

EFFECT OF INCUBATION TEMPERATURE AND pH ON *L. LACTIS* SSP *DIACETYLACTIS* AND THEIR VIRULENT PHAGES

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ABSTRACT

Two virulent phages HTK₁ and HTK₂ of *Lactococcus lactis* sub ssp *diacetylactis* isolated from indigenous dahi whey were examined for their activity at different pH and temperatures with respect to their host. It was observed that the lysis activity of both the tested phages varied with the tested pH values. Complete lysis of bacterial cultures was recorded after 2 hours at pH 6.5 in the case of phage HTK₁, while no lysis was observed at same pH in case of phage HTK₂. The effect of temperature on phage lytic pattern and titers was also monitored. It was observed that temperature at which complete lysis of host strain occurred by some phages was different from the temperature at which maximum phage titre was observed.

INTRODUCTION

Lactic acid bacteria are widely used for the preparation of different dairy products in which they are responsible for the development of acidity and characteristic flavour. For successful fermentation, these micro-organisms must produce acid rapidly. Bacteriophage infection of these cultures commonly prevent fermentation, resulting in great economic loss to the industry.

Different studies on various aspects of phages including morphological and structural characterization, host phage interaction, effect on lactic fermentation and product quality and genetic studies have been carried out in order to combat phage attacks in the dairy industry (Rhimani and Frieds, 1993; Catherine *et al.*, 1994).

No detailed study on the phages isolated from dahi is available despite their significance to the dairy industry. The present study was undertaken to compare the isolated phages HTK₁ and HTK₂ at different pH and temperatures with respect to their host.

MATERIALS AND METHODS

The bacteriophages used in this study were originally isolated from indigenous dahi whey samples. The genetic homogeneity of each phage stock was ensured by isolation twice of single plaques. *L. lactis* sub ssp *diacetylactis* was used as propagating strain for these phages at 37°C in M-17 broth (Difco). M-17 broth was further supplemented with 20m M Ca Cl₂ for phage propagation. Phage propagation, concentration and purification were carried out as described previously (Rizwana, 1994).

Effect of pH and Temperature on Bacterial Growth and Phage Infectivity

The M-17 medium was adjusted to different pH values namely: 6.5, 6.7, 7.0 in separate flask (50ml). Each flask was inoculated with 1 ml of overnight grown bacterial culture (*Lactococcus lactis* ssp *diacetylactis*) at 37°C incubated on a shaking incubator for two hours. The growth was recorded by using SHIMADZU Spectrophotometer. After two

hours 500 μ l 1M CaCl_2 was added in all flasks except those which were used for studying bacterial growth at different pH. Then after adding 500 μ l 1M CaCl_2 1 ml of each phage used in this study was added in the flask for each pH value. Reading from Spectrophotometer, at 650nm, for 6 hours were taken. Reading for bacterial growth at different pH values 6.5,6.7,7.0 were also recorded at 650nm by using same Spectrophotometer. Same procedure was applied in order to study temperature effect on bacterial growth and phage infectivity. Different temperature values were 32,37 and 42°C. pH value was 6.5 as it is recommended for M-17 media. (Metalon and Sandine, 1986).

RESULTS AND DISCUSSION

The starter culture for production of dahi is a heterogenous mixture of lactic acid bacteria. However, there is a large variation in the quality of the final product because crucial parameters such as milk quality, starter composition and the quality and quantity of inoculum and incubation temperature during the preparation are not appropriately monitored. This may add to the chances of starter culture failure due to phage contamination. However, the effects of phage contamination may not necessarily results in complete batch failure or an inferior product quality, because of the practice of using multiple strain starters.

Bacterial strain *L. lactis ssp diacetylactis* was grown in M-17 adjusted to different pH values i.e pH 6.5,6.7 and 7.0. It was observed that bacteria showed maximum growth at pH 7.0 as compared to growth of bacteria at pH 6.7 and 6.5 as indicated by OD values (Fig. 1). Culture at pH 7.0 became turbid after 3 hrs, while at pH 6.7 low turbidity was observed after 4 hrs. In culture at pH 6.5 no turbidity was observed although OD value increased. These results showed that bacteria grow well in medium adjusted to pH 7. It could, therefore, be concluded that pH affected bacterial growth. These results are in line with the findings of Catherine *et al.*, 1994. They reported that *Lactobacillus delbrueckii ssp bulgaricus* and *S. salivarius ssp thermophilus* grew well at pH 7 at 37°C than at pH 4.6. Similarly Furtado *et al.* (1990) described that *S. salivarius ssp thermophilus*

showed maximum growth rate at pH 7 at 42°C that at pH 5.5 at 42°C. This study, however, was based on monitoring the effects of pH and temperature conditions. In this study effect of pH on phage lytic pattern and titers was monitored using two phages HTK₁ and HTK₂. Phage HTK₁ caused lysis of host strain after two hours of phage inoculation. Phage titre of 10^{11} PFU/ml was observed. In culture at pH 7 lysis started after 3hours of phage inoculation but lysis was slow and culture was not cleared even after 4 hours of phage inoculation. Phage titre at pH 7 was 10^{12} PFU/ml (Fig.2).

Fig. 1 - Spectrophotometric analysis of bacterial culture (*Lactococcus lactis ssp diacetylactis*) at different pH

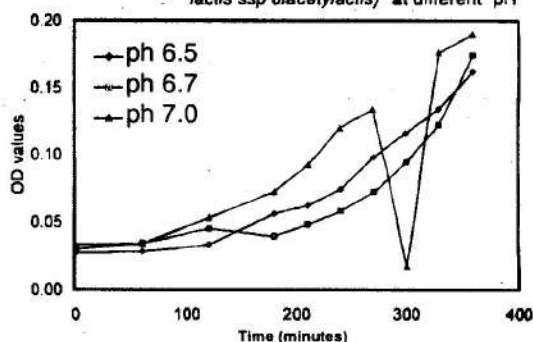
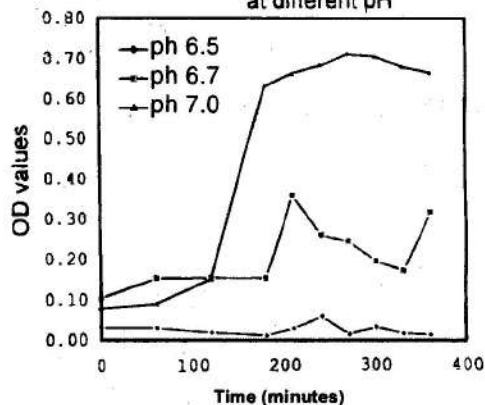


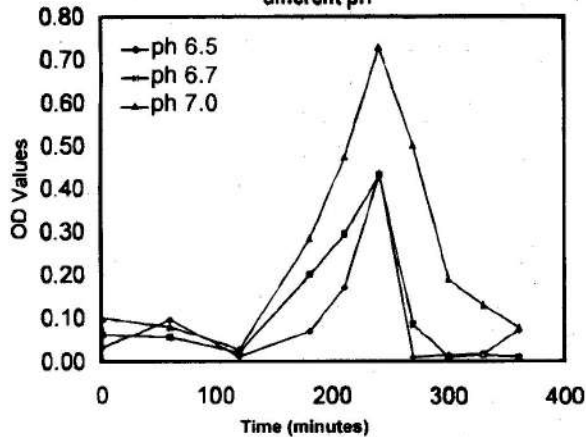
Fig. 2 - Spectrophotometric analysis of bacteriophage HTK-1 at different pH



On the other hand phage HTK₂ showed no lysis at pH 6.5 when low titre phage (10^8 PFU/ml) was observed, however with this titre lysis occurred in culture at pH 6.7 and pH 7. When titre was increased 10^{11} PFU/ml lysis was observed in culture at pH 6.5 after two hours and at the end phage titre

was 10^{11} PFU/ml (Fig. 3).

Fig. 3 - Spectrophotometric analysis of bacteriophage HTK-2 at different pH



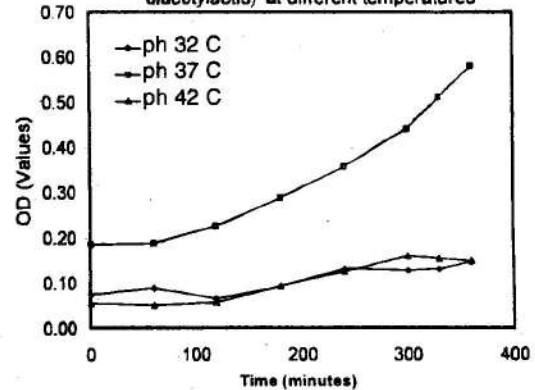
In culture at pH 6.7 lysis was compared after 3 hours of phage inoculation and phage titre of 10^{12} PFU/ml was observed. In culture at pH 7.0 turbidity of culture was lost due to lysis after 4 hours of phage inoculation and phage titre of 10^{13} PFU/ml was observed.

With the help of above information, it could be assumed that maximum phage titre is observed in culture at pH 7. Similar views were expressed by Rizwana. (1994) who observed that phages showed maximum titre at different pH and complete lysis of host strain occurred at different pH. It may be suggested that pH values affect the adsorption and lysis by delaying these process. These results are in accordance with the finding of Rhimani and Freids (1993). They reported that the host interactions of Lactococcal phages FRC1, FRC2, FRC3 and FRC4 were maximum at pH 7, 7.2 and 7.6 after 10 minutes. On the other hand Sijtsma *et al.* (1991) determined that when *Lactococcus lactis ssp cremoris* cell wall was subjected to low pH and high temperature, the strain losted its ability to resist phage attack. Furtado *et al.* (1990) observed that lactic acid bacteria at low pH value 5.5, 5.6 and 5.8 showed low sensitivity to phages.

Wu *et al.* (1990) used 4 subtypes of *Bacillus thuringiensis* and their 4 types of phages were studied and it was observed that these phages showed stability at pH 5 to pH 8 and were inactive at pH 3 and pH 11 to 12. The tested bacterial strain *L. lactis ssp*

diacetylactis was grown at three different temperature values i.e. 32°, 37° and 42°C. It was observed that bacteria grow better at 37°C as compared to growth at 32°C and 42°C as indicated by OD values (Fig. 4).

Fig. 4 - Spectrophotometric analysis of bacteria (*Lactococcus lactis ssp diacetylactis*) at different temperatures



Autolysis of *L. lactis ssp diacetylactis* was observed at 32°C after one hour of bacterial inoculation. Autolysis of host strain was not observed at 37°C and 42°C. These results are in line with the observations of Lortal *et al.* 1991; Mou *et al.* 1976 and Vegrud *et al.* 1983, that Lactococcal cells autolyzed spontaneously when they are transferred from M-17 broth to a buffer solution. The influence of temperature on autolysis of 14 Lactococcal strains in broth was studied by Vegrud *et al.* (1983).

In another study with *S. salivarius ssp thermophilus* it was observed that growth rate increased as temperature was increased. Growth rate at 42°C was 1.42 at 340nm, while growth at 42°C was 1.33 at 340nm (Furtado *et al.*, 1990). Goldberg *et al.*, (1994) observed that *E. coli* growth was inhibited when temperature was raised from 37°C to 41°C.

The effect of temperature on phage lytic pattern and titers was monitored by two phages which included HTK₁ and HTK₂ (Figs. 5,6). From the results, it was observed that temperature at which complete lysis of host strain occurred by some phages was different from the temperature at which maximum phage titre was observed. This effect of temperature may be due to the fact that temperature may delay phage-adsorption or affect phage development. It was

further observed that phage-adsorption restriction-modification and development were found to be temperature sensitive, permitting full lytic development at 40°C in contrast to greatly restricted phage development at 30°C (Chopin *et al.*, 1976). In another study on *Streptococcus cremoris* strains and its bacteriophages, it was observed that at 38°C, the titre of disturbing phage was 10¹ to 10⁴ times greater than observed at 25 to 30°C and that transition from reversibly adsorbed to irreversibly adsorbed phage occurred more rapidly on host bacteria grown at higher temperature (Eddy and Hull, 1987).

Fig. 5 - Spectrophotometric analysis of bacteriophage HTK-1 at different temperatures

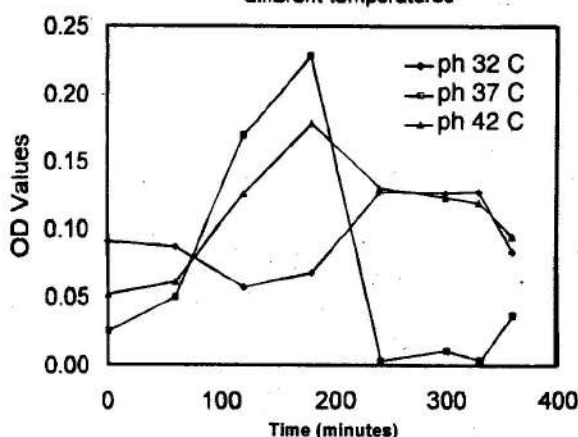
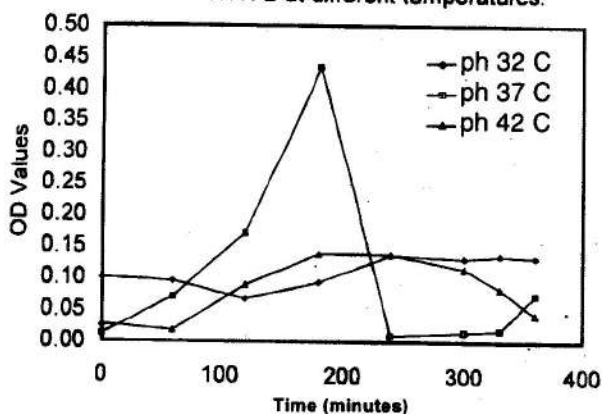


Fig. 6 - Spectrophotometric analysis of bacteriophage HTK-2 at different temperatures.



In a study of 19 phages from *L.lactis* and *S.cremoris* (Keogh, 1973), it was observed that at higher temperature 37°C latent period was decreased but the effect on the burst size was variable.

In another work by Mary and Klaenhammer

(1984) it was observed that when *S.lactis* ME2 a dairy strain that is insensitive to variety of phage including ϕ 18. The EOP of ϕ 18 on ME2 could be increased from $<1 \times 10^{-9}$ to 5.0×10^{-2} when host culture was sub cultured at 40°C before plating phage and phage assay plates were incubated at 40°C. Then at 30°C phage adsorption also increased at 40°C than at 30°C phages showed shorter latent period and larger burst size at 40°C than at 30°C after 4 hours of phage inoculation and phage titre of 10¹³ PFU/ml was observed.

CONCLUSION

On the basis of these studies it is concluded that pH and temperature conditions do affect bacterial growth and phage infectivity and development. Bacteria grew better in media at pH 7.0 as compared to other tested pH. Most of these phages showed earlier lysis of host bacteria in cultures at pH 6.5 and maximum phage titre was recorded at pH 7.0. In case of temperature bacteria showed better growth rate at 37°C as compared to 32°C and 42°C respectively.

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**PRODUCTION OF α -AMYLASE AND GLUCOAMYLASE
ENZYMES BY *ASPERGILLUS NIGER* IMMOBILIZED
WITHIN THE VEGETABLE SPONGE OF
*LUFFA CYLINDRICA***

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ABSTRACT

Production of amylolytic enzymes such as α -amylase and glucoamylase with *Aspergillus niger* hyphae immobilized within vegetable sponge of *Luffa cylindrica* was studied in batch and repeated batch cultures. Immobilized hyphae produced these extracellular enzymes in batch culture in amounts as were produced by free hyphae. In the repeated batch culture studies it was noted, however, that whereas free hyphae can be used for only two to three 5-day batches, the extracellular enzymes production by immobilized hyphae was maintained, without any significant difference, in successive batches during the 5-day 10 repeated batch cultures. Advantage of the immobilized fungal hyphae system over free suspension mycelium for enzyme production is discussed.

INTRODUCTION

Numerous studies have been reported on the development of techniques for efficient production of enzymes as industrial biocatalysts (Linko *et al.*, 1994). Screening, mutation and genetic engineering of microbes are among the tools used for the purpose. Production methodology of microbial enzymes has, however, basically remained the conventional submerged batch and solid-state surface cultures (Battaglino *et al.*, 1991; Pandey, 1992; Tsekova *et al.*, 1992; Sudo *et al.*, 1993). Immobilized biocatalyst technology has only recently been applied in the production of extracellular enzymes, taking advantage of the ability of microorganisms to secrete them while immobilization methods presently available, entrapment of biocatalysts within a porous polymeric matrix has wide applicability for microbial, plant and animal cells (Fukui and Tanaka, 1984). The entrapment media most often used are gels such as agar, Ca-alginate, K-carrageenan, and polyacrylamide (Li *et al.*, 1984; Chevalier and de la Noue, 1987; Kuek, 1991). However, their insufficient mechanical and chemical stability often limits the application and lifetime of the immobilized biocatalyst systems (Cheetham *et al.*, 1979; Wada *et al.*, 1980; Boyaval and Goulet, 1988). As an alternative to gels, a natural

fibrous network of plant origin (Iqbal and Zafar, 1994) has been successfully used for the immobilization of *Aspergillus niger* hyphae and used for the production of amylolytic enzymes. The results of these studies are presented.

MATERIALS AND METHODS

Growth medium for the immobilization of a locally isolated strain of *Aspergillus niger* consisted of: (g/l) starch, 10; $(\text{NH}_4)_2\text{HPO}_4$, 2; and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.25 in distilled water (pH 5.0). The enzyme production medium comprised of 5% soluble starch, 1.5% peptone, 1% yeast extract, 0.05% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.5% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, pH 6.0 at 28°C. The vegetable sponge, obtained from matured dried fruit of *Luffa cylindrica* was used as the entrapment matrix. The vegetable sponge was separated from soft tissues of the dried fruit and prepared for immobilization as described earlier (Iqbal and Zafar 1993). *A. niger* was precultured from test tube slants by inoculating in 250 ml Erlenmeyer flasks containing 70 ml growth medium and incubated at 30°C for 3-4 d on a rotary shaker (100 rev min⁻¹). The hyphal mass was separated as a pellet by centrifugation at 5000 rev min⁻¹ for 20 min. The pellet was rinsed with sterile distilled water,

resuspended in fresh culture medium containing glass beads (4.5-5.5 mm dia), and shaken in a rotary shaker (300 rev min⁻¹) for 30 min to provide a fragmented hyphal suspension. One ml of the suspension was inoculated in 250 ml Erlenmeyer flasks containing 70 ml culture medium and two pre-weighed reticulated vegetable sponge pieces (approximately 16x 18x3.5 mm) as the entrapment matrix. Culture flasks with no vegetable sponge piece in the medium served for free hyphal growth. The inoculated flasks were incubated at 30°C and shaken at 100 rev min⁻¹ for 24 h. The sponge pieces were removed from the flasks after 24 h and washed thoroughly with fresh culture medium to remove the untrapped free hyphal fragments. Hyphal immobilization was also observed microscopically. The sponge pieces with the entrapped hyphae were then transferred to 70 ml fresh medium contained in 250 ml Erlenmeyer flasks and incubated at 30°C on a shaker (100 rev min⁻¹) for subsequent studies on biomass and the production of biomass and amylolytic enzymes in the free and immobilized states.

For the determination of biomass, immobilized hyphae were washed with distilled water and oven-dried at 80°C to constant weight. Extracellular amylolytic enzymes, α -amylase and glucoamylase, released in the medium were determined according to Sudo *et al.* (1993) and Ozawa *et al.* (1994), respectively.

RESULTS AND DISCUSSION

Vegetable sponge of *Luffa cylindrica* is a unique open netted structure and provides very strong mechanical support for the immobilization of living cells (Iqbal and Zafar, 1994). Microscopic examination revealed that hyphal entrapment within the vegetable sponge occurred in 24 hours. The immobilized system was, thereafter, subcultured in fresh medium in batch culture for 5 days. Complete coverage of the vegetable sponge pieces with the hyphae of *A. niger* with 2-3 days. Biomass accumulation/loading, however, was noted to continue until the attainment of stationary phase at day 5. For determining the effect of immobilization on the growth pattern and the extracellular enzyme release of *Aspergillus niger*,

biomass production and enzyme activities of immobilized cultures were compared with those of free suspension cultures. Two parallel runs, each with 12 shake flasks, were carried out, all starting with the same initial conditions. Two shake flasks were harvested at each sampling time, everyday for 6 days.

Biomass yield, and the production of α -amylase and glucoamylase enzymes by *A. niger* free suspension cultures are respectively presented in Figs. 1 and 2 a. A continues increase in biomass was

Fig. 1- Growth of *Aspergillus niger* in batch culture

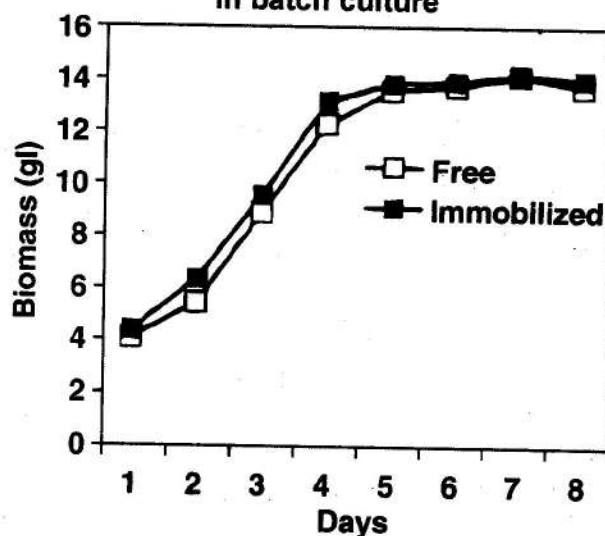
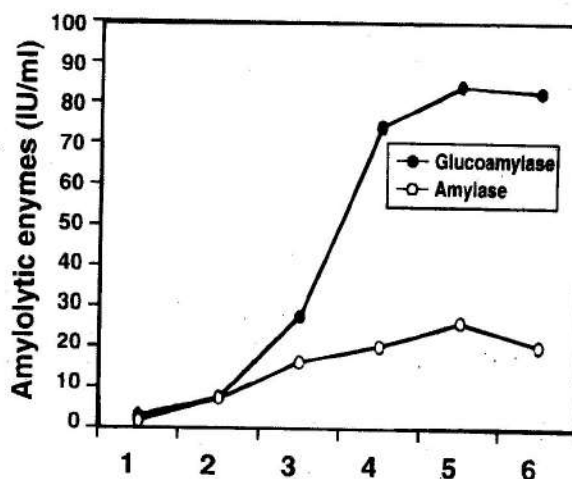


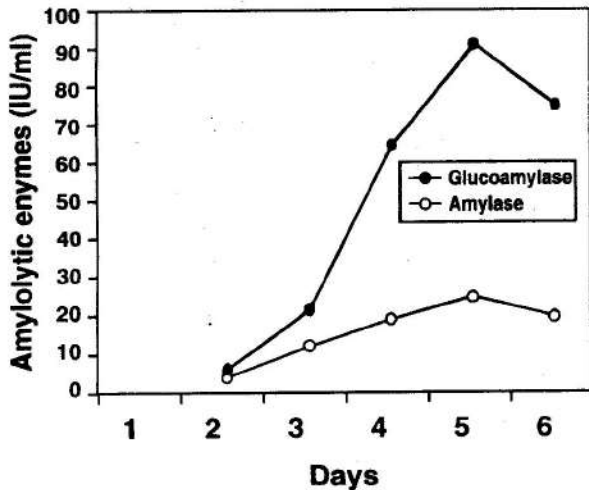
Fig. 2a- Amylolytic enzyme production by free *Aspergillus niger* hyphae.



observed upto 4 day of batch culture, after which fungal growth entered into stationary phase. Enzyme

activity of both α -amylase and glucoamylase, however, continued to increase for 5 days. The highest α -amylase and glucoamylase activities at this stage were found to be 26.1 IU/ml and 84.9 IU/ml, respectively. 6-day old cultures recorded a sharp decrease (22.6%) in α -amylase activity whereas no change in the level of glucoamylase activity was observed. It may be noted that the maximum level of activity of the two enzymes corresponded with the onset of stationary phase of growth. The same observation was recorded with immobilized hyphae, which also showed highest level of activities of these enzymes at this stage of growth on day 5 (Fig. 2b).

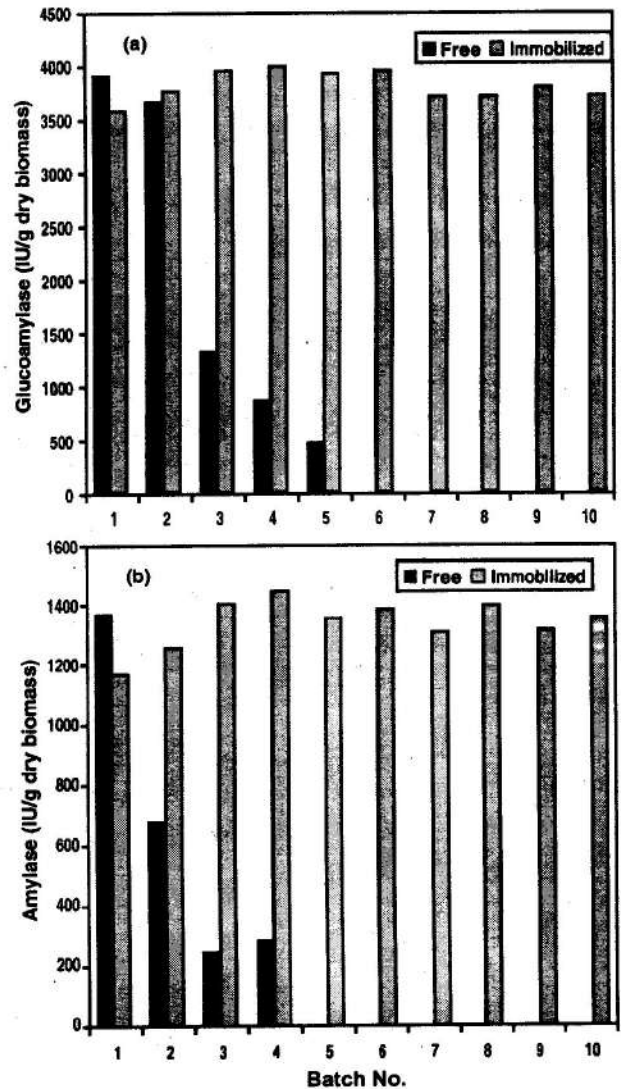
Fig. 2b- Amylolytic enzyme production by immobilized *Aspergillus niger* hyphae.



Enzyme production by immobilized hyphae showed maximum activity of 24.85 and 90.5 IU/ml, respectively, for α -amylase and glucoamylase at 5 days of fermentation, decreasing sharply thereafter.

Amylolytic enzymes production during repeated 5-day batch fermentations both for free and immobilized systems are presented in Fig. 3a. It is interesting to point out that with free hyphae both α -amylase and glucoamylase activities decreased drastically after the first and second batch, respectively, approaching zero in the next few batches. Fig. 3b. With the immobilized biocatalyst on the other hand, the level of enzyme production was maintained throughout the 10 consecutive batches investigated for 50 days. The volumetric productivity (Q_p) of the amylyolyti enzymes

Fig. 3- Production of (a) glucoamylase, and (b) amylase in repeated 5-day batch culture by free and immobilized hyphae of *Aspergillus niger*.



α -amylase and glucoamylase from *A. niger* hyphae immobilized within vegetable sponge were found to be comparable with those of free suspension cultures (Table-1). Maximum levels of enzymes production,

Table-1:- VOLUMETRIC PRODUCTIVITY (Q_p) OF α -AMYLASE AND GLUCOAMYLASE BY FREE AND IMMobilized ASPERGILLUS NIGER HYPHAE.

Culture Conditions	α -Amylase (IU.l ⁻¹ .h)	Glucoamylase (IU.l ⁻¹ .h)
Free Hyphae	83.3	216.7
Immobilized hyphae	82.0	218.0

furthermore, noted after the cessation of growth at stationary phase (Figs 02. 2a & b) suggest that it is possible to produce these enzymes on a continuous basis by immobilizing the biocatalyst within vegetable

sponge and replacing the growth medium with the growth limiting enzyme production medium.

A major advantage that the immobilized hyphae present is their ability to maintain these levels in numerous repeated batch cultures without any loss of metabolic activity related with extracellular amyolytic enzymes production, which contrarily drops to zero after a few repeated batches when *A. niger* was cultured in free suspension.

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STUDIES ON STORAGE OF ONION UNDER DIFFERENT STORAGE CONDITIONS

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ABSTRACT

Post harvest losses of onion occurring at various stages are enormous. Two onion varieties, namely, Red Desi and Phulkara were stored in mesh trays and cold storage chambers with controlled humidity and temperature. Data were recorded for weight loss and rotting percentage upto 105 days. It was found that weight loss and rotting were on higher side under ambient conditions i.e. 54 - 57 % and 14 - 15 %, respectively. In case of mesh trays weight loss was 28 - 30 % and rotting 9 - 13%, while under controlled temperature and humidity conditions the weight loss and rotting percentage was 11 - 13% and 1 - 2% respectively. Thus there is a need to study the shelf life of onion under different storage conditions in order to reduce the post harvest losses.

INTRODUCTION

Onion (*Allium cepa*) is the main crop in the genus *Allium*. It has unique characteristics as "onion-like" flavour and taste, onion is being consumed as human diet all over the world. It is mainly used in cooking and also eaten as raw, baked, french fries and in the form of pickle. It can also be dried or shredded into flakes. It is being grown in all provinces of Pakistan with the production of 1076.5000 tons in which Punjab's share is 22.7000 tons (Agri. Stat. of Pakistan, 1997-98). It has a great export potential but due to lack of post harvest techniques we could not get bloom in this business. It was observed that improper harvesting and storage techniques increased its post harvest losses (upto 35% of the total produce). During peak harvest season prices are lowered due to the glut in the market. To overcome this problem and to make the supply regular at reasonable prices storage of produce is necessary (Bhatnagar *et al.*, 1989). Storage methodology has great impact on the quality of the onion (Guer-Cahuzac, 1996). Keeping in view the said problems studies were conducted on storage of onion.

MATERIALS AND METHODS

Two onion varieties "Red Desi" and "Phulkara" were selected for this experiment. The crop

was harvested when about 25% tops were broken over (Salunkhe and Desai: 1984). After the manual harvesting and removal of the dried tops from bulbs, onions were thoroughly cured for 8 -10 days at ambient temperature at about 35° to 40°C (Maw *et al.*, 1997). After the completion of the curing period, bulbs were placed under storage conditions as following:

1. Primary storage
 - i. Conventional method (spread on ground)
 - ii. Mesh trays
2. Low temperature storage

RESULTS AND DISCUSSION

Data in respect of rotting and weight loss were recorded on fortnightly intervals for the storage period of 105 days. This study was conducted for three consecutive years (1994 - 95 to 1996 -1997). At ambient storage and mesh trays rotting and weight losses just started after the storage of 15 days in both the varieties. The maximum rotting 14 - 15% and weight loss 54 - 57% was observed at ambient stored bulbs in case of onions spread on the ground. (Figs. 1 to 4). It is evident from the given figures that weight loss in both varieties ranged from 28 - 30% during the storage of bulbs in the mesh trays, which was half as compared to conventional method. Rotting

Fig. 1- Effect of Treatment of Weight Loss % of Onion cv.red. Desi During Storage

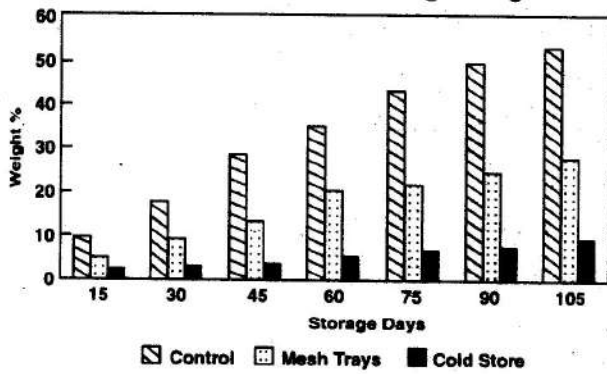
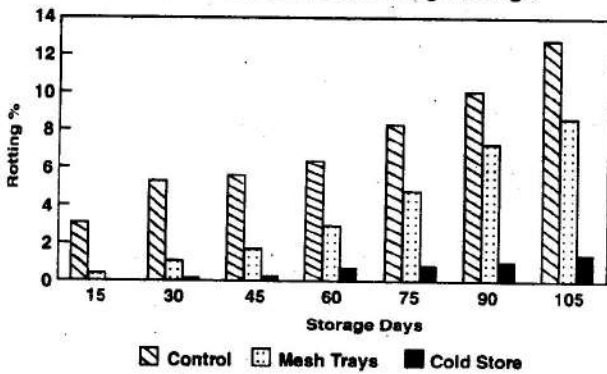


Fig. 2- Effect of Treatment on Rotting % of Onion cv.red Desi During Storage



in onion was 9 - 13% which is also on the lower side as compared to the conventional method. In case of low temperature storage at 0°C with 60 - 70% relative humidity (Tanaka *et al.*, 1985), rotting was only 1 - 2% which was seven times less as compared to conventional method and mesh trays storage. Weight loss was only 10 - 13% in both varieties which was 3 - 4 times less compared to other two methods of storage.

Fig. 3- Effect of Treatment on Rotting % of Onion cv.Phulkara During Storage

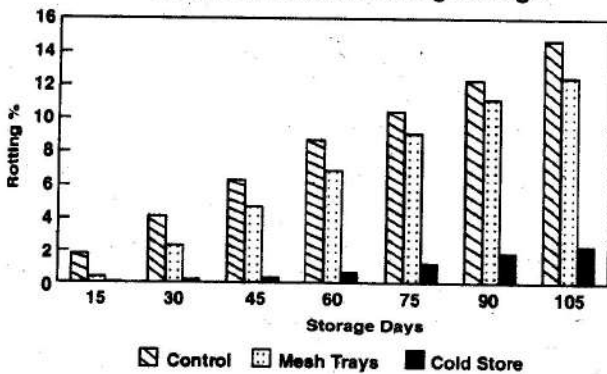
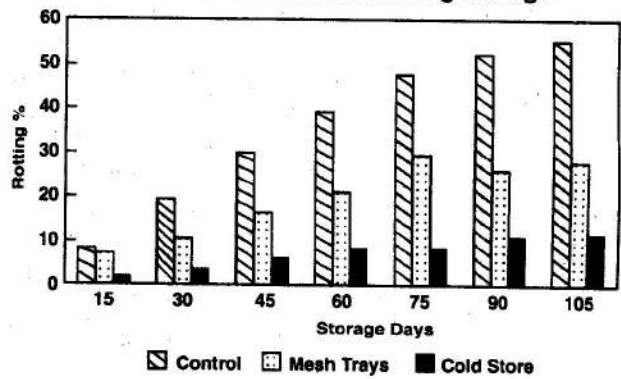


Fig. 4- Effect of Treatment on Weight Loss % of Onion cv.Phulkara During Storage



Low temperature proved better for onion storage than the other two conditions i.e. conventional (spread on ground) and mesh trays. The effect of storage methods was similar for each variety. The experiment was laid out in a RCBD with three replications. All the data were subjected to analysis of variance and means were compared by LSD test and results were found highly significant. (Tables 1, 2).

Table-1:- WEIGHT LOSS % DURING STORAGE FOR 105 DAYS

Treatments	Varieties		
	Red Desi	Phulkara	Means
Conventional	37.32a	34.48a	35.90a
Mesh Trays	20.10b	18.05b	19.08b
Low Temperature	08.05C	05.84C	06.95C
Means	21.82A	19.46B	

* LSD value for treatment 0.30
 * LSD value for varieties 0.24

Table-2:- ROTTING % DURING STORAGE FOR 105 DAYS

Treatments	Varieties		
	Red Desi	Phulkara	Means
Conventional	8.32a	8.01a	8.46a
Mesh Trays	7.24b	4.09b	5.66b
Low Temperature	1.08C	0.80C	0.94C
Means	5.7A	4.3B	

* LSD value for treatment 0.21
 * LSD value for varieties 0.17

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EFFECTS OF CULTURAL CONDITIONS ON THE LIPID PRODUCTION BY *RHODOTORULA RUBRA*

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ABSTRACT

Rhodotorula rubra was used to produce lipids of microbial origin. Cultural conditions related to oxygen supply, carbon source, pH of the medium and temperature were controlled. The possible contributing factors for high lipid content of *R. rubra* were examined. Among different carbon sources used glucose proved to be the best yielding 20.2% lipids while maltose and xylose gave 14.0% and 12.0% respectively. Due to certain varying conditions, the organism *R. rubra* modifies its biosynthetic capabilities resulting in an increase or decrease in the degree of unsaturation of its lipids. TLC results revealed that the major components of the total lipids were triglycerides, which enhanced in nitrogen limiting culture. The overall fatty acid composition of the lipids as studied by Gas Liquid Chromatography showed that there was an increase in the polyunsaturated acids C18:2 and C18:3 and a decrease in saturated C16:0 C18:0 and mono unsaturated C18:1 with increase in the growth rate.

INTRODUCTION

Microbial lipid production has long been a subject of research interest to industry. In recent years the energy crisis has triggered a search for in-expensive and less expensive raw materials from which energy can be derived. In this context both micro-organisms and waste materials have been considered for effective utilization. Research into the strain isolation of higher lipid producing microorganisms, the fermentation technology and the characterisation of microbial lipids has been carried out ever since the microbial production of lipids was considered as an alternative to conventional lipids from plants and animals.

Several yeasts and moulds are known to produce a large proportion of their biomass (upto 7% as lipids) Rattray *et al.*, 1975. To elucidate their unique oleaginicacy a number of fermentation and biochemical studies have been carried out and lipid accumulation mechanism in oleaginous yeasts has now been established to a limited extent.

Investigations on the lipid contents of *Rhodotorula gracilis* were first carried out by Steinberg (1954) and later by Enebo and Iwamoto

(1966) and Kassel (1968). All of these experiments were carried out in batch cultures but only Kassel consistently used a fermenter with abilities to control all of the relevant parameters during growth. Krumphanzl, *et al.* (1973) studied *R. gracilis* by growing it in a medium containing glucose or ethanol (Ratledge and Hall, 1977). The continuous cultivation of *Rhodotorula glutinis* was first performed by Ratledge and Hall (1977) to study the oxygen demands under nitrogen and carbon limited conditions. Despite the significant technological advancement of microbial lipid production with *R. glutinis* achieved in recent years, further research is required before practical applications of the production technology can be fully realised.

In the present work *Rhodotorula rubra* was used to find out any possible utilization of the lipid forming capacity. Keeping in view that work done so far it is of interest to evaluate the relationship between growth lipid productivity and other process parameters considered important for its microbial lipid production.

MATERIALS AND METHODS

Rhodotorula rubra was grown on a

medium of the following composition.

CaCl ₂ 6 ₂ 0	=	0.02%
MgSO ₄ 7H ₂ O	=	0.15%
KH ₂ PO ₄	=	0.7%
Na ₂ HOP ₂	=	0.2%
Yeast Extract	=	0.05%
(NH ₄) ₂ SO ₄	=	0.1%
Glucose	=	3.5%

Preliminary studies revealed that 0.1% was the growth limiting concentration of (NH₄)₂ SO₄ in the presence of 0.05% yeast extract. The pH was adjusted to 5.5 with 2N HCl or 2N NaOH before sterilization. The medium was sterilized by autoclaving. The glucose solution was sterilized separately and aseptically mixed with the salt medium before cooling.

Erlenmeyer flask (500 ml) containing 100 ml of the medium were inoculated from a culture slant and incubated for 60 hrs. at 30°C in a controlled incubator shaker.

A batch culture, in 1L flasks each containing 300 ml of the medium, was incubated with a 5% v/v inoculum obtained from shake culture.

Analytical Methods. Sample were removed at 24 hrs. intervals for biomass assay and the sugar consumed. The total and reducing sugars in the fermenting media were determined by the method of M. Somgyi as modified by M. Younas⁷. Residual ammonium nitrogen was determined by modified micro-kjeldahl method⁸.

Lipid Extraction. The dried cells were grounded with sand in a mortar and lipids were extracted with a solvent mixture (methanol, chloroform (1,2 w/v)) as described earlier⁹. The crude lipid extract was purified by the Folch *et al.*, (1975) washing method.

Thin Layer Chromatography. In carrying out TLC the precoated silica gel plates (20 cm x 20 cm x 0.25 u thickness) were used and the solvent systems were as follows:

Neutral Lipids = Petroleum ether/diethyl ether/acetone (100 : 15 : 1 v/v/v),

Phospho-lipids = Chloroform/methanol/water/18.4% NaOH 130 : 70 : 8 : 0.5 (v/v/v/v).

Gas Liquid Chromatography. The purified lipids, as obtained above, were then saponified to liberate the fatty acids. Esterification of the acids was achieved by reaction with a methanol benzyl chloride and acetyl chloride mixture.

The identity and percentage of the fatty acids was determined by the gas chromatographic analysis with the PEGS column at 210°C. Injector and detector temperatures were 200°C and 250°C respectively. Nitrogen was used as carrier gas with a flow rate of 40 ml per minute.

RESULTS

Effect of Temperature on the Lipid Production.

Different incubation temperatures viz. 20°, 25° 30°, and 40°C were used and the results indicate that highest yield of the dry weight lipid of *R. rubra* were obtained at 30°C. (Table-1). Change in the temperature above or below 30°C showed marked decrease in the above mentioned determination.

Table-1:- EFFECT OF TEMPERATURE ON LIPID PRODUCTION BY RHODOTORULA RUBRA

Temperature	Cell biomass g/l	Lipid %
20 C	5.4	15.4
25 C	8.0	19.4
30 C	8.3	20.2
40 C	4.8	14.0

Effect of pH and time on Lipid Production.

The effect of different pH values on lipid production by *R. rubra* at 30°C is shown in the Table-2. The maximum yield of dry weight lipids and lipid coefficient of *R. rubra* were recorded at pH 5.5. Change of pH towards acidity or alkalinity decreased the yield.

Table-2:- EFFECT OF DIFFERENT pH VALUES ON LIPID PRODUCTION BY R. RUBRA.

pH Values	Cell biomass	Lipid%
4.0	6.5	15.8
4.5	7.4	18.0
5.0	8.2	20.0
5.5	8.3	20.2
6.0	8.2	20.0
6.5	7.2	17.5
7.0	6.6	15.8

The lipid accumulation was initiated at 24 hr. after the beginning of fermentation and continued

upto 72 hours. The biomass concentration (8.3g/l), reached at the end of 72 hrs. fermentation, showed 20.2% lipid content. Lipid production also reached its maximum value after 72 hrs. fermentation time.

Effect of Shaking. The effect of shaking on *R. rubra* is shown in Table-3. Results indicate that the shaking increased the quantity of biomass to 8.3g/l (on dry wt. basis) while it was 6.8g/l, in the still cultures. The fatty acid composition of the lipids obtained were also affected by shaking.

Table-3:- EFFECT OF SHAKING ON LIPID PRODUCTION AND FATTY ACID COMPOSITION OF *R. RUBRA*

Cell biomass g/l	Lipid	FATTY ACID COMPOSITION								
		C _{12:0}	C _{14:0}	C _{16:0}	C _{18:0}	C _{18:1}	C _{18:2}	C _{18:3}	C _{19:0}	
Still cultures	6.8	15.0	0.79	1.0	6.4	3.0	17.8	15.9	12.0	4.4
Shaking cultures	8.3	20.5	0.83	0.95	8.2	1.9	15.4	16.4	15.8	4.3

Effect of Different Carbon Sources. Glucose, sucrose, maltose and xylose were used as carbon sources in these studies. The efficiencies of these carbon sources for lipid synthesis are shown in Table-4. The results indicate that glucose is consumed preferentially. The lipid percentage on dry wt. basis was 20.2% with glucose and 12.0% with xylose. No marked differences were observed in the component fatty acids with the change of carbon source.

Fatty Acid Composition. The characteristics of each lipid class were determined by TLC with comparison of R_f values of standard materials. The proportion of neutral lipids to phospholipids was 83.2% to 11.5%. The results indicate that steryl esters (7.9%), sterols (4.0%) free fatty acids (50%), monoglycerides (1.8%) and triglycerides (75.2%) were present in neutral lipid portion. The presence of triglycerides was more pronounced in nitrogen limited cultures.

Table-4:- EFFECT OF DIFFERENT CARBON SOURCES ON LIPID PRODUCTION BY *R. RUBRA*

pH Values	Cell biomass	Lipid%
Glucose	8.3	20.2
Sucrose	7.6	18.5
Maltose	5.0	14.2
Xylose	4.5	12.0

The fatty acid composition changed with change in incubation time. The results show that there was an increase in the polyunsaturated acids (C18:2 and C18:3) and a decrease in the saturated (C16:0,

C18:3) and mono unsaturated acids (C18:1) with increase in the growth rate (Table-5).

Table-5:- FATTY ACID COMPOSITION OF LIPID BY *R. RUBRA* AT DIFFERENT INTERVALS OF TIME.

Time hours	C _{12:0}	C _{14:0}	C _{16:0}	C _{18:0}	C _{18:1}	C _{18:2}	C _{18:3}	C _{19:0}
24	0.82	1.6	8.7	6.3	19.4	11.6	8.3	43.2
48	0.79	1.2	7.4	5.2	18.2	14.5	10.4	42.3
72	0.79	1.0	6.4	3.0	17.8	15.9	12.0	44.0
96	0.83	1.5	6.0	2.8	15.4	17.2	14.6	42.5

Table-3 also shows the effect of aeration on fatty acid composition. It is observed that the percentages of stearic and oleic acids decreased with aeration, whereas the polyunsaturated fatty acids, linoleic and linolenic, increased slightly. There was also an inversely proportional increase of palmitic acid to the stearic acid.

DISCUSSION

The results reported here show that *R. rubra* grew well at 30°C at pH of 5.5. Changes of temperature and pH towards acidity or alkalinity decreased the lipid yield. This may be attributed to (i) the production of small amounts of organic acids like succinic acid during growth (ii) the CO₂ production by the cells and its dissolution in the growth medium and (iii) The use of the basic compounds such as ammonia by the cells. Temperature is one of the main environmental agents influencing microbial growth. Results indicate that 30°C was the best suitable temperature for the growth and lipid production of *R. rubra*. It is also well known that the fatty acid composition of a variety of organisms, including bacteria and yeasts, varies in response to growth temperature.

Aeration also influenced the cell biomass and lipid production. The results show that the fatty acid composition was affected by aeration in *R. rubra*. Although there was an increase of linoleic and linolenic acids with a decrease of stearic and oleic acids yet this change of fatty acids did not significantly affect the overall degree of unsaturation. In contrast, lipid production by *Candida utilis*, under similar conditions, shows an increase in the overall degree of unsaturation 9. It therefore, suggests that the effect is strain specific. It may also be deduced that unsaturated fatty acids are permanent constituents of normal yeast

cells and the effect of aeration is to stimulate both the quantity and the degree of unsaturation of the cellular lipids.

As the Table-4 shows glucose proved to be the best carbon source producing 20.2% lipids on dry wt. basis whereas *R. rubra* growing on xylose when used as carbon source gave 12.0% lipids.

It may be concluded that lipid production by fermentation is attractive for several reasons. One of these reasons is that it should be possible to obtain lipids with a preferable fatty acids composition. It depends on the strain specificity of the organism and on the growth condition. Among the other factors affecting lipid production are the reaction of the medium, phosphate concentration, degree of aeration, nature of the carbon source and the presence of certain metallic ions. Not only there are great differences in the potentialities of different micro-organisms for lipid production but also the composition of the medium and the environmental conditions likewise have a profound effect on lipid production by a culture.

In the fermentation system to obtain more microbial lipids, the cultural medium should involve the minimum amount of nitrogen source for cell proliferation and channel excess carbon to lipid synthesis. It is also seen that a knowledge of nutritional requirements is necessary for a better planning of physiological and biochemical studies on micro-organisms.

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STUDIES ON THE PHYSICO-CHEMICAL CHARACTERISTICS, TOTAL POLYPHENOLS AND PROTEIN DIGESTIBILITY OF VARIOUS LEGUMES

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ABSTRACT

Three legumes (mash, mung and lentils) were evaluated for their physico-chemical properties and cooking quality. These legumes fell into two categories (smooth and rough) according to texture and varied in seed volume and weight. Water absorption capacity, swelling capacity and seed coat percentage of whole seeds were within a range of 33.09 to 47.31%, 57.9 to 90.1% and 5.6 to 11.5% (w/w) respectively. Chemical composition of whole seeds, seed coats and kernels was individually studied. Seed coats of the legumes contained higher amount of ash and fibre than kernels. However, kernels were rich in protein and fat. Highest amount of total polyphenols was present in the seed coats. In-vitro protein digestibility of the whole seeds of these legumes ranged from 38.71 to 44.25% which increased from 12 to 32% after the removal of seed coats and from 46 to 56% as a result of cooking. Cooking time of these legumes was reduced by 36-45% on soaking in water for 4 hrs.

INTRODUCTION

Legumes occupy an important place in human nutrition as a source of dietary proteins, carbohydrates and minerals in several countries. These are free from cholesterol but they contain considerable amount of polyphenols (Akroyd and Dought, 1964; Uebersax *et al.*, 1991). These poly-phenolic compounds are responsible for the low digestibility of proteins (Ramadan *et al.*, 1994). It has been observed by many workers that the removal of polyphenols from the legumes improved the digestibility of proteins (Deshpande *et al.*, 1984). Soaking and cooking methods are generally used for the partial removal of polyphenols as well as for improving the digestibility of proteins and other cooking qualities (Chavan *et al.*, 1979). Some physical properties such as seed size or weight, swelling capacity and seed coat percentage of certain legumes are associated with their cooking quality (Akinyele *et al.*, 1986; Phirke *et al.*, 1982).

The objective of this study was to provide information on the association between physico-chemical properties and the cooking quality of three

locally available legumes, i.e. Mash, Mung and Lentils. Effect of soaking on cooking time and water absorption by the legumes, was also studied.

MATERIALS AND METHODS

Samples of whole seeds of Mash (*Phaseolus radiatus*), Mung (*Phaseolus mungo*) and Lentils (*Lens esculenta*) were obtained from Ayub Agricultural Research Institute, Faisalabad (Pakistan). The samples were cleaned to remove broken seeds, dust and foreign matter manually before being used in the present study.

PHYSICAL CHARACTERISTICS

a) Seed Weight, Seed Volume and Seed Density. One hundred randomly selected seeds of each legume were weighed separately and recorded as 100 seed weight.

Seed volume and seed coat percentage was determined as described by Phirke *et al.* (1982). Twenty-five weighed seeds were immersed in a cylinder containing water and the amount of water

displaced was recorded as volume of seeds. After 2 hrs soaking in water, seed coats were removed manually, drained on filter paper, weighed after drying to constant weight by hot air oven and percentage of seed coats were calculated. Apparent seed density was calculated from the values obtained for weight and volume.

b) Swelling Capacity. Swelling capacity of the legumes was estimated using Akinyele *et al.* (1986) method. Fifty seeds of each legume were weighed followed by cooking in boiling distilled water for 20 minutes. The seeds were drained and reweighed. The final weights plus the leached solids were taken as the cooked weights. The swelling capacity was the difference between the raw seed weight and the cooked weight per 100 g seeds.

c) Water Absorption Capacity. Water absorption capacity of the legumes was determined by the method followed by Safa-Dadeh and Stantley (1979). Whole seed samples (10g) were soaked at room temperature (30°C) in distilled water at a seed to water ratio of 1:5. At predetermined time intervals the seeds were removed from the soaking water, drained, surface dried with filter paper and reweighed. Gain in weight was taken as the amount of water absorbed and expressed as a percentage of the initial dry weight of the seeds.

d) Cooking Time. Presoaked whole seeds were cooked in a beaker in five times their weight of distilled water and while being cooked, sub samples were withdrawn periodically and tested for their softness by pressing them between fingers and thumb and were considered cooked when the seeds were softened to a uniform mass (Singh *et al.*, 1991).

Chemical Analysis. Ash contents of legume samples and crude protein contents after digestion with conc sulphuric acid according to Micro-Kjeldahl method were estimated as described in A.O.A.C. (1984). Fat contents were determined after extracting with hexane whereas crude fibre was measured after digestion with 1.25% sulphuric acid and 1.25% NaOH solutions (A.O.A.C., 1984). Total polyphenols were determined by the Vaillin - sulphuric acid method

on spectrophotometer at 500 nm as described by Wilson and Blunden (1983). Protein digestibility was estimated in-vitro after digestion with pepsin HCl solution at 37°C for 24 hrs. (Price *et al.*, 1979).

All determination were carried out in triplicate and standard deviations were calculated using Steel and Torrie (1980) methods.

RESULTS AND DISCUSSION

Physical Characteristics. Physical characteristics data for the three legumes (Mash, Mung and Lentils) are presented in Table-1. The seed volumes and weights of 100 seeds of mash and mung were significantly high compared to those of lentils ($P < 0.05$). Similarly, the seed coat percentage of mash and mung was also high (11.5 & 9.4%) compared to those of lentils (5.6%) which might be due to smooth seed coat surfaces of these legumes. Studies of Akinyele *et al.*, (1986) also showed higher seed coat percentage in smooth coated cowpea cultivars (11.19%) than in rough coated cultivars (4.5%). Water absorption capacity for mash, mung & lentils was 47.31, 41.27 & 33.09% respectively after 16 hrs soaking in water while swelling capacity of these three legumes were found to be 90.0, 89.5 and 57.9% respectively. Apparent seed density of these legumes varied from 1.19 to 1.24 g/ml. Deshpande *et al.* (1984) also found that physical characteristics of the seeds such as seed size and seed coat percentage influenced water absorption of the legumes.

Table-1:- PHYSICAL CHARACTERISTICS OF THE LEGUMES

Legumes	100 Seed weight (g)	100 Seed volume (ml)	Seed coat (%)	Apparent seed density (g/ml)	Water absorption capacity (g/100g)	Swelling capacity (g/100g)
1	2	3	4	5	6	7
Mash	4.35±0.2	3.5±0.01	11.5±0.4	1.24±0.02	47.31±1.1	90.1±1.3
Mung	4.54±0.4	3.8±0.03	9.4±3.0	1.19±0.01	41.27±1.0	89.5±1.2
Lentils	2.18±0.3	1.8±0.01	5.6±0.3	1.21±0.02	33.09±1.1	57.9±1.4

Chemical Composition of Whole Seeds, Seed Coats and Kernels of Legumes. Wide variations in the chemical composition of whole seeds, seed coats and kernels of the three different legumes were observed (Table-2). Protein content varied from 23.12 to 24.00% in the raw whole seeds, 14.60 to 17.31% in the seed kernels and 6.69 to 8.90% in the

seed coats of these three legumes. These results are in fair agreement with the findings of Laurena *et al.* (1984), who found about 22-25% proteins in whole seeds while 9.00 to 16.00% in seed coats of cow-peas.

it is suggested that legumes should be used after removing the seed coats to get maximum benefit of protein. However, by doing so fibre content of the pulses would be reduced, consequently a good quality dietary fibre would be lost.

Table-2:- CHEMICAL COMPOSITION OF WHOLE SEEDS, SEED COATS AND KERNELS OF THE LEGUMES (DRY WT. BASIS)

Parameters (%)	MASH			MUNG			LENTILS		
	Whole seeds	Seed coats	Kernels	Whole	Seed sees	Kernels coats	Whole	Seed seed	Kernels coats
Protien	23.12±1.2	7.43±1.0	15.69±1.2	23.50±1.1	8.90±0.4	14.60±1.4	24.00±1.3	6.69±0.7	17.31±1.5
Fat	8.79±0.9	2.16±0.3	6.63±0.4	8.30±0.7	1.81±0.2	6.42±1.0	6.31±1.0	2.25±0.3	4.05±0.5
Fibre	16.45±1.4	25.50±1.1	7.38±0.8	19.66±0.8	29.38±1.3	9.93±0.8	17.52±1.12	6.66±1.7	8.37±0.8
Ash	3.73±0.3	2.29±0.2	1.44±0.08	4.67±0.7	3.80±0.5	0.87±0.1	3.95±0.7	2.16±0.3	1.79±0.3

Fat contents in whole seeds of mash, mung and lentils varied from 6.31 to 8.79% while minimum fat (1.18 to 2.25%) was present in the seed coats in all these legumes, whereas kernels contained 4.05 to 6.63% fat. It was interesting to note that seed coats of these legumes contained the highest amount of fibre varying from 25.50 to 29.38% while kernels retained only 7.38 to 9.93% fibrous matter. Ash contents of mash, mung and lentils varied from 3.73 to 4.67% whereas seed coats and kernels retained ash contents ranging from 2.16 to 3.80% and 0.87 to 1.79% respectively. These results indicate that seed coats had higher amount of ash and fibre than kernels. On the other hand, kernels were significantly rich in proteins and fat contents compared to seed coats of all these legumes.

Total Polyphenols and Protein Digestibility.

Table-3 shows that total polyphenols and in-vitro protein digestibility (IVPD) of the legumes have significant correlation with each other ($P < 0.05$). Before cooking, IVPD of whole seeds of mash, mung and lentils was 38.71, 44.25 and 42.93% repetitively, whereas IVPD of the kernels of these legumes ranged from 49.63 to 52.20% and proteins digestibility became 12 to 32% more due to removal of seed coats. It clearly indicates that polyphenols present in seed coats were responsible for the low digestibility of proteins of whole seeds. Sathe and Salunkhe (1981) reported improvement in protein digestibility of winged beans after removal of seed coats. It has also been observed by Singh (1993) that partial removal of polyphenols from pigeon peas caused marked improvement in protein digestibility. Therefore,

Table-3:- TOTAL POLYPHENOLS AND IN VITRO PROTEIN DIGESTIBILITY BEFORE AND AFTER COOKING THE LEGUMES (DRY WT. BASIS)

Legumes	Total proteins (%)		In Vitro Protein Digestibility (IVPD%)	
	Before cooking	After cooking	Before cooking	After cooking
	1	2	3	5
Mash				
Whole seeds	1.83±0.2	1.63±1.05	38.71±1.3	51.37±1.6
Seed coats	1.67±0.4	1.55±0.4	23.88±1.1	29.78±1.4
Kernels	0.16±0.05	Nil	51.37±1.1	67.33±1.4
Mung				
Whole seeds	1.47±0.3	1.21±0.2	44.25±1.6	64.86±1.4
Seed coats	1.20±0.7	1.06±0.2	24.46±1.2	31.06±1.0
Kernels	0.27±0.04	Nil	59.63±1.1	65.50±1.3
Lentils				
Whole seeds	1.59±1.5	1.33±0.4	43.93±1.5	63.00±1.7
Seed coats	1.41±1.1	1.20±0.3	21.61±1.2	27.11±1.3
Kernels	0.18±0.04	Nil	52.20±1.2	69.01±1.5

Effect of Cooking on Protein Digestibility. IVPD of these legumes was further improved ($P < 0.05$) as a results of cooking (Table-3). Cooking process caused an improvement in IVPD of whole seeds, seed coats and kerbels by 46.0 to 55.7%, 24.7 to 27.0% and 48.0 to 74.0% respectively. It is also evident from these results that the total polyphenolic compounds were reduced upto 17.68% after cooking. Improvement in IVPD could be the result of partial removal of polyphenolic compounds from the legumes or due to some structural changes which might have occurred in protein profile and have increased accessibility of the proteins to enzymic attack. Similar observations have already been made many other workers. (Laurena *et al.* 1984; Van der Poel, 1990).

Effect of Soaking on Water Absorption. The amount of water absorbed per unit weight of the legumes increased gradually with soaking time ranging from 2 to 16 hrs (Table-4). The absorption of water was 12.87, 14.91 and 9.89% after 4 hrs soaking from mash, mung and lentils respectively. Prolonged soaking increased water absorption values but it also caused putrefaction of the legumes to some extent. Therefore,

it is not beneficial to soak legumes for more than 4 hrs. Variation in water absorption values of the legumes could be attributed to structural changes in seed coat thickness, seed size and helium size. These results suggested that it is not feasible to soak legumes for longer period (more than 4 hrs) under tropical conditions without microbial spoilage and subsequent production of an offensive odour by the legumes soaked in water.

Table-4:- EFFECT OF SOAKING TIME ON WATER ASSORPTION CAPACITY AND COOKING TIME OF THE LEGUMES

Legumes	Water Absorption Capacity (% w/w)			Cooking Time (Minutes)			
	2hrs	4hrs	8hrs	16hrs	0hrs	2hrs	4hrs
Mash	7.25±0.9	12.87±1.0	21.23±1.2	47.11±1.6	50	45	32
Mung	9.07±0.7	14.91±0.9	24.09±1.1	41.27±1.4	47	42	26
Lentils	7.00±0.8	9.89±0.6	18.08±1.0	33.09±1.1	53	50	29

Effect of Soaking on cooking Time. Cooking time of mash, mung and lentils was markedly reduced ($P < 0.05$) on soaking in water (Table-4). Cooking time of unsoaked whole seeds of mash, mung and lentils was 50, 47 and 53 minutes respectively. Cooking time was reduced by 5.66 to 10.63% and 36.0 to 45.0% as a result of soaking in water for 2 and 4 hrs respectively. Prolonged soaking (more than 4 hrs) did not cause further reduction in cooking time of the legumes. Onayemi *et al.* (1986) reported a reduction of 23.0 to 25.0% in cooking time of cowpeas whereas 55.62% reduction in cooking time for various cultivars of dry beans was observed as a result of soaking in water by Phirke *et al.* (1982). Reduction in cooking time could be the result of absorption of sufficient water from the soaking media which ultimately decreased the hardness of the legumes, especially the seed coat portion of the seeds.

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STUDIES ON CONTROL OF SWEETENING PHENOMENON IN DIFFERENT POTATO VARIETIES AT LOW TEMPERATURE STORAGE

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ABSTRACT

One of the major problem of low temperature storage is development of sweetness in potato resulting in lowering culinary properties of the produce. Studies regarding control of sweetening in different potato varieties were carried out at different temperature regimes under low temperature storage with application of different sprout suppressants. Observations regarding weight loss, sprouting, rotting and reducing sugars content, were recorded fortnightly, while the organoleptic evaluation was carried out at the end of storage period of 90 days in three potato varieties; namely, Cardinal, Diamant and Desiree. The data revealed no sprouting and rotting in treatments. But a little increase in reducing sugars was observed at 5°C and 9°C storage (0.12 to 0.19%) but it was higher at private cold store (1 - 2°C) while weight loss was less as compared to other storage temperatures.

INTRODUCTION

Potato (*Solanum tuberosum L.*) is an important international crop which enjoys top most position in vegetable culture of Pakistan. It is used in traditional oriental dishes singly or in combination with meat, chicken and vegetables. From an area of 104 thousand hectares about 1224 thousand tons of potato are produced in the Punjab province (Agri. Stat. of Pakistan, 1997-98). Due to lack of awareness and technical know-how of post harvest problems amongst the growers and traders, about 30 to 40% of the produce is wasted from farm gate till it reaches the consumer (Farooqi, 1996). This loss which tunes to approximately 2.21 billions of rupees annually can be minimized if post harvest handling of the produce is improved. This straight away wastage influences the cost of production and marketing, ultimately lowering the return to the farming community and higher price for common man.

Presently main problems of potato industry is poor cold store management because optimal conditions are not followed in the cold stores. As a result, substantial increase in sugar contents in potato takes place (Isherwood, 1973). In order to control

the sweetening phenomenon in potato, a trial was carried out by selecting three cultivars of potato by following different temperature regimes in combination with sprout suppressants application to test their efficacy on potato sprouting.

MATERIALS AND METHODS

Studies were conducted on three cultivars of potato namely, Cardinal, Desiree and Diamant for controlling sweetening phenomenon during storage conditions. These cultivars were procured from Vegetable Research Institute, Faisalabad. After sorting and grading potatoes were washed with water to remove adhering soil and following sprout suppressants were used (Boe *et al.*, 1974 Harris, 1992).

1. CIPC @ 1500 ppm (Chloro Isopropyl N-phenyl Carbamate)
2. CIPC @ 2000 ppm
3. IPC @ 1500 ppm (Isopropyl N-phenyl Carbamate)
4. IPC @ 2000 ppm
5. Combination of CIPC & IPC (Decco 276 EC)

The treated samples were kept in cold store chambers maintaining the temperature of 5°C and 9°C with relative humidity 85 to 90% for the storage period of 90 days. The sample of each variety was placed in privately owned cold store (1 - 2°C) for the sake of comparison which served as control. Physico-chemical studies like weight loss, rotting, sprouting and reducing sugars were carried out at fortnightly intervals. The organoleptic evaluation was carried out after the storage period of 90 days.

RESULTS AND DISCUSSIONS

Weight loss. Table 1 shows the weight loss data in all potato varieties during low temperature storage. The loss in weight was less in case of Desiree (1.25%) followed by Diamant and Cardinal (1.32 & 1.36%) respectively at storage temperature of 5°C. But slight increase in weight loss was noticed in case of Desiree at 9°C which was 2.81% followed by Cardinal and Diamant which ranged from 3.12 and 2.84%. While minimum weight loss was observed in all treatments stored at 1 - 2°C privately owned cold store within the range of 0.14 - 0.19% (Table-1).

Table-1:- EFFECT OF VARIOUS TREATMENTS ON WEIGHT LOSS IN POTATOES DURING STORAGE

Treatment	Temp	Storage in Days					
		15	30	45	60	75	90
		(Weight loss in percentage)					
Cardinal	1 - 2°C	0.04	0.06	0.08	0.11	0.14	0.18
	5°C	0.13	0.27	0.59	0.84	0.99	1.36
	9°C	0.35	0.87	1.50	2.10	2.53	3.12
Desiree	1 - 2°C	0.02	0.06	0.08	0.10	0.13	0.14
	5°C	0.11	0.24	0.32	0.62	0.95	1.25
	9°C	0.25	0.27	1.34	1.91	2.13	2.81
Diamant	1 - 2°C	0.04	0.05	0.07	0.12	0.16	0.19
	5°C	0.16	0.37	0.70	1.10	1.25	1.32
	9°C	0.39	0.95	1.45	1.96	2.34	2.84

Rotting. No rotting was observed in all treatments at 5°C, 9°C and 1 - 2°C after storage period of 90 days.

Sprouting. There was no incidence of sprouting in all the potato cultivars upto 45 days at temperature regimes of 5°C. Desiree showed no sprouting at 5°C upto 60 days, but it was initiated after 60 days of storage. Sprouting was less in case of Desiree variety at 9°C after 90 days of storage when compared to other varieties. No. sprouting was observed in all varieties stored at private cold store. The effect of sprout suppressant was almost same in all the treatments but CIPC was cost effective.

Reducing sugars. Data regarding reducing sugar content (Table - 2) reveal that there was slight increase in reducing sugar content in case of Cardinal, Desiree and Diamant potato varieties which ranged from 0.13 to 0.19 percent after 90 days of storage at 5°C and 9°C. But it was a little on higher side in tubers stored at private cold store at 1 - 2°C within the range of 0.13 to 0.39% (Table - 2).

Table-2:- EFFECT OF VARIOUS TREATMENT ON REDUCING SUGARS IN POTATO DURING STORAGE

Treatment	Temp	Storage in Days					
		15	30	45	60	75	90
		(Weight loss in percentage)					
Cardinal	1 - 2°C	0.13	0.18	0.20	0.23	0.27	0.34
	5°C	0.13	0.14	0.16	0.15	0.15	1.16
	9°C	0.12	0.13	1.14	0.15	0.16	0.17
Desiree	1 - 2°C	0.14	0.19	0.21	0.24	0.27	0.31
	5°C	0.15	0.15	0.16	0.16	0.17	0.17
	9°C	0.14	0.15	1.17	0.18	0.18	0.19
Diamant	1 - 2°C	0.15	0.21	0.26	0.30	0.34	0.39
	5°C	0.15	0.16	0.17	0.18	0.19	0.19
	9°C	0.3	0.3	0.6	0.7	0.7	0.8

Organoleptic evaluation. The panel of ten judges evaluated organoleptically after the storage period of 90 days. No sweetening was detected in all treatments stored at 5°C and 9°C. In case of tubers stored at 1 - 2°C were suspected to sweetening. It was found that Desiree variety showed better storage life in terms of quality after storage period of 90 days as compared to Cardinal and Diamant.

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UTILIZATION OF FOOD INDUSTRY WASTE (PEA POD) IN QUAIL FEED

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ABSTRACT

Pea pod waste was procured from a local processing unit and analysed for its chemical constituents. Pea pod contains protein 12.80%, fat 1.70%, fibre 20.0%, ash 6.0% and nitrogen free extract 49.50%. This waste was incorporated in quail feed at the rate of 2.5 and 5.0% by replacing rice polish. Results of this investigation indicated that incorporation of pea pod improved weight gain and feed efficiency of quail chicks.

INTRODUCTION

In Pakistan fruits and vegetables are produced in large quantities (Pakistan Economic Survey, 1998). Surplus are processed for preservation. Pea is a commonly used vegetable in Pakistan. It occupies an important place in human diet and is utilized for the manufacture of a number of processed foods. The waste (pea pods) left after processing contains sufficient quantity of proteins, fat, minerals and other nutrients with high biological value that can be utilized to replace expensive nutrients in feed of all classes of livestock. According to Donald *et al.* (1996) pea pod contains proteins 10.80%, ether extract 11.0%, crude fiber 35.60% and ash 5.3%. Many workers have evaluated the nutritive value of fruit and vegetable industry wastes and suggested their incorporation into livestock feed (Majum and Payne 1977; Oltgen *et al.* 1977; Ali *et al.* 1989, 1992a, 1992b and Zia-ur-Rehman, 1994).

Present study was conducted to determine the nutritive value of pea pods and to observe their positive effect through incorporation into poultry (quail) feed.

MATERIALS AND METHODS

Pea pod was procured from a local commercial unit, dried and analysed for protein, fat, ash and moisture following A.O.A.C. (1984)

methods. Three experimental feeds were formulated, which were designated as A, B and C. Feed A served as control feed B contained 2.5% and feed C contained 5.0% pea pod (Table-1).

Table-1:- COMPOSITION OF EXPERIMENTAL FEEDS

Ingredients	Feeds		
	A 0.00% (Control)	B 2.50% (Pea pod waste)	C 5.00% (Pea pod waste)
Maize	2.00	22.00	22.00
Wheat Waste	10.00	10.00	10.00
Rice Broken	20.00	20.00	20.00
Rice Polish	8.00	5.50	3.00
Cotton Seed Meal	5.50	5.50	5.50
Sesame Cake	4.00	4.00	4.00
Corn Gluten Meal 60%	3.50	3.50	3.50
Soyabean Meal	10.00	10.00	10.00
Pea Pod	—	2.50	5.00
Fish Meal	10.00	10.00	10.00
Blood Meal	2.50	2.50	2.50
Molasses	3.00	3.00	3.00
Dicalcium Phosphate	0.50	0.50	0.50
Lime Stone	1.00	1.00	1.00
Vitamin-Mineral	0.50	0.50	0.50
Total	100.00	100.00	100.00

One hundred and eighty quail chicks of day old age were acquired and randomly divided into three groups.

Experimental room was white washed, cleaned and disinfected prior to starting the experiment. Experimental room was partitioned into different compartments. Saw dust was used as bedding material. Fresh and clean water was made available round the clock. Feed was offered *ad libitum*.

During the experimental period of six weeks;

following parameters were observed: weight gain, feed consumption, feed efficiency, dressing percentage and weight of internal organs. Data collected were subjected to analysis of variance (Steel and Torrie, 1980).

RESULTS AND DISCUSSIONS

Results of chemical analysis of pea pod after drying are presented in Table-2. It contained protein 12.80 ± 1.12 , ash 6.0 ± 1.41 , ether extract 1.7 ± 0.54 , fibre 20.50 ± 2.17 and nitrogen free extract 49.50 ± 2.23 percent. High fibre content was the main factor which can affect the performance of quail chicks. Fibre content varies with the stage of maturity of the plant. Due to its high protein content (20.50%) it can be safely used for feeding. Results of present studies are in accordance with the findings of Ranjan (1981) and Donald *et al.* (1996).

Table-2:- CHEMICAL COMPOSITION OF PEA POD

Constituents	Pea Pod
Moisture %	10.00 \pm 1.06
Protein %	12.80 \pm 1.12
Ash %	6.00 \pm 1.41
Ether extract %	1.70 \pm 0.54
Fibre %	20.50 \pm 2.17
Nitrogen free extract %	49.50 \pm 2.23

Average of three replicates

Average weight gained by the quail chicks fed on experimental feeds A, B and C was 102, 113 and 123 grammes respectively (Table-3). Maximum weight was gained by the chicks, fed on ration C (significant at 5 percent level) replacing rice polish. It seems that the weight gained was due to the presence of well balanced amino acids and fatty acid profile in pea pod as reported by Dale (1996) and Ghol (1975).

Table-3:- AVERAGE WEIGHT GAIN, FEED CONSUMPTION, FEED EFFICIENCY, DRESSING PERCENTAGE AND WEIGHT OF INTERNAL ORGANS OF QUAIL GIVEN FEED WITH PEA POD

Particulars	Groups		
	A (Control)	B 2.50% (Pea pod)	C 5% (Pea pod)
Number of Chicks/group	60	60	60
Duration of experiment (days)	35	35	35
Weight at the start of the Chick (g)	8.50	8.50	8.50
Weight at the termination of experiment/Chick (g)	108	113	120*
Feed consumed/Chick (g)	390	399	410
Feed efficiency	3.61	3.50	3.41
Dressing Percentage	40.64	40.01	40.98
Weight of internal organs			
Heart weight (g)	1.37	1.40	1.35*
Liver weight (g)	3.67	5.30	4.99
Gizzard weight (g)	4.25	4.31	3.69

* Significant at 5 percent level

Average feed consumed by quail chicks is presented in Table-3, which indicated that addition of pea pod increased feed consumption which reflected the good palatability effect of pea pod. Feed efficiency of different experimental groups indicated that addition of pea pod improved feed utilization in the conversion of meat due to its high biological value. Feed efficiency (Table-3) was maximum in case of birds fed on ration C, but the difference in feed among different groups was found to be statistically significant. Similarly dressing percentage data indicated non significant differences. Heart, liver and gizzard weight indicated no abnormalities. No loose droppings were observed due to high fiber content. It may be concluded that pea pod can safely be incorporated in poultry feed replacing rice polish.

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ANTIOXIDANTS (As food supplements)

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Antioxidants are compounds, which hinder or slow down the oxidation reaction rate in the human body and in the presence of these compounds the formation of free radicals also, become slow. Free radicals are created through oxidation. These are the compounds that have lost an electron and are unstable toxic compounds generated every day in the body through the metabolic process. Small amount of these compounds in the body is normal but poor eating habits, pollution, stress and other factors have led to a dangerous increase in the number of free radicals created by our bodies. An unhealthy, full of processed foods, high in fat or sugar, toxins in the air we breath and present day stress all lead to an increase in free radical production. These free radicals travel through the body, stealing the electrons from vital tissues, normal healthy cells are damaged as molecules are forced to surrender their election to a free radical. This can impair the intracellular DNA and RNA cell membranes and numerous other important molecules in cellular environment. Damage caused by free radical is closely linked with many degenerative diseases.

You may think of your self as a peaceful sort but I am sorry to report that there is an all out endless war going on in your body, but inevitable defeat of the body can perhaps be delayed. Oxygen on the other hand is very useful and essential for human life. The oxygen we breath is life giving, make a complex with hemoglobin to oxyhemoglobin which later on take to every part of the body by blood circulation and where it reacts with compounds (glucose, amino acid, fat, etc.) to give energy, carbon dioxide and water vapour. But in the filed of battle which is going on in our body oxygen is a major player in the battlefield and it is also toxic. It sets off a process that creates a long time enemy known as free radical. This is a molecule lacking an electron in its outer most orbit making it extremely unstable. In an attempt to reclaim their electron, free radicals attack our cells to free up electron, damaging the cells and boosting the

probability of disease.

Free radical mechanisms have been implicated in the pathology of several human diseases including cancer, atherosclerssis, malaria and rheumatoid.

Neuroregenerative Diseases. Superoxide radical (O_2^-) and hydrogen peroxide are known to be generated in the brain and various systems in vivo.

Anti fungal defense to remove O_2 and H_2O_2 , exists. Superoxide dismutases (SOD) remove O_2^- by greatly accelerating its conversion to H_2O_2 .

Catalysis in peroxisomers convert H_2O_2 into water and O_2 help to dispose H_2O_2 generated by the action of the oxidase enzyme that are located in these organelles. Other important H_2O_2 removing enzymes in human cells are the glutathione peroxides when produced in excess. Reactive oxygen species (ROS) can cause tissue injury, however tissue can itself cause ROS generation (e.g. by causing activation of phagocytes or releasing transition metal ions from damaged cells).

Think of the entire body, including your DNA under going this endless assault, Think of your cells including brain cells each getting thousands of free radical hits every day. This violent process in called oxidation.

Body produces in minutes quantity of alpha lipoic acid, a powerful antioxidant. The increase in free radicals directly causes muscle soreness and antioxidant counter the effect. Anti-oxidants are also called anti aging agents.

Here come the reinforcements and the antioxidants, which can be classified into three groups:

- 1- Vitamins A, C, E
- 2- Minerals

3- Fruits and vegetables.

These compounds are powerful source of these valiant protectors. Their role is to limit the damage, may slow down the disease and keep us living longer. They do this by giving up their very own electron to those desperate free radicals and saving the cells from attack. Anti-oxidants give you a boost right now by eating lots of fresh fruits and vegetables. Vitamins A, C, E and beta carotene are all antioxidants. These are found in the foods that you eat. Good sources of these nutrients include fruits and vegetables.

Vitamins A. (fish oil, egg yolks, milk, orange and yellow vegetables). Vitamin A is a unique in that it is not destroyed when it quenches a free radical. Effective in both fat and water-soluble are 100% of recommended daily allowance (RDA). Beta-carotene is effective in both fat and water-soluble areas. It is converted into vit. A in the body as it is required. Studies show that beta-carotene deficiency can lead to many serious conditions.

Vitamin C. (amla, guava, green pepper, citrus, etc.) It is essential in variety of processes fundamental to our existence over 100% of RDA. The most important antioxidant. It is a water soluble vitamin. The fluid that surrounds all over cell is comprised of mostly water. Vitamin C is responsible for scavenging of naturally free radicals before they reach fatty cell membrane. Vitamin C has a secondary, yet equally vital role in conjunctions with a vitamin E, when vitamin E is inactivated after quenching a free radical, vit. C if present will recharge it. Studies show deficiencies can lead to many serious conditions.

Vitamin E. (vegetable oil, whole grains, nuts, wheat germ, sesame seed, etc.) It is an antioxidant that scavenges from essential fats and oils including heart over 100% RDA. Vitamin E is fat soluble. Since free radicals have a particular affinity for fat (especially poly unsaturated fat in nerve cells, brain cells and cell membranes), this is a vital nutrient for protection against free radical damage. It is particularly effective against the lipid peroxide radicals that attack fat. Studies show that vit. E deficiencies lead to many serious conditions.

MINERALS

Zinc. Zinc carnosine chelate and zinc citrate also act as antioxidants. These compounds specifically absorb the thyroid in its function. Zinc is a mineral which is involved in over 300 enzyme reactions including those that produce and repair DNA. Zinc sulphate with amino acid showed good antioxidant activity.

Selenium. A natural mineral that acts as catalyst to speed up and intensify the action of primary antioxidation (vitamins C,E,A etc.). This mineral has shown to protect the body against heart disease and cancer; and does not exist in sufficient quantities in our daily diet. Supplementation of this element is most essential. It is a mineral that acts as a co-factor in the process of free radical quenching, and is basically a catalyst that speeds up and intensifies the action of primary antioxidant.

Vegetables and Herbal Antioxidants. The best promising antioxidant in the vegetable origin is Fenugreek seed (Methary) which is usually used in the preparation of "achar". These seeds are commonly used in Germany and Europe as herbal tea. It is available in developed countries in the form of tea bags.

The presence of phenolic compounds in nature works as antioxidant and their role in prevention of cancer and cardiovascular diseases is well established.

Sesame seed (Till) and its oil is a very good antioxidant. Even its minute application on roasted peas increases their shelf-life significantly. The peptide present in soya bean also works as an antioxidant.

Gallic acid and tannic acid and their salts act as antioxidant. These compounds are present in Jambul (jamaun). Wheat bran dietary fiber has also the properties of antioxidant and the "OH" free radical scavenging activities.

Ajwain, Fennel, Mulberry leaves and Termeric are natural potential sources of antioxidants. Antioxidant properties of the major poly phenolic

compounds are present in broccoli.

List of Antioxidant Herbs.

- * *Alpinia officinarum*
- * *Areca Catechu*
- * *Brassica Alba*
- * *Cannabis Sativa*
- * *Curcuma longa*
- * *Engenia Caryophyllata*
- * *Evodia officinalis*
- * *Paeonia suffruticosa*
- * *Rhaphanus Sativus*
- * *Rheum Palmatum*
- * *Trapa Bispinosa*
- * *Zanthoxylum piperitum*
- * *Citrus aurantium*
- * *Cornus officinalis*
- * *Gleditsia Japonica*
- * *Lindera Strychinifolia*
- * *Phragmites Communis*
- * *Prunus mume*
- * *Schizandra Chinesis*
- * *Terminalia Chebula*

ANTIOXIDANT TEAS

Lemon grass, Licorice, Alfalfa, Dandelion root, Grape seeds extrat, Amla extrat, Jasmine green tea leaf, etc.

FRUIT ANTI OXIDANTS

Strawberry, White grapes, Melon, Plum, Banana, Red grape, Apple, Tomato, Pink grapefruit, etc. Prunes and prune juice may provide a source of dietary antioxidants.

The optimal supply of antioxidants, vitamins

and secondary plant metabolites should be acquired from normal diet, not by supplementation.

A high intake of antioxidant diet may decrease the risk of developing cardiovascular diseases.

IMPORTANT MODES OF ANTIOXIDANT'S ACTIONS

When a player (cricket, hockey, football, baseball, etc.) feel soreness which indicates a flag of defeat in an internal muscle, it may be prevented by supplementation of antioxidants.

When a weight lifter takes antioxidants he could lift more weight with less pain.

Exercise increases the feisty molecules free radicals. These unstable oxygen molecules have one or more unpaired electron in its outer orbit. It looks always other molecules to share with. Free radicals some time damage or kill the cells they latch on to.

Smoking depletes your body's supply of these antioxidants very quickly. Free radicals that are allowed to go unmonitored slowly are damaged. This can result in heart ailments or lead to various types of cancers.

The antioxidant balance is an important determinant of immune cell function including maintaining integrity and functioning of membrane lipids, cellular proteins, nucleic acids and for control of signal transduction and gene expression in human cells. Optimal levels of antioxidants are needed for maintenance of the immune response across all age groups.

INVOLVEMENT OF PRIVATE SECTOR IN MARKETING OF FRUITS AND VEGETABLES IN PAKISTAN

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ABSTRACT

Total fruit production in Pakistan is discussed with special emphasis on citrus and mango since they are the main fruits with respect to production. Citrus is elaborated with a detail of the marketing lines through grading unit, juice processing and local market. Export markets for both fruit and juice are pointed out. Mango production and export markets for both fruit and juice are presented. Vegetables production and especially export markets for potato and onion are pointed out. Processing industry activities are summarized and the financial situation of two role models, Shehzan and Mitchells are depicted. In the end, problems and recommendations are given to improve the situation for better profits to the growers, processors, exporters and achieve a healthier economic situation in the country.

FRUIT IN PAKISTAN

Pakistan is blessed with vast agricultural resources on account of its fertile land, well-irrigated plains, extremes of weather, and centuries old tradition of farming. Pakistan is one of the few countries of the world where fruits grown in cool temperate climate (apples, pears, plums, cherries), warm temperate (apricots, grapes, pomegranates and melon), and subtropical climate (citrus, mango, banana, dates, and guava) are available, As Table - 1 shows that citrus and mango are the major fruit production in Pakistan (32% and 15% respectively), they shall be discussed in detail.

marketing and processing line will show the steps taken from the grower to the consumer. The citrus grower tends to provide his fruit to the contractor which may give the fruit either to grading units or juice processing plants. Still, it is estimated that 30-40% of the fruit goes waste due to post-harvest losses resulting from disease, unfavourable weather, delay in harvesting, absence of proper roads, glut formation in the market, and other factors. The juice processing factories are usually given B and C type fruit, sometimes A, while the local "Mandi" and grading units take only A and B type. In the grading units, the fruit is washed, waxed, and graded. They are placed in wooden crates for sea shipment and in cardboard boxes for air-freight.

Table-1:- FRUIT PRODUCTION IN PAKISTAN, 000MT,1997-98

Fruit	Citrus	Mango	Apple	Dates	Guava	Apric of	Pomegr anate	Banana	Plum	Grape	Peach	Other	Total
Production	2037	916.8	573.1	537.5	454.9	189	104.2	93.6	78.4	74.3	46.5	1189	6294.6
% of total	32	15	9	9	7	3	2	1.5	1.2	1.2	0.7	19	100

Source: Federal Bureau of Statistics (1999)

When worldwide production of citrus is compared for different countries, Pakistan is ninth (Table-2). Thus, we have ample citrus resources to utilize for local consumption and export.

Thus, Pakistan markets citrus both locally and in the international market. A description of the

A temperature of +5 to +10 deg. C must be maintained in the cold store and onward transit to the customer. There are over 40 grading units in the Sargodha, Bhalwal area doing a very good business in the short four-month season. In the juice processing unit, fruit is graded, washed, juice extracted, centrifuged to remove excess pulp, pasteurized, and the water evaporated to concentrate it 6 times to a honey-like

Table-2:- CITRUS PRODUCTION WORLDWIDE, IN 000 MT, 1997

Country	Brazil	USA	Mexico	Spain	China	India	Italy	Egypt	Pakistan	Other	Total
000MT	22960.82	11500	3917.94	2729.3	22684.22	2000	1995	1552.09	1410	14106	64854.4
% of total	36	18	6	4	4	3	3	2	2	22	100

Source: FAOSTAT database (1999)

consistency. This is filed in polyethylene bags in drums. It must be frozen at -18 deg. C in the cold store and during onward transit to the customer by ship. This frozen concentrated orange juice (fcoj) is also diluted with water to make juice or drinks for local or export purpose.

The cost of a citrus juice processing plant may be 6 to 8 million \$ (Exchange rate of 1\$=52Rs. used). The price of fruit during the season varies from \$ 30 to \$ 60 per ton. Usually, 11.5 Brix juice is obtained with 50% juice recovery. One ton of 66 Brix fcoj may fetch a price of \$ 1000 to \$ 1500. Also, other by-products, such as peel oil are saleable. Citrus fruit export markets for Pakistan are given in Table-3.

Table-3:- EXPORT OF ORANGE JUICE FROM PAKISTAN, TONS, 1997-98

Country	Netherlands	Japan	Bangladesh	Other	Total
Tons exported	1912	295	56	164	2427
% of total	79	12	2	7	100

Source: Export Promotion Bureau & Federal Bureau of Statistics

Regarding citrus grading units, the imported machinery from Spain cost around \$250,000 and works at a capacity of 10 tons per hour. The local version may cost \$38,000 to \$48,000. Cost of fresh fruit varies \$3-\$6 per 100 kg. The processing cost may be \$25-\$30 per 100 Kg. In Europe, Far East, and Gulf the price of the fruit may be \$60-\$70 or even \$100 per 100Kg in the USA (SMEDA, 1999). Export markets for citrus fruit (Kino) are given in Table-4.

Table-4:- CITRUS FRUIT (KINO) EXPORT FROM PAKISTAN, TONS, 1997-98

Country	Dubai	Indonesia	Sri Lanka	Saudia Arabia	Singapore	Nether lands	Afghanistan	Philippines	UK	Others	Total
Tons	26858	6679	6371	5479	3300	2192	1620	1355	954	3784	58592
% of total	46	11	11	9	6	4	3	2	2	6	100

Source: Export Promotion Bureau and Federal Bureau of Statistics, 1999

Pakistan is also a major producer of mangoes in the world (Table-5). Clearly, India leads the world

production with about 55% of the total. Mango is processed into juice puree and also exported as fruit

Table-5:- MANGO JUICE EXPORTS FROM PAKISTAN, TONS, 1997-98

Country	Bangladesh	USA	UK	Japan	Others	Total
Tons	1161	83	50	29	63	1386
% of total	83	6	4	2	5	100

Source: Export Promotion Bureau and Federal Bureau of Statistics, 1999

Table-6:- MANGO FRUIT EXPORT FROM PAKISTAN, TONS, 1997-98

Country	Dubai	UK	Saudia	Others	Total
Tons	31959	11810	762	1302	45833
% of total	69	26	2	3	100

Source: Export Promotion Bureau and Federal Bureau of Statistics, 1999

similar to citrus. Export markets for mango juice are given in Table-6. There is a strong need to develop new markets and penetrate existing ones more efficiently.

VEGETABLES IN PAKISTAN

Pakistan produces sufficient quantities of vegetables for local consumption, with 4,371,000 tons of production estimated in 1997 (Table-7). The main two vegetables produced are potato (33%) and onion (26%). The export of these two vegetables are shown in Table-8, indicating that most of them were exported to Sri Lanka and Dubai.

The figures indicate that 2.7% of the total production of potatoes and 5.7% of total onion

production was exported from Pakistan. Another opportunity is the export of dehydrated vegetables.

Table-5:- MANGO PRODUCTION WORLDWIDE, IN 000 MT, 1997

Country	India	China	Mexico	Pakistan	Phillippines	Indonesia	Brazil	Others	Total
000 MT	12,000	2,142	1,461	914	700	605	455	343	23,063
% of total	55	10	7	4	3	3	2	1.6	100

Source: FAOSTAT database (1999)

Table-7:- VEGETABLE PRODUCTION IN PAKISTAN, 000 MT, 1997-98

Vegetable	Potato	Onion	Tomato	Other	Total
Production	1425	1131	325.3	1490.3	4371.8
%	33	26	7	34	100

Source: FAOSTAT, 1999

Table-8:- EXPORT OF VEGETABLE FROM PAKISTAN, TONS, 1997-98

1	2	Sri Lanka	Dubai	Singapore	Malaysia	Other	Total
Potato	Tons	35118	2755	310	288	445	38916
	% of total	97.94	7.08	.8	.74	1.14	100
Onion	Tons	38259	21020	3737	1105	876	64997
	% of total	58.8	32.33	5.7	1.7	.13	100

Source: Export Promotion Bureau and Federal Bureau of Statistics, 1999

Presently, Pakistan is contributing to less than 1% of this huge market, while China is a world leader (Table-9).

Table-9:- EXPORT OF DEHYDRATED VEGETABLES, 000 \$, 1992

Country	China	USA	Taiwan (China)	Germany	France	India	Pakistan	Others	Total
Value	382,990	103,748	58,469	50461	44,462	14,834	8,416	393,515	1,056,895
% of total	36	9	6	5	4	1	1	38	100

Source: International Trade Centre, 1993

Fruit and vegetables are being processed by many companies in Pakistan. For citrus, Cargill, and Indus are operating. Mitchells and Shezan are role models of profitable companies. Tripple-Em is the largest potato chip producer in the North. Nestle's fruit drinks (Frost) and orange juice are quite popular. Ketchup is being made by Rafhan Best Foods, National Foods and many other companies. Icepac is packing and exporting frozen vegetables. Thus, the private sector activity is quite high in this sector.

Shezan was established in the 1960's and presently, in 1997, exported around 13% of its products. Its sales are now around \$11.39m with a profit after tax of \$0.42m with fixed assets of \$1.16m, according to the Annual Report. The Company foresees great potential in Pakistan on account of its population growth and also seeks new markets abroad for its products.

Mitchells was established around 1930's and has its own farms from where fresh fruit and vegetables are utilized for operations. The company makes jams, jellies, squashes, juices, drinks and cans vegetables. It also has confectionery products. According to the Annual Report, 1998, sales were \$9.7m while profit after tax was \$0.82m with fixed assets of \$0.92m.

PROBLEMS AND RECOMMENDATIONS

The fruit and vegetable industry is facing a lot of problems, which are mostly inter-related. The grower needs credit, advice and assistance in marketing of the products as soon as possible since

these are perishable products. The processors need rebates, subsidies and relaxation on taxes to improve the profitability. Proper cold chain in the form of cold

stores at markets, refrigerated trucks, PIA cargo, railway cold transport, and other infrastructure must be improved to alleviate the situation. Pakistan has ample fruit and vegetable resources at the present and needs to manage them properly for economic benefits to all players in the agribusiness.

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SHOOT REGENERATION FROM CULTURES OF LENTIL (*LENS CULINARIS* MEDIK.) *IN-VITRO*

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ABSTRACT

Shoot explants from a 4 days old aseptically grown seedlings of *Lens culinaris* Medik. were cultured for callogenesis on MS medium. The culture medium supplied with various concentrations of BA (2.5 - 10.0 mg/L) alone or in combination with an auxin i.e. IAA/2, 4-D (0.25- 5.0 mg/L) BA (2.5 mg/L) alone was found more effective for callus initiation and growth as compared to its combinations with the IAA/2, 4-D (0.25 mg-5.0 mg/L each). Calli growing on medium containing 2.5 mg/L of BA were green, compact and nodular. Transfer of these calli to the media containing various amounts of BA lesser than 2.5 mg/L resulted in regeneration of shoots i.e. on medium supplied with 0.05 mg/L of BA.

INTRODUCTION

Many crop species are included in the legume family. Despite their vast economic potential, however, crop improvement efforts based on manipulative biotechnology have remained limited due to the general difficulty associated with the *in-vitro* regeneration, particularly of grain legumes (Jain and Chopra, 1998). The tissue culture of legumes for crop improvement has been comprehensively reviewed (Mroginski and Kartha, 1984) and not withstanding the difficulties, regeneration has been reported in some, such as peanut (Atreya *et al.*, 1984), winged bean (Wilson *et al.*, 1985), pea (Rublua *et al.*, 1984), soy bean (Li *et al.*, 1985), moth bean (Godbole *et al.*, 1984) and pigeon pea (Kumar *et al.*, 1983). Nevertheless, the problem is more aident in pulse legumes, as a class where there are only a few examples of successful and reliable plant regeneration from non-organized cells in culture (Williams and McHughen 1985).

The Lentil plant (*Lens culinaris*), a pulse legume, has been reported to be regenerated from cultured shoot tips (Bajaj and Dhanju, 1979), from embryo rescue (Cohen *et al.*, 1984), and organogenesis followed by rhizogenesis from regenerated shoot (Williams and McHugen, 1986).

Limited studies have reported embryogenesis from ambryo-derived (Sexana and King, 1987) and root-tissue derived calli (Zaidi *et al.*, 1989). The studies have been further extended to describe somatic embryogenesis from the shoot-tissue derived calli treated with new sets of growth regulators comprising of IAA, BA and 2,4-D not reported heretofore.

MATERIALS AND METHODS

Plant Material. Seeds of *Lens Culinaris* cv. Masoor-85 were obtained from Ayub Agricultural Research Institute, Faisalabad, Pakistan. Rinsed with 90% ethanol. Surface sterilization of seeds was done with 0.1% (w/v) mercuric chloride solution containing a few drops of Tween-20 for 20 minutes. It was followed by three washings with sterile distilled water. The seeds were aseptically germinated on pre-soaked filter papers placed in petri plates in darkness at $25 \pm 2^\circ\text{C}$, after germination cultures were grown under 16h photoperiod of 3000 Lux cool white fluorescent light.

Growth Medium and Growth Regulators. The growth medium was based on MS formulation (Murashinge and Skoog, 1962) supplied with 0.55% (w/v) agar and 3% (w/v) sucrose adjusted at pH 5.8

with 0.1 N NaOH/HCl prior to the addition of agar. The medium was sterilized at 15 p.s.i. for 20 minutes. For callus initiation and maintenance, this medium was supplied with growth regulators i.e. BA at 0.25 - 10.0 mg/L alone (A) or in combination with 0.25 - 2.5 mg/L of IAA(B) or with 0.25-5.0 mg/L of 2, 4-D. (C) Lesser amounts of BA (0.05 - 0.25 mg/L without IAA/2, 4-D were added to the medium for the induction of somatic embryogenesis.

Culture Conditions. Explants from 4 days old seedlings were excised, 5-10 mm (approximately) in size, inoculated on 30 ml of growth medium in 100 ml culture flasks and incubated at $25 \pm 2^\circ\text{C}$ in 16 h photoperiod of 3000 Lux cool white fluorescent light. Subculture of the calli was done at 4 weeks interval.

RESULTS AND DISCUSSION

Shoot explants developed green calli after 2 weeks in culture, when grown in medium containing BA alone at the concentration of 2.5 and 5.0 mg/L respectively appearing as well-developed and nodular as against less developed and soft (Table-1/A) At

Table-1:- EFFECT OF AUXIN AND CYTOKININ INTERACTION ON CALLOGENESIS IN *LENS CULINARIS* TISSUE IN-VITRO.

Growth Regulators (mg/l)		Callus Initiation Period (Weeks)	Callus Characteristics		
BA	Auxin		Colour	Texture	Quantity
A					
2.5	-	2	Green	Nodular	+++
5.0	-	2	Green	Soft	++
10.0	-	-	-	-	-
B					
	IAA				
2.5	0.25	3	White	Soft	+
5.0	0.25	2	White	Soft	++
10.0	0.25	1	G. White	Nodular	++
2.5	2.5	3	Green	Nodular	+
5.0	2.5	2	Green	Soft	-
10.0	2.5	1	Green	Soft	+++
2.5	5.0	1	W.Green	Compact	++
5.0	5.0	1	White	Soft	++
10.0	5.0	3	White	Hyline	+
C					
	2, 4-D				
2.5	0.25	3	Green	Nodular Soft	+++
5.0	0.25	3	G. White	Nodular Compact	++
10.0	0.25	2.5	Amber	Compact	++
2.5	2.5	2	Green	Nodular	+++
5.0	2.5	3	Amber	Soft Nodular	++
10.0	2.5	3	Amber	Soft hyline	+
2.5	5.0	3	Amber	Soft	+
5.0	5.0	-	-	-	-
10.0	5.0	-	-	-	-

* ++++ - Very Good
+++ - Good
++ - Fair
- - Negligible

higher concentration of 10.0 mg/L of BA however, no callus formation occurred. Addition of IAA or 2,

4-D in combination with BA affected the callus initiation period and colour as well as texture of the calli (Table-1/B,C).

It is evident from Table-1/B that BA supplied at a rate of 2.5 mg/L in combination with 0.25 or 2.5 mg/L IAA delayed callus initiation upto 3 weeks, whereas in combination with 5.0 mg/L IAA callus formation was induced earlier after one week (Table-1/B) as compared to BA alone which took 2 weeks for callus initiation (Table-1/A). Colour of the calli also varied from green to greenish-white or white at different concentrations and combinations of auxin and cytokinin. Callus formation was not observed when 5.0 mg/L BA was supplied in combination with 2.5 mg/L of IAA on the other hand balanced ratio of BA and IAA (5.0 mg/L each) induced white coloured soft and slow growing calli which was initiated within one week (Table-1/B). IAA at a rate of 0.25 mg/L in combination with 5.0 mg/L of BA did not affect the initiation period or texture except that the colour and growth rate of the calli while compared with the cultures grown on medium containing 5.0 mg/L of BA alone (Table-1/B). Higher concentration of cytokinin alone (BA 10.0 mg/L) did not induce callogenesis in stem explants. Early callus initiation (1 week) was observed when 0.25 or 2.5 mg/L of IAA were incorporated alongwith 10 mg/L of BA (Table-1/B). Calli were greenish-white or green respectively. But higher concentration of both auxin and cytokinin (IAA-5.0 mg/L \pm BA-10.0 mg/L) induced callogenesis after 3 weeks. Calli produced were hyaline and slow growing. Growth rate of callus was affected by the amount of auxin as well as cytokinin in the medium which directly influenced the quality of the calli also.

Table-1/C shows callogenic response of the stem explants under the influence of BA alongwith 2,4-D. Explants showed delayed callus initiation when 2, 4-D was incorporated in the medium alongwith BA. No callus formation was observed when 5.0 mg/L or 10.0 mg/L of BA was combined with 5.0 mg/L of 2, 4-D. Quality of the callus was affected as the amount of 2, 4-D increased from 0.25 mg/L to 2.5 mg/L. Texture of the calli changed from compact nodular to soft whereas the colour of the calli also turned amber (Table-1/C). Quantity of the callus mass

was lesser as compared to the amount of callus produced by the explants grown on BA alone (Table-I/A). Maximum amount of the callus was formed by the explants grown on medium containing BA alone (2.5 mg/L). Addition of the auxin decreased the growth rate and ultimately the amount of the callus produced was less at the end of the incubation period.

Calli growing on medium containing 2.5 mg/L of BA were selected for regeneration studies. After three successive subcultures (12 weeks), pieces or calli were recultured on medium containing lower concentrations of BA (2.5 - 0.05 mg/L). Results are shown in Table-2. With 3-4 weeks in culture, dark

Table-2:- EFFECT OF BA CONCENTRATION ON REGENERATION POTENTIAL OF *LENS CULINARIS* CALLI *IN-VITRO*

BA mg/L	Callus Colour	Characteristics Texture	Morphogenesis	Remarks
0.05	Green	Nodular	Small shoots regenerated	Growth of calli was slowed but regeneration of shoots was achieved.
0.1	Green	Compact nodular	-	Growth of Green nodular calli continued.
0.2	Brownish green	Compact	-	Very poor growth.
0.25	Green	Compact nodular	-	Green nodular calli which had regeneration potential.
2.5	Bright Green	Compact	-	Growth of compact and healthy calli continued.

green structures emerged at the surface of the calli grown on medium containing 0.05 mg/L or 0.1 mg/L of BA. In case of calli grown on former combination of medium small young apices became prominent with growing clusters of shoots. The procedure of Lentil caulogenesis used during present study differs from that of Williams and McHughen (1986). As well as Saxena and King (1987). In this study caulogenesis in lentil has been achieved by a simpler method. In this case, different concentrations of BA alone were found effective for callogenesis as well as morphogenesis in two stage, whereas Williams and McHughen (1986) used KIN and GA₃ and Sexana and King applied a three stage procedure - callus formation on medium containing 2, 4-D, its maintenance on medium supplied with BA and IAA combination and finally regeneration in the presence of glutathione. So the new procedure reported in this paper involves less time and only one growth regulator

for shoot regeneration in a legume i.e.lentil.

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NEWS AND VIEWS

PROMOTIONS:

Pakistan Society of Food Scientists and Technologists (PSFST) congratulates the following members on their well deserved promotions.

A. *Department of Food Technology, Agricultural University, Faisalabad:*

Promotions to the Posts of Professors:

1. Dr. Javaid Aziz Awan (Ex-Secretary-PSFST)
2. Dr. Faqir Muhammad Anjum (Secretary-PSFST)
3. Dr. Ghulam Mohyuddin

Promotion to the Post of Associate Professor:

1. Dr. Saleem-ur-Rehman (Treasurer - PSFST)

Promotions to the Posts of Assistant Professors:

1. Dr. Masood Sadiq Butt
2. Mr. Sarfraz Hussain

Appointed to the Post of Lecturer:

1. Mr. Tahir Zahoor

B. *Pakistan Council of Scientific and Industrial Research (PCSIR)*

Promotions to the Posts Principal Scientific Officers:

1. Dr. Faqir Muhammad Khan (PCSIR-Peshawar)
2. Dr. Shahnaz Hamid (PCSIR-Lahore)

Promotions to the Posts of Scientific Officers:

1. Ms. Zahida Nasreen (PCSIR-Lahore)
2. Mr. Muhammad Ashraf (PCSIR-Lahore)

RETIREMENTS:

PSFST wishes happy and healthy future to the following members on their retirements from active services of various institutions:

1. Prof. Dr. Muhammad Saeed (Ex-President-PSFST) from NWFP Agricultural University, Peshawar.
2. Prof. Dr. Amjad Ali from Agricultural University, Faisalabad.
3. Prof. Dr. Muhammad Ismail Siddiq from Agricultural University, Faisalabad.
4. Prof. Dr. A.K. Baloch (Ex-Vice President) from Gomal University, D.I. Khan.
5. Dr. Mumtaz Ali (Ex-Vice President) from NIAB, Faisalabad.
6. Dr. Muhammad Yaquab Ch. from PCSIR, Lahore.
7. Mr. Hamid Ahmad (Chairman-PSFST Lahore Chapter) from PCSIR, Lahore.

PLACEMENTS AND TRANSFERS:

1. Prof. Dr. Muhammad Shahbaz Bhatti (Chairman-PSFST Rawalpindi/Islamabad Chapter) of Arid Agricultural University, Rawalpindi has shifted to England.
2. Mr. Asadullah H. Bhatti (Vice Chairman-PSFST Lahore Chapter) has rejoined Tripple Em (Pvt) Ltd. as Factory Manger.
3. Mr. Sajjad Hussain (Treasurer-PSFST Lahore Chapter has left Vita Food Industries (Pvt) Ltd. and has gone abroad.
4. Mr. Atta Muhammad Naz has rejoined Vita Food Industries (Pvt.) Ltd. Lahore.

REVISION OF WEST PAKISTAN PURE FOOD ACT 1965:

Department of Health Government of the Punjab has constituted a committee to revise and update the West Pakistan Pure Food Act 1965 in the light of present scientific developments related to new foods and food products. Appart from other members, the following PSFST members have been nominated/included in this committee:

1. Prof. Dr. Muhammad Shafiq Ch.
2. Dr. A.F.M. Ehteshamuddin
3. Dr. Wazir Hussain Shah
4. Dr. Shahnaz Hamid
5. Mr. Hamid Ahmad
6. Mr. M. Arshad Karim

FOREIGN VISITS OF PSFST MEMBERS:

1. Prof. Dr. Faqir Muhammad Anjum (Secretary-PSFST) of Agricultural University, Faisalabad has been awarded a fellowship in a prestigious University of U.K. He has been working on advanced techniques on the effect of starch proteins on the quality of bread. He would be back soon after spending almost a year in England.
2. Mr. Hamid Ahmad, Principal Scientific Officer (Rtd.) PCSIR Lahore (Chairman-PSFST Lahore Chapter) visited, Federal German Meat Research Centre, Kulmbach and Federal German Centre for Health and Veterinary Medicine; Berlin, on invitation of the President German Senior Academic Exchange Service (DAAD) for two months (August/September, 1999).
3. One of our member, Dr. Muhammad Iqbal, Senior Scientific Officer, PCSIR Laboratories, Lahore who availed the German Humboldt Postdoctoral fellowship has been provided scientific equipments worth DM 50,000/- for his research and development project relevant to environment biotechnology. Mr. Landes, Press and Cultural Attaches, German Embassy, Islamabad came to PCSIR Lahore to present this equipment on behalf of the German Government.
4. Mr. Arshad Mahmood (Secretary-PSFST Lahore Chapter) Chief Executive M/s. Maki Corporation, visited Singapore and participated in the following international activities organised by Singapore Exhibition Services (Pvt) Ltd. from 30 Nov-3 Dec. 1999, held at International Convention & Exhibition Centre (SICEC); Singapore:
 - **ChemAsia 99** (The 11th Asian International Chemical & Process Engineering & Contracting Exhibition).
 - **InstrumentAsia 99** (The 9th Asian International Instrumentation, Control, Measurement & Testing Exhibition & Conference).
 - **AnaLabAsia 99** (The 7th Asian International Laboratory & Analytical Technology & Equipment Exhibition).
 - **Environmex Asia 99** (The 5th Asian International Environmental Manager Technology, Equipment & Control Systems Exhibition & Conference).
 - **Watermex Asia 99** (The 5th Asian International Water Management Technology, Equipment & Control Systems Exhibition & Conference).

Mr. Arshad Mahmood availed the opportunity to discuss with leading international companies about the "Role of Food Industry & its problems in Pakistan, regarding ISO-9000 (Quality Standard) & ISO-14000 (Environment Standard)" and about high tech instrumentation and advanced equipments technologies transfer in Pakistan at cost effective price packages.

ATTRIBUTE TO THE SENIORS:

It is a matter of pride for me to write these lines regarding the contributions of Prof. Dr. Mohammad Shafiq Chaudhry (President-PSFST: 1990-1997) and Prof. Dr. Javaid Aziz Awan (Secretary-PSFST: 1990-1999). They have brought up the Pakistan Society of Food Scientists and Technologists to its present level. Though the Society came into existence in early eighties, yet it was revived in 1990 under the leadership of these dedicated professionals. The Society has flourished and gained its present status due to their sincere efforts as well as scientific and technological inputs. Now that they have encouraged us to run the affairs of the Society we assure them that efforts will be made to farther the level of progress in future (Insha-Allah) and we hope that their guidance and patronage would be available to us in future also.



(Dr. Wazir Hussain Shah.)

NEWS FROM THE CHAPTERS:

1. PSFST-Peshawar Chapter:

Members of PSFST welcome the news regarding formation of PSFST-Peshawar Chapter and congratulate Dr. Abdus Sattar, Chief Scientific Officer, NIFA as its founder Chairman. It is hoped that the Chapter would have positive contribution towards the promotion of technical/educational activities in the field of Food Science and Technology.

2. A letter from Rawalpindi/Islamabad Chapter is reproduced:

Dr. Wazir Hussain Shah
P.C.S.I.R. Lahore.

Ref. No. R-7/99
Dated: 04-10-99

1. During Sept 99, the Executive Council has held its meeting at PCSIR Lahore. It has been decided by the Executive Council to hold the coming Annual General Meeting at PCSIR Laboratories Complex, Lahore during first week of December 1999. Exact date will be communicated later.
2. Dr. Muhammad Shahbaz Bhatti--The President-PSFST Rawalpindi/Islamabad Chapter has shifted to England. In his absence the Senior Vice President Mr. Shafaat Ahmed will act as President of Rawalpindi/Islamabad Chapter.
3. Wife of Mr. Shafaat Ahmed. Vice President PSFST Rawalpindi/Islamabad Chapter, Factory Manager Dane Foods Hattar has died last month. **إنا لله وإنا إليه راجعون** May Allah shower all the blessings upon her soul in Heaven, Ameen. Her "Chehlum" would be on 18th Oct, 99 at their residence in Okara. Tell. Factory (Hattar) 0995-617230, 617018 Tel: Res. (Haripur) 0995-611368.

Thanking you,

Yours faithfully,



(IJAZ HUSSAIN)
Chapter Secretary

GLIMPSES OF THE 10TH ALL PAKISTAN FOOD SCIENCE CONFERENCE AND ANNUAL GENERAL MEETING, 1999



From left to right : Top: Dr. J.A. Awan presenting the Annual Report; 2. Dr. A. Sattar (NIFA) addressing the audience. II-row: Dr. Quratul-Ain (PCSIR) presenting her paper; 2. Dr. M. Shafiq Ch. along with Dr. Shafiq Ahmad (Synarome) & Mrs. Qamar Shaheen (Public Analyst) presiding over the Technical Session-I, III-row: Dr. M. Khalid Farooq (PCSIR) along with Mr. M. Haroon (SMC), Mr. Aurang Zeb (NIFA) & Dr. A.K. Baloch (Gomal Univ.) presiding over the Inaugural Session; 2. Dr. S.I. Zafar (PCSIR) along with Dr. Mumtaz Ali (NIAB) & Mr. Asadullah H. Bhatti (Tripple-Em) presiding over the Technical Session-II. Bottom: Dr. M. Shafiq Ch. awarding Shield to Dr. Awan; 2. Dr. W.H. Shah receiving his Shield.

GLIMPSES OF THE 10TH ALL PAKISTAN FOOD SCIENCE CONFERENCE AND ANNUAL GENERAL MEETING, 1999



From left to right: Top: Mr. Fida Hussain (Dawn Bread) inaugurating the industrial exhibition; 2. Dr. A.K. Baloch (Gomal Univ.) along with Mr. M. Haroon (SMC) and Dr. W.H. Shah (PCSIR) presiding over the Business Session. II-row: Audience of the meeting; 2. Industrial Stall. III & IV-row: Stalls of the industrial exhibition.