolic (tannin) compounds to the extent of 0.528 to 0.724%.

4. The safflower proteins were deficient in an essential amino acid, methionine. The methionine content ranged from 1.02 to 1.76 g/16g N while the tryptophan content varied from 1.11 to 1.50 g/16g N.

5. There were significant differences in the acid value and peroxide value which ranged from 0.391 to 0.626% and 2.40 to 3.66 meq/kg oil, respectively. These values were nutritionally under safe limits.

6. The iodine value of safflower oil ranged from 134.18 to 145.93 and the saponification value varied from 186.25 to 190.32.

The results of this study indicated that the oil and protein quality parameters were under nutritionally safe limits.

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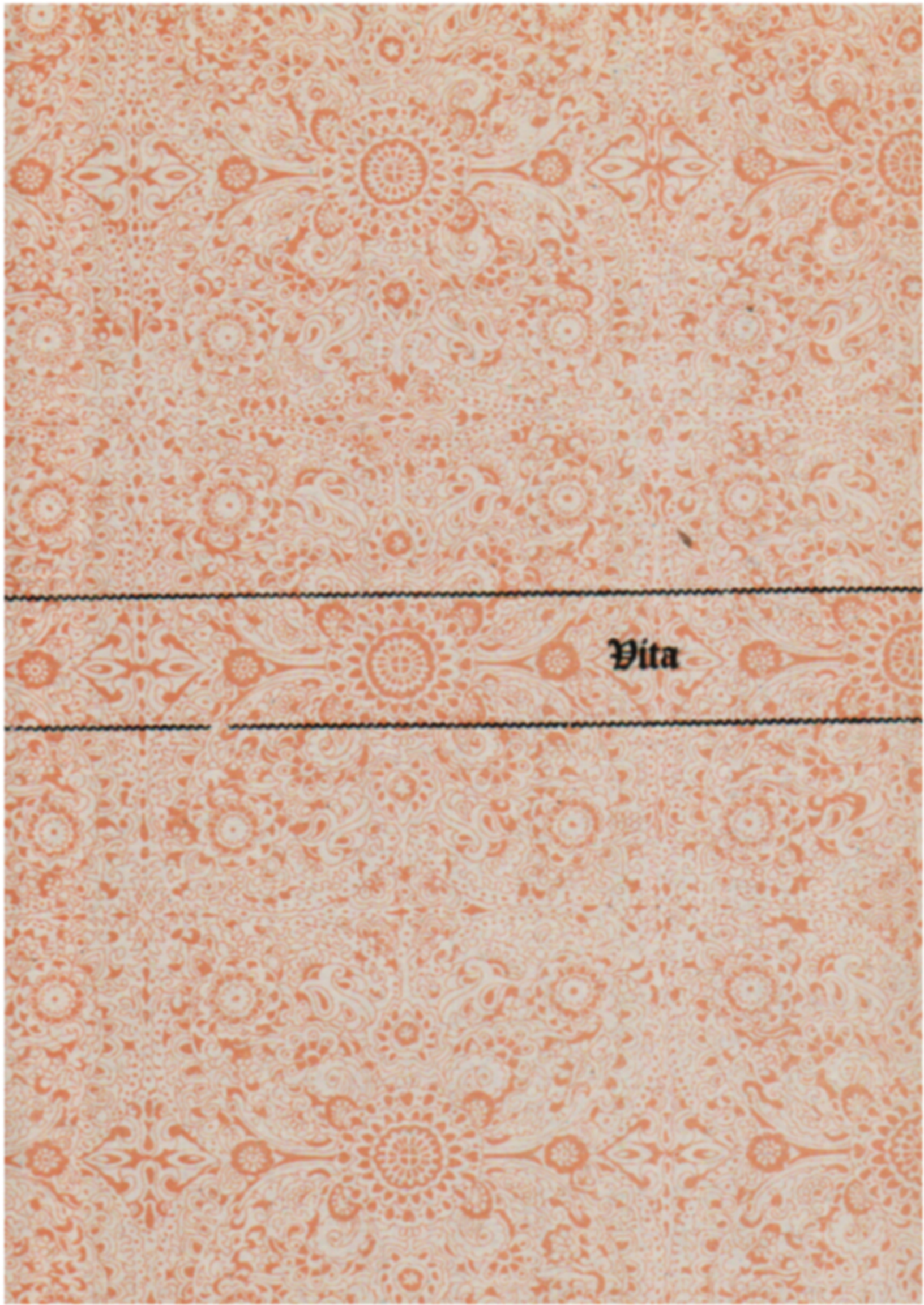
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7. VITA

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