STUDIES ON THE STATUS OF STARCH PHOSPHORYLATION IN DEVELOPING TUBERS FROM VARIOUS POTATO CULTIVARS

A
DISSERTATION
By

Ms. Teena Shukla
Roll No.3020126

Submitted in partial fulfillment of the requirement for the award

of the degree of Masters of Science in Biotechnology

Department of Biotechnology and Environmental Sciences

Thapar Institute of Engineering and Technology

Patiala –147004

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CANDIDATE'S DECLARATION

I, hereby declare that the work presented in the dissertation entitled**, "Studies on the Status of Starch Phosphorylation in Developing Tubers From Various Potato Cultivars "** in partial fulfillment of the requirement for the award of the degree of Masters in Biotechnology, Department of Biotechnology and Environmental Sciences, Thapar Institute of Engineering and Technology, Patiala; is an authentic record of my own work during the period of five months from January 2004 to May 2004, under the supervision of Dr. N. Das ,Assistant Professor, Thapar Institute of Engineering and Technology. I have not submitted the matter embodied in this dissertation for the award of any other degree or diploma.

Place: Patiala TEENA SHUKLA
Date
This is to certify that the above statement made by the candidate is correct and true to the
best of our knowledge.

(Dr. N. Das) (Dr. Sunil Khanna)
Assistant professor Head, DBTES,
Supervisor T.I.E.T., Patiala

CERTIFICATE

This is to certify that the thesis entitled "**Studies on the Status of Starch Phosphorylation in Developing Tubers From Various Potato Cultivars** " submitted by Teena Shukla in partial fulfillment of the requirements for the award of Degree of Masters of Science in Biotechnology, to Thapar Institute Of Engineering and Technology (Deemed University), Patiala, is a record of student's own work carried out by her under my supervision and guidance. The report has not been submitted for the award of any other degree or certificate in this or any other university or institute.

(Dr. N. Das) (Dr. Sunil Khanna)
Supervisor Head
Deptt. of Biotech. & Env. Sciences Deptt. of Biotech. & Env. Sciences
Thapar Institute Of Eng. and Tech Thapar Institute Of Eng. and Tech.
PATIALA PATIALA

Dr. D.S. Bawa
DEAN (ACADEMIC AFFAIRS)
THAPAR INSTITUTE OF ENGINEER. AND TECH.
PATIALA

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DATE: (TEENA SHUKLA)

ABSTRACT

Potato is one of the most important food crops of the world. It is cultivated for its tubers, which are modified stem structures formed by the enlargement of the tips of underground stems. A crucial feature of the post-harvest physiology of potato tubers is an undesirable process called cold-induced sweetening. A comprehensive biochemical and molecular approach need to be undertaken to overcome such problem in the cultivars suitable to our agro-climatic conditions. Currently, phosphorylation status of starch in potato tubers is believed to play a crucial role in the above process as phosphate groups attached to glucose residues at C-6 and C-3 positions alter overall secondary structure of starch hence its degradability at low temperature. We need to have an insight to find correlation between starch phosphorylarion and cold sweetening process. Keeping in view with the above aim a few basic experiments were done and executed in this dissertation work. The study was done on several potato cultivars namely Kufri Chipsona-1 (CS-1), Kufri Chipsona-2 (CS-2), Kufri Chandramukhi (KCM), Kufri Jyoti (KJ), Kufri Pukhraj (PR), Kufri Ashoka (AS) and Cultivar Desiree (DE), which were routinely micropropagated in our laboratory. After proper hardening and acclimatization, the potato plantlets of the above mentioned varieties were cultivated in the field in the second week of November 2003. Developing mini-tubers were collected time to time through out the entire period of cultivation. Mature tubers from various cultivars were also transferred to 20^0C for a period of four weeks as required in this study. A rapid and convenient method was adopted during isolation of starch granules from various tuber samples and overall yield was calculated in gm per 100 gm of tuber weight. Starch-bound phosphates were converted to inorganic phosphates by complete ashing procedure.

A very sensitive ascorbic acid-ammonium molybdate assay method was adopted for estimation of total phosphorus. The level of free phosphates was also checked in various starch preparations. The purpose was to see the status of starch phosphorylation in the tubers at various stages of development. This study was extended to the several potato cultivars that were routinely micropropagated in our laboratory. With respect to the above biochemical attribute profile was made for each cultivar variety and finally a comparison also made among the cultivars. This type of study could help further to find correlation between starch phosphorylation and cold sweetening phenomenon.

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1. INTRODUCTION

The potato is the most popular and widely used vegetable in the world. It belongs to family Solanaceae**.** The potato is the only member of the Solonaceae of outstanding agricultural importance. This family includes herbs, shrubs, trees, or vines. The plants in this family are dicotyledons and are of considerable importance for food, drugs, weeds, and poisonous plants**.** The genus *Solanum* comprises some 2000 species and is very variable in habit, about 170 species produce underground stem tubers**.** The potato forms an indispensable item of daily food and is an important source of nutrition parity. Today potato is the fourth most important food crop in the world. Potato is one of the highest calorie-yielding crops in the world. Such root crops are particularly valuable in the tropics where most of the population depends on carbohydrate foods as dietary staples.

1.1 ORIGIN

The first edible potatoes are considered to have been originated in the Peruvian and Bolivians Andes of South America, where it was consumed for many millennia. The South American Indians were in fact able to select alkaloid-free potato varieties. Later, Spanish explorers carried these potatoes to Europe in the late 16th century and from there, they spread potatoes throughout the world. By the 19th century it had spread throughout the Indian sub-continent.

Initially, the crop was used as a medicinal plant and grown by pharmacists, in Spain in particular. Merchants and kings, who encouraged the cultivation of this efficient plant to increase local agricultural production, later introduced it to other parts of Europe. The successful introduction of this new crop did not only require changes in the dietary habits of the people, but also a biological adaptation of the crop to a new climate. In fact, the

potato plant being originally adapted to short day conditions of the tropical highlands, it
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would yield very little under the long summer days in Europe. Breeding over more than
150 years led to plants tolerating long day conditions. The modern breeding of potatoes
began approximately in 1780, where crossings were performed between local varieties. At
the beginning of the 19th century, the introduction of new potato germplasm, especially
from Chile, contributed highly to the breeding of modern varieties. Towards the end of the
last century, there was already a large array of breeding varieties available to the breeders.
However, because of the need for new resistance genes against pests and diseases, the 20th
century brought about the use of a large population of wild- and cultivated potato species
from South America for backcrossing into European varieties. The potatoes of today in
Europe are largely the result of the intensive breeding programs of the 19th century, but
have benefited greatly from the improvements in breeding techniques of the 20th century to
improve traits like disease resistance, tolerance to environmental factors.

1.2 THE BIOLOGY OF POTATO PLANT

The Scientific name of potato is ***Solanum tuberosum* L.** It belongs to family Solanaceae,
genus *Solanum* and species *tuberosum.* The first cultivated potato species were diploid. The
development of the modern varieties was related to the spontaneous occurrence of
tetraploid species that were superior in yield.
The common potatoes (*Solanum tuberosum* L.) consist of two subspecies. The most
common cultivated species is a tetraploid, with chromosome number of 48 (4n=48), which
is thought to have arisen as a hybrid between the diploid species ***S. stentotomum*** and the
diploid weed ***S. sparsipilum*** with subsequent chromosomal doubling.

1.3 CHARACTERISTICS OF POTATO PLANT

Solanum tuberosum L. is an herbaceous cultivated as an annual crop, and is susceptible to

frost and freezing. It is a cool season crop. The commercially significant portion of the
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plant is the tuber, which is a fleshy, swollen, rounded, oval or oblong distal portion of
underground axillary or adventitious branches. Underground stem is called as stolon.
Swelling causes when stolon ceases to elongate. Swollen region of an underground stem is
modified due to the translocation and storage of photosynthates (carbohydrates), which
occurs as the aerial portion of the plant reaches maturity. Tubers are used in commercial
propagation since the true seeds are heterozygous and highly variable but used primarily in
crop improvement.

Root System - Potatoes produce a fibrous root system arising from initials along the
underground portion of the stem. These roots can extend to a depth of around 20 inches,
but are not highly effective in penetrating the soil layers in search of water. True seed
potatoes produce a tap root system

Eyes - The eyebrow is a leaf scar, which always faces the apical end of the potato.
Eyebrows are highly concentrated at the apical end. The eyes on a potato are the growing
points for new plants. Each tissue layer is connected to each eye. Eyes appear in higher
concentration nearest the "rose end" of the tuber, decreasing in size and concentration
towards the "heel end" where a stolon scar is visible. During the growth of the tuber, the
eyes remain dormant.

Stem (basal) end - the stem (basal) end is the attachment point between the tuber and the
plant. The stem cannot produce new plants. As the plant matures, the stem weakens and lies
prostrate, eventually yellowing and dying back at the end of the growing season.

Leaf – leaf pattern is pinnately compound, alternate, with 7-9 ovate leaflets (one terminal
leaf). The margins are serrated or entire.

Flower - the potato has a perfect flower, which contains both male and female flower parts.
It can be white, purple, lilac, or violet depending on the variety of potato. A significant
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amount of potato cultivars are either pollen sterile or fail to set fruit because of some other
means.
True seed - If fertilization is successful, a small green fruit ball (to 1 ¼" diameter) is
produced. The fruit is a berry with seeds in a mucilagenous pulp. Seeds are flattened and
ovate, around 300 seeds per fruit. Cross-pollination is manipulated for crop improvement in
quality and insect or disease resistance. However, many potato cultivars are pollen sterile,
which creates difficulty in breeding programs.
Temperature - Potatoes are affected by differences in temperature. Tuberization occurs
earlier at lower temperature. The optimal temperature for tuberization is 55°F, the process
decreases above 70°F and with certain cultivars, may stop at 85°F. Higher temperatures
may often induce knobbiness and secondary growth in tubers. Maximum yields may be
obtained with an average growing temperature between 60° and 65°F. These cooler
temperatures cause the rate of respiration to be lower than the rate of photosynthesis,
resulting in more accumulation of carbohydrates.
Photoperiod - A majority of potato cultivars are not day length sensitive. Cool night
temperatures are important because they affect the accumulation of carbohydrates and dry
matter in the tubers. A misconception is that potatoes contain alkaloid solanine, while the
fact is that only green potatoes contain it Potato plants and tubers contain the toxic
glycoalkaloids, alpha-solanine, and alpha-chaconine, which act as cholinesterase inhibitors.
When tubers are exposed to light, chlorophyll along with the glycoalkaloids is synthesized.
The amount of glycoalkaloids formed depends on exposure length, intensity and light

quality (mostly ultraviolet), and temperature; little is synthesized at temperatures below
41°F. These compounds taste bitter, and ingestion can cause illness even death in extreme
cases, toxicity depends on the amount ingested.
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1.4 NUTRIENT PROFILE OF POTATO

The main nutritional value of and tubers lies in their potential ability to provide one of the
cheapest sources of dietary energy, in the form of carbohydrates Potatoes are the most
important 'vegetable' in the world today, after cereals the tubers provide the main source of
carbohydrate. Potato contains high nutritive value. Potatoes yield 17 - 21% fresh weight of
starch and 0.5 - 1.2 % of pure protein.

Food Value Minerals and Vitamins

Moisture - 74.7 % Calcium - 10 mg
Carbohydrates - 22.6 % Phosphorus - 40 mg
Protein - 1.6 % Iron - 0.7 mg
Minerals - 0.6 % Vitamin C - 17 mg
Fat - 0.1 %
Fibre - 0.4 %
*Values per 100 gm edible portion

Small amount of Vitamin B complex, Vitamin A and P is also present. Misconceptions
regarding the nutritional value of potato need to be dispelled to increase domestic
consumption. Most people think that potatoes cause obesity, while it has only 0.1 per cent
fat. It absorbs fat during frying. The potato can be boiled, baked, steamed and cooked with
other vegetables. But it should be cooked in such a way so as to retain all its excellent
qualities. To secure its maximum value, it should always be cooked with its skin as the
most nutritive part of the potato lies just below the skin and this particular layer is very rich
in protein and mineral salts´
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1.5 Natural Benefits and Curative Properties

The potato contains several medicinal virtues. As it is one of the most strongly alkaline of

all foods, it is, therefore, very helpful in maintaining the alkali reserve of the body and a
natural antidote for an overdose of acid or acidosis.
However, obese people should avoid fried potatoes as they are fattening. They should also
be omitted from the diet of those suffering from venereal diseases and those afflicted with
aphrodisiac tendencies. The potatoes green in color contains an alkaloid toxin known as
solanine, which affects the nerves controlling the sexual organs.
An exclusive potato diet is considered valuable in the treatment of certain disorders such-as
chronic constipation, intestinal toxaemia, uric acid diseases, renal calculi or stone and
dropsy. The potato is regarded as an excellent food remedy in scurvy. Raw potato juice
helps to eliminate an “an acid condition” and relative rheumatism, skin blemishes and
swellings. Potato juice also relieves gastritis. Stomach ulcers are treated with juice of pink
potatoes.
Potato starch is administered as an anti-inflammatory for gastro-intestinal diseases and
toxins. The skin is exceptionally rich in vital mineral salts and the water in which the
peelings have been boiled is one of the best medicines for the ailments caused by excess of
acid in the system. Raw potato juice externally applied is valuable in the treatment of
swelling and other disordered conditions of the joints and muscles.

1.6 MICROPROPAGATION AND CULTIVATION

Tissue Culture is currently being used to perpetuate disease free seed stock, which can then
be stored "*in vitro*" until needed. This method of micropropagation is meant for rapid
distribution maintenance of existing cultivars and to bulk up new breeding lines for
germplasm storage and transport. Furthermore, the molecular analysis of plantlets, which

propagate and stored *in vitro*, shows that micropropagation gives genetically stable
plantlets. Another approach for micropropagation of potatoes is the *in vitro* induction of

micro-tubers. Although considerable research has been performed on micro-tubers, their
use on commercial basis in the potato industry has been rarely formulated. They can be
stored several months and easily transported unlike *in vitro* plantlets. Thus, they are ideal
for germplasm distribution as they can conform to long quarantine procedures. They can be
directly sown into the soil. Unlike propagation of *in vitro* plantlets, they do not need
subculturing to fresh media. They can be produced in bulk in any season.
Nevertheless, the cost of producing one micro-tuber is relatively higher than that of an *in*
vitro plantlet and has thus restrained its uses only for gene resource preservation.
Consequently, several experiments have been aimed on the factors controlling the
tuberization process in view of implementing those findings in the development of rapid
and cost effective methods of producing micro-tubers on a large-scale basis. These factors
are environmental, hormonal, nutritional and physiological in nature.
The plants growing *in vitro* have tender, young root lacking root hairs, which need to be
hardened. Hardening and acclimatization comprise of transition from completely controlled
conditions to uncontrolled field conditions.

1.7 POTATO PROCESSING

The potato (*Solanum tuberosum*) is a major world food crop, surpassed by only wheat, rice,
and corn in world production for human consumption. Potato is used in livestock feed and
many processed foods, such as potato chips, curls, flakes, fires, thickening agents, and
alcoholic beverages. Potato tubers also have a number of industrial uses like potato starch
provides a tough resilient coating for paper and textiles.

In general, tubers with a high dry matter, high amylose to amylopectin ratio, small cell size
and low sugar content is preferred for most processing uses, including baking or frying.
Potatoes with a low dry matter are best used boiled because they tend to remain intact. The

starch composition tends to have a low amylose to amylopectin ratio. Such potatoes, when
baked, tend to have a moist texture. However, not all potato varieties available in country
are suitable for processing.
Potatoes are typically classified into three main types: round whites, red-skinned and
russets, red-skinned cultivars generally have low dry matter content (low specific gravity)
and are used for fresh market consumption. Round white cultivars with high specific
gravity and fewer reducing sugars are used for chip processing, whereas round white types
with lower specific gravity are used for table stock. Long russet types (high specific
gravity) are used for frozen processing and dehydration. The long russet is the major type
used for table stock. In addition, all fresh market types should be uniform and have an
attractive appearance: good size, smooth shape and no external defects.

1.8 BIOSYNTHESIS AND OTHER ATTRIBUTES OF STARCH

Keeping in view with the objective of present dissertation work, several attributes of potato
starch including its biosynthesis are discussed briefly in the following few sections. The
discussion is mostly focused on potato starch.
Starch is very important substance, both to plants and humans. For humans, it is a vital
component of our diet, and an important commercial product. Starch and its derivatives are
already widely employed in the manufacture of paper, textiles and adhesives, and due to
their biodegradable and renewable nature they are increasingly being considered as an
environmentally-friendly alternative to the use of synthetic additives in many other

products, including plastics, detergents, pharmaceutical tablets, pesticides, cosmetics and
even oil-drilling fluids.
Starch is the major storage carbohydrate (polysaccharides) in higher plants, being the end
product of photosynthesis. As during the growing season, the green leaves collect energy

from the sun. In potatoes this energy is transported as a sugar solution down to the tubers,
and down there, the sugar is converted to starch in the form of tiny granules occupying
most of the cell interior.
It is believed that starch biosynthesis in potato tubers is developmentally regulated process
in the amyloplast of the tubers. The synthesis of starch in plant cells begins with the
enzyme ADP-glucose pyrophosphorylase (AGPase), which catalyses the reaction of
glucose-1-phosphate with ATP to form ADP-glucose (liberating pyrophosphate). The
ADP-glucose is then used a substrate by starch synthase enzymes, which add glucose units
to the end of a growing polymer chain to build up a starch molecule (releasing the ADP in
the process). Branches in the chain are introduced by starch branching enzymes (SBEs),
which hydrolyse 1,4-glycosidic bonds, and in their place, create 1,6 bonds with other
glucose units. Starch is composed of two polymers, an essentially linear polysaccharide
amylose, and a highly branched polysaccharide - amylopectin.

Amylose - The constituent of starch in which anhydroglucose units are linked by
∝-(1∅ 4)-D glucosidic bonds to form linear chains. Amylose molecules are typically
made from 200-2000 anhydroglucose units. Aqueous solutions of amylose are very
unstable due to intermolecular attraction and association of neighboring amylose
molecules. This leads to viscosity increase, retrogradation and, under specific conditions,
precipitation of amylose particles.

Amylopectin - A constituent of starch having a polymeric, branched structure. In addition
to ∝-(1∅ 4)-D bonds that are present in amylose and the linear segments of amylopectin,
the amylopectin molecule has ∝-(1∅ 6) bonds, which occur every 20-30 anhydroglucose
units. Aqueous solutions of amylopectin are characterized by high viscosity, clarity,

stability, and resistance to gelling.
For many commercial uses it is desirable to alter the proportions of amylose and
amylopectin found in starch. High-amylose starches are useful in confectionery (because
they thicken rapidly), in fried snacks (because they resist the penetration of cooking oil),
and in photographic film (because of their toughness and transparency). It has also been
suggested that the nutritional properties of bread can be improved by the use of flour high
in amylose. Amylopectin is preferred in paper-making and adhesives (because its branched
chains give it greater binding power), and in frozen food (because it enhances stability and
shelf-life.
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1.8.1 Composition of Potato Starch

Potatoes are well known for being starchy, and a potato tuber comes close to being 100%
starch storage parenchyma**.** Potato starch is second most important and abundant starch of
the world. It is the major carbohydrate reserve in plant tuber in the form of granules. Starch
is used as a raw material in the food, pharmacy, cosmetics, paper and plastics industries.
The composition of potato starch is given below:

Starch, dry substance 80 %
Water 20 %
Ash 0.3 %
Sand 0.02 %
Protein 0.09 %
Phosphor, P 0.07 %
Calcium, Ca 0.03 %
Iron, Fe 3 ppm
Cold water solubility 0.1 %

1.8.2 Phosphorylation of starch

Starches contain phosphorus in some form or another. The nature of bound phosphorus
affects starch performance. The phosphate content in potato tubers is exceptionally high.
Small starch granules contain 25 % more ester-bound phosphate per glucose residue than

large starch granules. The phosphate groups are located as monoesters at mainly C-3 & at
the C-6 position of the glucose monomers. It is believed that the phosphate content
influences the overall secondary structure of starch granules.
Starch biosynthesis is accomplished by different form of starch synthase, which polymerize
the glucose monomer using ADP- glucose, and isoforms of branching enzymes, which
introduce the branch points. Other enzymes are needed, to determine the final starch

structure, e.g. the presence of a debranching enzyme is a prerequisite to synthesize the
semi-crystalline starch granules. More phosphorylation in the starch is related to its more
degradability, particularly at low temperature leading to more cold sweetening. However
the mechanism of starch phosphorylation is not completely known.

1.9 NEED AND PROBLEMS

Potato plants are grown in moderate climate, therefore, continuous production of tubers is
not possible round the year. In summers as the temperature rises sprouting, microbial
spoilage and senescence-induced sweetening, weight loss, rottage etc. cause the damage to
the tubers. As a result, major part of potato tubers need to be stored at lower temperatures
for a certain period of time to prevent damage.
A crucial feature of the post-harvest physiology of tubers of the potato plant
is a process called cold-induced sweetening, i.e. the accumulation of the reducing sugars
fructose, glucose and sucrose during tuber storage at low temperatures. It results from
partial starch breakdown. Potatoes with high reducing sugar levels pose problem for the
food industry. The reducing sugars react with proteins and amino acids to form dark
products in a non- enzymatic browning reaction after frying. Hence high levels of these
sugars cause discoloration, changes in flavor and loss of essential amino acids in processed
products made from stored tuber slices. Therefore, due to the growing demand of the

processed potato products, important parameters for the selection of raw material in potato
processing industries should have dry matter of the tuber up to 21 to 23 % and reducing
sugar content below 150 mg / 100 g fresh tuber weights.
At present we need to focus on agricultural research to ensure adequate productivity of
potato crop, both for fresh consumption and processed potato products. At the same time
we need to find out proper means to overcome cold storage problems of the harvested

tubers. At the moment, we need to understand the phenomena of cold-sweetening process
at biochemical levels. We should focus on the potato cultivars suitable to Indian agroclimatic
conditions.

2. REVIEW OF LITERATURE

As potato is not grown round the year, a major portion is required to be stored at low
temperature (2- 4^{0}C). This storage prevents sprouting, microbial spoilage and senescence
induced sweetening, weight loss and rottage etc.
Potatoes are the most important 'vegetable' in the world today - after cereals the tubers
provide the main source of carbohydrate. Secondary uses include the production of starch
and dextrose, industrial alcohol by fermentation, and spirits. Potatoes yield 17 – 21 % fresh
weight of starch and 0.5 - 1.2 % of pure protein. The importance of this crop was realized
soon after independence. The Central Potato Research Institute, Shimla since its
establishment in 1949 has been doing comprehensive potato breeding programme to
develop improved cultivar varieties. They bred and released many high yielding varieties
so far, which are suitable for different agro-climate regions. Most of these varieties have
been bred for consumption as fresh potatoes. However fresh varieties required for
processing should have the following criteria. Tuber dry matter in the range of 21 to 23 %.

Reducing sugar content below 150 mg per 100 gm fresh tuber weight and reasonably good
yield to provide economic returns to the farmer (Singh *et al*., 1999).
In general, the potato tubers from different cultivars vary with respect to the following
characteristics: dry matter content, starch content, protein content, starch yield and mean
particle diameter (μm) of the starch granules (Jansen *et al*., 2001) etc.

2.1 STARCH IN POTATO TUBERS

Starch is the major predominant storage form in potatoes. Potatoes starch is, in many ways,
a superior starch and its many interesting properties make it attractive for both food and
industrial application (Alexander, 1995). Starch synthesis in storage organs occurs over a

long developmental period, during which there are usually considerable changes in the
complement of starch-synthesizing enzymes (Smith and Martin, 1993; Burton *et al.*, 1995).
Starch is composed of two polymers amylose and amylopectin. The amylose molecules are
essentially linear ∝-(1∅ 4)D glucan chains, whereas the amylopectin molecules are highly
branched and often contain covalently-bound phosphate (Hizkuri *et al.*, 1970).

2.2 PHOSPHATE CONTENT IN STARCH

In this section phosphorylation of potato starch is mainly discussed. The phosphorus in
tuber starches is in the form of phosphate monoesters. The phosphate groups are located as
monoesters at the C-6 and at the C-3 position of the glucose residues in the amylopectin are
phosphorylated (Hizkuri *et al.*, 1970).
The low degree of phosphorylation has a profound effect on the qualitative properties of
the starch changing e.g. the gelatinization temperature, viscosity, retrogration and solubility
(http://www.plbio.kvl.dk/plbio/starch.htm). Starch phosphorylation proceeds concurrently
with the *de novo* biosynthesis of starch in potato tubers (Nielson *et al*., 1994).
Small starch granules contain approximately 25 % more bound phosphate per glucose
residues than large granules, whereas the overall level of phosphorylation does not depend

upon the tuber size (Nielsen *et al*., 1994). Majority of the phosphate is bound to the
amylopectin fraction of starch (Hizkuri *et al*., 1970).
The starch-bound phosphate constitutes a major part of the total phosphate pool in the
potato tubers (Quick *et al*., 1976). The level of phosphorylation may vary approximately
two folds among different potato cultivars and depends upon the growth conditions (Nikuni
et al., 1969). On an average, one of every 200-500 glucose residues is phosphorylated
(Nielsen *et al*., 1994).

2.3 COLD INDUCED SWEETENING

Potatoes are stored in cold-stores (2-4oC). This storage prevents the moisture loss, decay,
and early sprouting while removing respiratory heat. Storage extends the availability and
thereby assists with orderly marketing, distribution, and utilization. Whereas storage can
extend the usefulness of harvested potato crops, quality does diminish proportionally to the
length of storage. As during storage at low temperatures, a process known as **cold
sweetening** occurs in the tuber, i.e. starch is broken down to soluble sugars such as sucrose,
glucose and fructose. Hence, it is very desirable to reduce cold-sweetening as potatoes with
high sugar levels pose problem for the food industry. It is thought that the cold-induced
hexose accumulation is caused by an imbalance between starch breakdown and glycolytic
activity. Several explanations are also there related to cold-induced sweetening in the
potato tubers.

´ ^ An increased concentration of hexose phosphates as a result of cold lability of
phosphofructokinase and other glycolytic enzymes.

´ ^ The starch is thought to be e degraded mainly by the phosphorylytic route, based on
the relative activities of phosphorylase and amylolytic enzymes. In other words, an
increase in the activity of one or more starch degrading enzymes leads to above

process.
´ ^ Increased activity of invertases or the enzymes involved in sucrose synthesis.
(Isherwood, 1973; Pollock and Rees, 1975; Guy *et al.*, 1992; Deiting *et al.*, 1998)
The only reported example of turnover (the simultaneous occurrence of synthesis and
degradation) in storage organs is in transgenic potatoes in which the flux of carbon into
starch was increased 6-fold by elevating ADP-Glc pyrophosphorylase activity 17
(Sweetlove *et al*., 1996). However, little, if any, turnover was observed in the wild-type
tubers. This is consistent with earlier findings (Dixon and ap Rees, 1980).

2.4 Possible correlation between starch phosphorylation and cold-sweetening

Various attempts have been made currently in order to identify enzymes/proteins
involved in starch synthesis and its modification. For this, starch bound proteins were
isolated from potato tubers and antisera raised against them for screening of potato cDNA
expression libraries. Recently, a cDNA clone has been isolated and characterized. It is 4851
bp in size encoding starch-granule-bound protein R1, found to cause phosphorylation.
Starch-granule-bound R1 protein involved in starch biosynthesis and modification is an
alpha-glucan, water dikinase (GWD). The enzyme catalyses the phosphorylation of starch
by a dikinase-type reaction in which the beta-phosphate of ATP is transferred to either the
C-6 or the C-3 position of the glycosyl residue of amylopectin. Starch phosphorylation
seems to be an integrated activity in starch biosynthesis. Moreover, potato tubers with
repressed GWD showed significant changes in starch structure.
The R1 protein is involved in determining the phosphate content of potato starch. A major
observation made is the reduction of the phosphate monoester content of the starch
synthesized in the transgenic lines down to 10 % as compared with wild-type plants. This

indicates that the R1 protein is responsible for the phosphorylation of starch, which is also
supported by the fact that the expression of the protein in *Escherichia coli* leads to elevated
phosphate content of the synthesized glycogen. Similarities to PEP synthase in the
C-terminal part of the R1 sequence could be taken as an indication that R1 mediates an
ATP-dependent starch phosphorylation by a Dikinase type of reaction.
The biochemical mechanism by which the R1 protein phosphorylates amylopectin remains
to be elucidated. It is not known which substrate acts as phosphoryl donor, nor which type

of glucans are phosphate acceptors. Evidence that phosphoenol pyruvate may act as a
phosphoryl donor comes from slight sequence homology of the R1 protein to the PEP
synthases from different bacteria.
The overall secondary structure of starch from transgenic tubers with lowered levels of R1
protein is altered, that led to less starch degradation during cold storage. It was found that
the level of reducing sugars was up to nine folds less after two months of storage at 4$_{o}$C as
compared to the wild type tubers. In other words, repression of cold sweetening was noted
in transgenic tuber (Loberth *et al*., 1998).
In our country, no comprehensive biochemical and molecular approaches have been
adopted so far to understand and inhibit the process of cold-induced sweetening in potato
tubers suitable to our own agro-climatic conditions. However, the details of starch
phosphorylation in the developing potato tubers are not clearly known at biochemical and
molecular level. Now rigorous attempts should be made to understand the cold sweetening
phenomenon at biochemical level. Attempts to be made to understand the overall starch
phosphorylation status in the developing mini-tubers from the above varieties and to find
out the varietal differences with respect to this attribute. At the same time, we need to

understand the metabolic aspects of starch phosphorylation at biochemical and molecular
level.
19
Keeping in view with the cold sweetening problem and its possible correlation with
starch phosphorylation status in potato tubers, the following objectives were formulated for
this dissertation work:
´ ´ ^ ^ Micropropagation of various potato cultivars, hardening, acclimatization and
cultivation in the field
´ ´ ^ ^ Harvesting of developing and mature mini-tubers from field grown plants
´ ´ ^ ^ Isolation of starch granules from the mini-tubers of various potato cultivars
´ ´ ^ ^ Determination of total phosphorus in starch samples among different potato cultivars
´ ´ ^ ^ Comparison of the status of the starch phosphorylation in the various developing tubers
with in a particular variety as well as among different varieties
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3. MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 PROCUREMENT OF THE MATERIALS

The germplasm of various potato cultivars such as Kufri Chipsona-1 (CS-1), Kufri
Chipsona-2 (CS-2), Kufri Chandramukhi (KCM), Kufri Jyoti (KJ), Kufri Pukhraj (PR)
Kufri Ashoka (AS) were procured from central potato research institute (CPRI), Shimla
except cultivar Desiree and routinely maintained in our laboratory on modified MS
medium.
The required chemicals were purchased from Sisco Research Laboratory Pvt. Ltd. Mumbai,
Qualigens Fine Chemicals, Merck, CDH, New Delhi, HiMedia Laboratories Pvt. Ltd.
Mumbai and Various glasswares from Borosil and plasticwares from Tarson Pvt. Ltd.
Kolkata. All the chemicals used in this study were of analytical grade (AR).

3.1.2 MEDIA USED IN MICROPROPAGATION

Shoot Multiplication Medium (SM-1):

Major salts concentration (mg/l)

KNO_3 1900
NH_4NO_3 1650
$MgSO_4.7H_2O$ 370
$CaCl_2.2H_2O$ 440
KH_2PO_4 170
Minor Salts
$MnSO_4.H_2O$ 22.3
$ZnSO_4.7H_2O$ 8.6
H_3BO_3 6.2
21
KI 0.83
$Na_2MoO_4.2H_2O$ 0.25
$CuSO_4.5H_2O$ 0.025
$CoCl_2.6H_2O$ 0.025
Na_2Fe-EDTA 30.0
Additives
Thiamine HCI 0.4 mg
Nicotine Acid 0.5 mg
Pyridoxin HCI 0.5 mg
Ca-Pantothenate 2.0 mg
Glycine 2.0 mg
Myo-inositol 100 mg
Sucrose 30 mg
NAA 0.01 mg
BAP 0.01 mg
GA_3 0.25 mg
Agar 0.7 % (w/v)
pH adjusted to 5.8

3.1.3 Phytohormones

For Cytokinins: Dissolve cytokinin such as BAP (benzylaminopurine), in 2.5 ml of 0.5N HCl, heat gently and made to the volume. Adjust the pH to about 5.0. Concentration of stock solution of BAP was 0.1 mg/ml.

For Auxins: Dissolved auxin such as 2,4-D (2,4-Dichlorophenoxyacetic acid) and NAA (α-Naphthaleneacetic acid) in 2.5 ml of 95 % ethanol or 2.5 ml of 1N KOH, stirred gently and made up the volume by adding distilled water. Adjusted the pH to 5.0 and stored at 4^oC. Concentration of stock solution of 2,4-D was 1 mg/ml and concentration of stock solution of NAA was 0.1 mg/ml.

Gibberellic acid was prepared by dissolving it in distilled water.

3.2 METHODS

3.2.1 PREPARATION OF SHOOT MULTIPLICATION MEDIUM

→SM-1 is a modified MS medium and was prepared by adding all the components in
required amounts.
→The pH of the medium was adjusted at 5.8 and then agar was added to it at a
concentration of 0.7 % (w/v).
→This was then melted and ~50.0 ml of the medium was dispensed in each tissue
culture bottle.
→These bottles were then autoclaved at 121^{0}C for 20 minutes at 15 psi, followed by
their storage at 25^{0}C.

3.2.2 MICROPROPAGATION OF POTATO CULTIVARS

Micropropagation of different potato cultivars such as Kufri Chipsona-1, Kufri
Chipsona-2, Kufri Ashoka, Kufri Pukhraj, Kufri Chandramukhi, Kufri Jyoti, and CV
Desiree was done on SM-1 media on a routine basis.
→The laminar airflow chamber was thoroughly cleaned with alcohol.
→Under aseptic conditions, young and tender plantlets of the above said varieties
were taken out on a sterile glass plate, with the help of sterile forceps. These
plantlets were not surface sterilized as these were already being maintained under *in*
vitro conditions.
→Using a sterile scalpel, the roots of these plantlets were excised.
→The leaves were removed and finally the shoot part was cut into small segments,
each segment retaining at least one node.
→Maintaining the correct polarity of the cut fragments, these were individually
inoculated in the solidified MS-1 medium (About 10-12 such explants were
inoculated per bottle).

→The bottles were sealed and labeled carefully and were finally kept in the culture
room under maintained conditions of temperature (25ºC) and light (16 hrs. light,
8 hrs. dark) and the growth of the inoculated explants was monitored regularly.

3.2.3 HARDENING AND ACCLIMATIZATION

→All the above rooted potato varieties maintained by tissue culture was gently
washed with warm water to remove the agar from the roots.
→These plantlets were treated with 1 % (w/v) Bavistine for 5- 10 and were transferred to the plastic protrays containing the potting mix Solirite.
→These plantlets were further shifted to highly humid conditions in a polyhouse for
two weeks with the temperature maintained at 25^0C and relative humidity of 85 to
90 %.
→These hardened plantlets were then shifted to shade house for 6-7 days with
temperature maintained at 25^0C and relative humidity of 65 to 70 %.

3.2.4 CULTIVATION OF PLANTLETS IN THE FIELD

→These plantlets were then cultivated in the field in different bed for different
varieties.
→Their growth was monitored regularly along with the application of water, fungicides and fertilizers whenever required.
→Initially after two months, minitubers of varying sizes from each variety were
harvested, and then tubers were harvested after regular interval of time of 14-20
days with their increasing developmental stages.
→After harvesting, every time minitubers were washed gently with water, dried on
the folds of blotting paper carefully, to avoid mixing.
→Finally various studies were carried out on them.

All the potato varieties were cultivated in the same agroclimatic conditions of sunlight,
relative humidity, water balance, fertilizer dose etc.

3.2.5 ISOLATION OF STARCH GRANULES FROM POTATO TUBERS

The protocols that were followed for the isolation of starch granules is as follows:
→Weighed 6.0 g – 10 g of potato tubers and crushed them using pestle and mortar to
form a paste; formed a uniform using ice-cold double distilled water.
→Filtered the homogenous slurry through a double-layered muslin cloth (which was
washed with double distilled hot water) into a 50 ml of centrifuge tubes and made
up the volume to 50.0 ml using ice-cold water.

→ Incubated the tubes in ice for atleast 4-5 hours and then decanted the supernatant
carefully to avoid any loss of starch granules.
→ The white colored starch granules were then mixed with nearly 30.0 ml of ice-cold
double distilled water, mixed thoroughly and centrifuged at 5000 to 6000 rpm for
10 minutes to give the first water wash.
→ Similarly gave a second water wash and decanted the supernatant without disturbing the pellet.
→ To the pellet added 15.0 ml of acetone, mixed and vortexed the solution and centrifuged at 6500rpm for 15 minutes to give the first acetone wash. Similarly
gave second wash and finally decanted the supernatant carefully.
→ Air-dried the starch granules by incubating the tubes at 37$_o$C overnight.
→ Finally weighed the dried starch granules and quantified them per 100 g fresh
tuber weights.

3.2.6 EXPERIMENT TO CHECK THE PRESENCE OF ANY FREE PHOSPHATE IN STARCH SAMPLES

The phosphate content in potato tubers is exceptionally high as compared with
other plant storage organs. The phosphate groups are bound as monoesters at the C-6 and
at the C-3 positions of the glucose residues. Therefore, the phosphate present in the
25
potato starch is the bound phosphate. However, to check if there is any free phosphates in
starch, following experiment was carried out:
→ Nearly10.0 mg of starch powder was thoroughly suspended in water and kept for
20-25 minutes at room temperature, so that the free phosphate, if any, would come in to the solution.
→ Then the suspension was centrifuged at full speed and the supernatant was assayed for phosphorus by ascorbic acid-ammonium molybdate assay. However,
the results showed that amount of free phosphate in the supernatant was negligible
and that was almost comparable to the blank sample.

3.2.7 DETERMINATION OF TOTAL STARCH-BOUND PHOSPHORUS

In this method the phosphomolybdate complex is reduced by ascorbic acid.
Essentially, this method is based on the principle of colorimetric estimation of inorganic

phosphate. Inorganic phosphate reacts with ammonium molybdate in an acid solution to
form phosphomolybdate complex, addition of a reducing agent like ascorbic acid, reduce
the molybdenum, in the phosphomolybdate complex, to give a blue color. In this reaction,
the uncombined molybdic acid will remain unaffected. This ascorbic acid method is very
sensitive, by which one can easily determine inorganic phosphate as low as 0.01
micromole.
Here, we need to determine the total starch-bound phosphate. For this ashing procedure of
starch was adopted to determine bound phosphorus. Complete ashing enables the
conversion of organic phosphates to inorganic phosphates. Basically, the ashing procedure
was coupled with the very sensitive inorganic phosphate method.
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(A) REAGENTS

REAGENT A: Ascorbic acid, 10 %
Weighed 1.0 g of ascorbic acid and dissolved in 10 ml of distilled water.
REAGENT B: 0.42 % Ammonium molybdate.$4H_2O$ in 1 N H_2SO_4.
Weighed 0.084 g of ammonium molybdate, to it added 0.572 ml of concentrated H_2SO_4
and then volume made up to 20 ml.
MIX: Mixed 1.0 ml of reagent A and 6.0 ml of reagent B before use.
STANDARD KH_2PO_4 (100 mg P in 1.0 liter solution i.e. 100 ppm)
Dissolved 87.9 mg of KH_2PO_4 in 80 ml of distilled water, then added 5.0 ml. of 7 N H_2SO_4 to it and then volume made up to 200 ml.
MAGNESIUM NITRATE: Weighed 0.4 g of Magnesium nitrate [$(MgNO_3)_2.6H_2O$]
dissolved in 4.0 ml of 95 % alcohol.

OTHERS (as describe in the methods):

0.1 N H_2SO_4, Conical flask, Pipettes, Micropipette, Water bath, Dispensor, Centrifuge
tubes, Glass rod, microfuge tubes, Centrifuge, Test tubes, Bunsen burner, Weighing
balance, Butter paper, Spatula etc.

(B) PROCEDURE

→ Weighed 5.0 to 7.0 mg of starch granule, isolated from potato tubers, and suspended
in 1-2 drops of water. The material was taken to dryness and completely charred

over a strong flame of gas burner.
→ The tubes were then allowed to cool and 0.4 ml of 0.1 N H_2SO_4 was added. The
tubes were tightly capped with plastic caps and heated in boiling water bath for 10
minutes, to ensure solubilization as well as conversion of any pyrophosphate formed during ashing, to inorganic phosphate.
27
→ The solution was allowed to cool and then transferred to 1.5 ml microfuge tubes
from glass tubes, then rinsed the glass tubes by addition of 0.1 N H_2SO_4 and volume
made up to 0.5 ml.
→ Sequential addition was done in the following way, for a reaction volume of 0.5 ml:
˜ ˆ 300 µl aliquots of the sample taken for assay.
˜ ˆ 180 µl of 0.1N H_2SO_4
˜ ˆ Made up the volume to 900.0 µl by addition of water
˜ ˆ In case of blank, 900 µl of 0.1N H_2SO_4 and 900 µl of water were mixed together
→ To each reaction tube, added 2.1 ml of the MIX and to the blank 4.2 ml of MIX,
vortexed the tubes and incubated in water bath at 45^0C for 20 minutes.
→ Cooled the tubes and read absorbance at 820nm.
The procedure was repeated for three times with almost reproducible results.
NOTE:
(i) The Standard Curve: For the preparation of standard curve KH_2PO_4 (100 ppm)
solution was used and the following amounts of phosphorus were taken in different tubes as
given here: 1.5 µg, 3.0 µg, 4.5 µg, 6.0 µg, 7.5 µg, 9.0 µg. The other ingredients and
reaction volume in each case were same as followed in the unknown samples.
(ii) Other ashing procedures:
→ Few starch samples were treated with few drops of water or conc. H_2SO_4 and then
charred in the muffle furnace at very high temperature of 600^0C – 615^0C for two
hours using silica crucible. The results were almost comparable as obtained by the
ashing procedure discussed in the previous section.
→ The charring of a few starch samples was also carried out by making suspension in

alcoholic magnesium nitrate [$Mg(NO_3)_2.6H_2O$] solution followed by assay of Pi as
described earlier. The purpose was to check the sensitivity of the assay procedure.
Nearly comparable data was obtained in all these ashing procedures.
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4. RESULTS AND DISCUSSION

Tuber development in potatoes is a very complex biological event. Various types of
metabolic activities are operational, till date detailed mechanism both at biochemical and
molecular level is not known clearly. In addition varietal differences are also there. Starch
phosphorylation occurs during tuber development and status of starch phosphorylation is
believed to play a crucial role with respect to cold-sweetening phenomenon in the potato
tubers during prolong storage at low temperatures. So far we do not know clearly the status
of starch phosphorylation in the tubers of our own potato cultivars. Keeping in view with
the above, here attempts have been made to know the status of starch phosphorylation in
the developing as well as mature tubers. In addition, the starch phosphorylation was also
studied in the potato tubers after transferring to 20^0C for four weeks. Mini-tubers at various
stages of development were harvested from field-grown seven cultivar varieties for this
study. The results obtained are given in the following sections.

4.1 MICROPROPAGATION

The following potato varieties namely Kufri Chipsona-1, Kufri Chipsona-2, Kufri Ashoka,
Kufri Pukhraj, Kufri Chandramukhi, Kufri Jyoti, CV Desiree were routinely
micropropagated on the shoot multiplication medium (SM-1), which was modified MS
medium. It contains various phytohormones such as auxin, cytokinin and gibberlin. Profuse
shoot growth was observed within three weeks of inoculation, along with the root growth to
some extent. Young and tender leaves started appearing on the stem within the same time
period as shown in the **Fig. 1, 2, 3 and 4.** The growth of the inoculated explants was

observed regularly and these were checked for contamination. Usually contamination was
less frequent. Further subculturing was carried out for every four to five weeks.
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30
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4.2 HARDENING, ACCLIMATIZATION AND CULTIVATION

The micropropagated plantlets with tender roots were hardened and acclimatized in the
potting mix-soilrite for two weeks. Soilrite consisted of peat moss to absorb water and
vermiculite along with perlite helps in aeration. While this step was carried out, the
planlets were carried under high humid conditions, to prevent dessication. Finally, these
were planted on the trial plots within our own campus. Weeds were manually removed
when required. The moisture level was maintained by spraying water almost twice a day,
under the dry weather conditions.
Growth characteristics of different cultivars were noted time to time are given below:
Kufri Chipsona-1 (CS-1): Green short plants with moderately thin stems, tripinnately
compound leaves with smooth lamina.
Kufri Chipsona-2 (CS-2): Dark green, erect and tall plants with compound leaves,
undivided and rough lamina and smooth margins. Dense growth was observed.
Kufri Chandramukhi (KCM): Short and light green plants with thin stem, tripinnately
compound leaves and plane lamina.
Kufri Jyoti (KJ): Short to moderate tall, light green plants with thin stem, small to very
large, shiny, compound leaves. Lamina was curved inside with pointed apex.
Kufri Pukhraj (PR): Moderately tall, dark green plants with thick stem compound leaves
with thin and large lamina and smooth margins. Apex was slightly blunt.
Kufri Ashoka (AS): Light green plant with bushy appearance, thick stem, thin compound
leaves with pointed apex and smooth lamina and margins.
Cultivar Desiree (DE): Short to moderately tall, light green plants with both simple and

compound leaves. Lamina was not smooth.
A few field-grown plants are shown in **Fig. 5, 6, 7 and 8**
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33
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4.3 HARVESTING OF MINI-TUBERS AT VARIOUS STAGES OF DEVELOPMENT

In this study seven important potato cultivars were selected to carry out experiments. Minitubers
were harvested from the field-grown plants of the above varieties. It may be noted
here that properly hardened and acclimatized plantlets of various potato cultivars served as
planting material in the field. Plantation was carried out in the second week of Nov. 2003.
The first maturing tuber samples (referred to as TS-01) from various cultivars were
collected after nearly eight weeks of cultivation. The weight of these TS-01 mini-tubers
from different varieties was in the range of 0.3 g – 0.4 g. Similarly, at different time
intervals growing mini-tubers from all these varieties were collected (referred to as TS-02
to TS-07). The final harvesting of tuber samples (referred to as TS-08) was done in the first
week of March 2004 (the entire period of cultivation in the field was nearly four months).
The mature tubers were also transferred to 20^0C for 4 weeks, which are referred to as
TS-09. A few harvested mini-tubers are shown in **Fig. 9,10,11 and 12.** Various potato tuber
samples as harvested above along with their range of weights are given in the **Table 4.1**

Table 4.1 Various potato mini-tuber samples

S.No.	Tuber Sample	Range of Tuber Weight
1.	TS-01	0.3 g - 0.4 g
2.	TS-02	0.8 g - 1.0 g
3.	TS-03	1.3 g - 1.5 g
4.	TS-04	2.5 g - 2.7 g
5.	TS-05	10.0 g - 11.0 g
6.	TS-06	14.0 g - 15.0 g
7.	TS-07	20.0 g - 22.0 g
8.	TS-08	30.0 g - 32.0 g
9.	TS-09	28.0 g to 30.0 g

35
36
37
In each potato variety, the weight of the developing minitubers was found to be
proportional to their size. Morphologically differences were noted with respect to the
shape and size of minitubers from different cultivars. The minitubers were more or less
spherical in shape with delicate tuber skin and light brown in color, except in the case of
cultivar Desiree of which tubers were reddish in color.

4.4 QUANTIFICATION OF STARCH GRANULES IN THE TUBERS

Here a rapid, convenient and simple method was adopted for isolation of good quality of
starch granules (section 3.2.5) from various tuber samples (TS-01 to TS-09) of each potato
cultivar. It may be mentioned here that extensive washing steps were employed using both
cold water and organic solvent like acetone during the isolation of starch granules. This
helped to remove various organic and inorganic impurities in the starch preparations.
Weight of each starch preparation was taken after extensive drying at 37$_0$C. The starch
granules recovered were clean, white-colored, and powdery in form. The starch yield was
then calculated in gm per 100 gm of tuber weight as shown in the **Table 4.2.**
.

Table 4.2 Starch yield from potato tubers at different developmental stages

(Calculated in gm per 100 gm of tuber weight)

Variety	TS-01	TS-02	TS-03	TS-04	TS-05	TS-06	TS-07	TS-08	TS-09
CS-1	5.7	4.5	4.5	4.8	6.5	8.9	9.8	13.9	6.3
CS-2	5.6	4.7	6.2	7.0	6.6	10.8	12	6.8	7.5
KJ	5.0	3.8	4.7	7.8	4.2	6.5	6.8	6.1	4.7
KCM	3.4	5.6	5.3	5.9	7.7	11.6	9.3	8.8	7.8
PR	3.0	5.3	3.7	4.1	4.9	5.3	8.0	7.7	7.5
AS	3.5	3.0	7.5	4.2	7.3	8.1	6.6	11.5	9.0
DE	4.1	5.0	4.7	4.5	7.8	9.8	9.6	12	7.6

TS – Tuber Sample

Close inspection of the data given in the above table shows that in almost all varieties
starch yield was found to increase in the later stages of development. The maximum starch
yield of was noticed in TS-08 of Kufri Chipsona-1 (13.9 g /100g). Whereas minimum
starch yield (3.0 g /100 g) was noted in TS-01 of Kufri Pukhraj and TS-02 of Kufri Ashoka.
However, suitable processing varieties like Kufri Chipsona 1, Kufri Chipsona 2 and Kufri
Ashoka showed more starch yield as tuber dry matter could be directly correlated with
starch content.
In all the above varieties storage of tubers samples at 20^0C leads to decrease of starch yield
as compared with the tubers before storage. Although it is not true for all, the low yield is
possibly due to starch degradation, respiration and other metabolic activities functional in
the potato tubers.

4.5 DETERMINATION OF TOTAL PHOSPHORUS FROM STARCH GRANULES

Starch is composed of two polymers, amylose and amylopectin. The amylose molecules are
essentially linear, where as amylopectin molecules are highly branched and often contain
some amount of covalently bound phosphate. In addition, amylopectin depending upon the
plant organ where it is manufactured contains different level of phosphate monoesters. The
phosphate content in potato tubers is exceptionally high as compared with other plant
storage organs. The phosphate groups are localized as monoesters at the C-6 and C-3
positions of the glucose residues in starch. Here attempts have been made to determine the
total starch-bound phosphate in the tubers of different potato cultivar in its various
developmental stages. The purpose is to know the phosphorylation status in various starch
preparations. The total bound-phosphate i.e. organic phosphates were converted into

inorganic phosphate through complete ashing procedure on the gas burner. Then phosphate
39
was determined by the Ascorbic acid-ammonium molybdate method. Another ashing
procedure carried out was by, charring of few starch samples in the muffle furnace by using
silica crucible at 600-612$_0$C for two hours. Nearly comparable data was obtained in both of
the ashing procedures The starch bound-phosphate was estimated in the tubers at various
stages of development from seven varieties. Here, the amount of total starch-bound
phosphorus was determined per 100 g of starch granule preparations. For each variety as
discussed earlier, the amount of bound phosphorus was measured from all starch
preparations as shown in **Table 4.2.**
In case of Kufri Chipsona-1, the amount of total phosphorus was found to vary
considerably as shown in **Fig. 13.** The bound phosphorus content was found to be higher in
the starch preparations isolated from very early maturing tubers (around 60 mg per 100 g of
starch). In case of Kufri Chipsona 2, the phosphorus content was found to be in the range of
30 mg to 40 mg per 100 g of starch (**Fig. 14).** The four cultivars namely Kufri Jyoti, Kufri
Chandramukhi, Kufri Pukhraj and Kufri Ashoka showed similar pattern. The phosphorus
content more or less was lying in the range of 30 to 50 mg per 100 g of starch as shown in
Fig. 15, 16, 17 and 18. It may be noted that phosphorus content appeared to be in the
higher side as compared with Kufri Chipsona-1 except its early maturing stages. In the case
of Kufri Ashoka maximum phosphorus content was noted at the early stages of tuber
development. It is of interest to note that phosphorus content was highest i.e., 70.0 mg in
the very early stage and then decreased notably in the other stages of development in case
of cultivar Desiree as shown in **Fig. 19.** All these data collectively suggest that varietal

difference does exist with respect to starch-bound phosphorus content. It is apparent that
starch phosphorylation occurs throughout the tuber development. The level of this
biochemical activity seems to be higher in the early stage of development. At present the

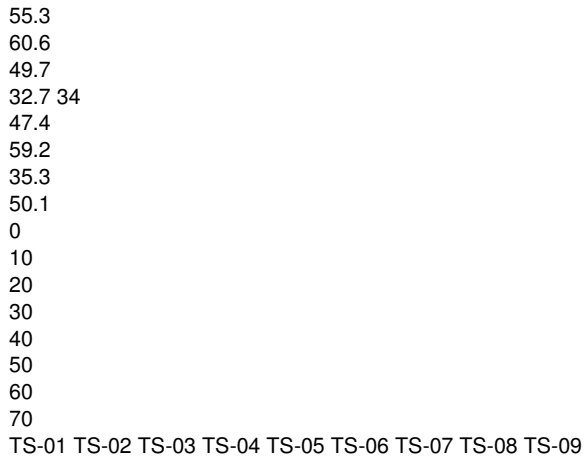

Fig. 13. Comparison of total phosphorus content in various starch samples

Cultivar: Kufri Chipsona-1

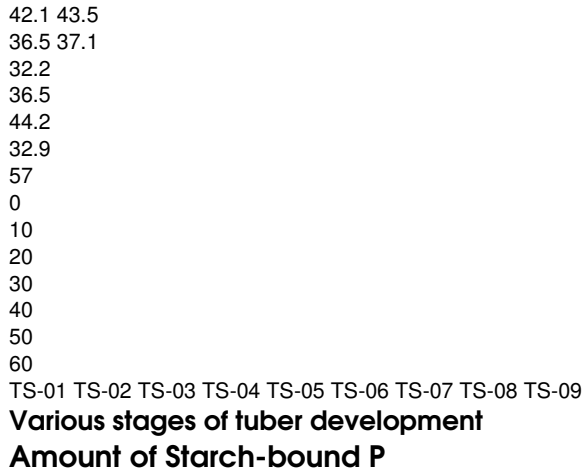

Fig. 14. Comparison of total phosphorus content in various starch samples

Cultivar: Kufri Chipsona-2

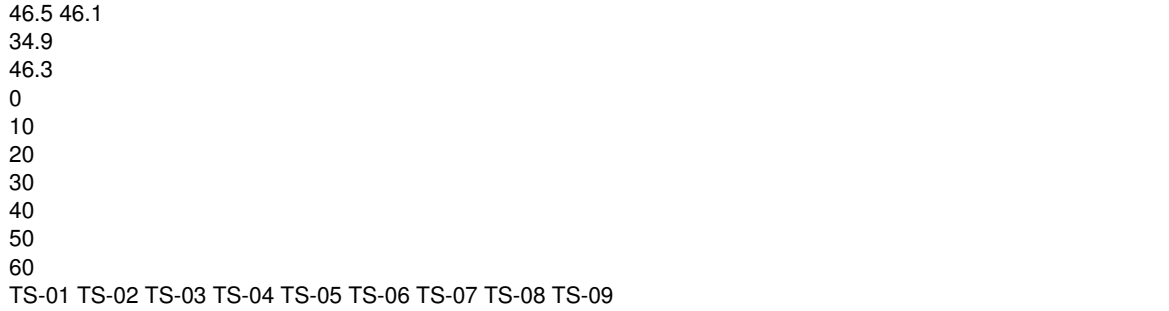

Fig. 15. Comparison of total phosphorus content in various starch samples

Cultivar: Kufri Jyoti

Fig. 16. Comparison of total phosphorus content in various starch samples

Cultivar: Kufri Chandramukhi

25
30
35
40
45
TS-01 TS-02 TS-03 TS-04 TS-05 TS-06 TS-07 TS-08 TS-09
Various stages of tuber development
Amount of Starch-bound P
(in mg)

Fig. 17. Comparison of total phosphorus content in various starch samples

Cultivar: Kufri Pukhraj

50.8 51.4
35.2 35.5
44.9
41.4 41.2
50.7
46.5
0
10
20
30
40
50
60
TS-01 TS-02 TS-03 TS-04 TS-05 TS-06 TS-07 TS-08 TS-09
Various stages of tuber development
Amount of Starch-bound P
(in mg)

Fig. 18. Comparison of total phosphorus content in various starch samples

Cultivar: Kufri Ashoka

43
70.6
50
43.4 42
35.6
38.2
40.5
31
43
0
10
20
30
40
50
60
70
80
TS-01 TS-02 TS-03 TS-04 TS-05 TS-06 TS-07 TS-08 TS-09
Various stages of tuber development
Amount of Starch-bound P
(in mg)

Fig. 19. Comparison of total phosphorus content in various starch samples

role of starch granule-bound protein (R1) possibly the known member causing phosphorylation of starch. Apart from this, other genes could be functional during tuber development. As R1 protein is known, we may focus on its expression both at the level of transcription and translation in the developing tubers. It may answer partly the varietal difference with respect to starch-bound phosphorus content. Starch-bound phosphorus content appeared to in higher side if potato tubers stored at 20^0C (here TS-09) for a few weeks. The molecular mechanism pertaining to starch degradation as well as phosphoryaltion under storage condition of potato tubers needs to be further understood.

The differential degradation of amylose and amylopectin fractions may be involved during prolonged storage. Finally, a comparative study was also made among different cultivars as shown in **Fig. 20**. In all the potato cultivars, the amount of total bound phosphorus was found to be higher along with considerable variation in the early stages of tuber development if compared with respective mature tubers. The bound phosphorus content in the starch preparations isolated from 20^0C stored tubers (TS-09) were almost comparable among the varieties and similar to the trend noted in the very early maturing tubers (TS-01). The variation within a variety may be due to alteration of the amylose to amylopectin ratio. Apart from this, it may be considered that during tuber development some other components or non-phosphorus impurities could be associated with starch granules, which were co-purified with starch preparations. The present study gives us an idea on the status of starch phosphorylation in the developing tubers from potato cultivars suitable to our own agro-climatic conditions. This will help us further to undertake biochemical and molecular studies on starch phosphorylation at developmental stages and

eventually to find out possible correlation between the status of starch phosphorylation and
cold-induced sweetening.

45
0
10
20
30
40
50
60
70
80
CS-1 CS-2 KJ KCM PR AS DE
Potato Varieties
Amount of Starch-bound P
(in mg)
TS-01
TS-08
TS-09

Fig. 20. Comparison of total starch-bound phosphorus content in the tubers of various potato cultivars

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